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Research Article

Silver Nanoparticles Synthesized with Extracts of Leaves of *Raphanus sativus* L, *Beta vulgaris* L, and *Ocimum basilicum* and Its Application in Seed Disinfection

Cristina Garcidueñas-Piña, Carolina Tirado-Fuentes, Julio Ruiz-Pérez, Roberto Carlos Valerio-García, and José Francisco Morales-Domínguez

Centro de Ciencias Básicas, Universidad Autónoma de Aguascalientes, Av. Universidad #940, Ciudad Universitaria, Aguascalientes 20131, Ags, Mexico

Correspondence should be addressed to José Francisco Morales-Domínguez; francisco.morales@edu.uaa.mx

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Silver nanoparticles (AgNPs) were produced by green synthesis using *Raphanus sativus* (*Rs*AgNPs), *Beta vulgaris* (*Bv*AgNPs), and *Ocimum basilicum* (*Ob*AgNPs) leaf extracts as reducing agents. Plant phytochemical composition analysis indicated that they contain phenolic compounds that can participate in the synthesis reaction as flavonoids. Synthesized AgNPs presented maximal absorption peak at 430 nm (*Rs*AgNP), 440 nm (*Bv*AgNP), and 420 nm (*Ob*AgNP) in ultraviolet-visible spectrophotometry analysis. Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis showed that *Rs*AgNPs are 76 nm diameter spheres made of up to 54.1% silver, the *Bv*AgNPs are 78 nm diameter spheres with 39.76% silver, and the *Ob*AgNPs are cubes of 99 nm edges with 69.74% silver. The found *Z* potential values indicate that all the obtained AgNPs are stable in phosphate buffer. A disinfection of 100% and 90% was achieved for the *in vitro* culture of *Arabidopsis thaliana* and *Psidium guajava* (guava) seeds, respectively, with all AgNPs synthesized. Treatment with these AgNPs showed no negative effects on germination, and on the contrary, in guava, a higher germination percentage was observed when the seeds were exposed to *Rs*AgNPs. Only at high concentration (10 mg/mL) of AgNPs, the growth of *A. thaliana* was decreased, while at low concentration (0.01 mg/mL) of *Ob*AgNP and *Bv*AgNPs, higher growth was showed, specifically 60 and 40% more than the control, respectively. All AgNPs showed antimicrobial activity against *Escherichia coli* and *Klebsiella oxytoca* which are bacteria of clinical interest, and against *Agrobacterium tumefaciens*, a bacterium used in the genetic transformation of plants.

1. Introduction

Silver nanoparticles (AgNPs) are currently used in different products such as textiles, cosmetics, medical supplies, household items, food additives, and for water treatment [1]. The synthesis of AgNPs can be carried out either by chemical reactions, using ascorbic acid or hydrazine as reducing agents or through green synthesis, replacing these chemicals with extracts from bacteria, fungi, algae, or plants [2]. Green synthesis is cheaper and does not generate toxic residues, and recently many publications have exposed the high efficiency of different plants in the synthesis of AgNPs (Table 1) [1–14]. Leaf extracts from cultivated plants with different purposes are used for the synthesis of AgNPs. For example, with the help of extracts of plants with nutritional elements like *Cynara scolymus* [3]; ornamental plants like *Gleichenia pectinata* [4] and *Murraya paniculata* [5]; or plants considered weeds like *Cyperus rotundus* [6], AgNPs with antimicrobial, anti-inflammatory, or anticancer activity have been synthesized.

Organic compounds from plant material, such as flavonoids, saponins, steroids, terpenoids, tannins, and phenol, provide reducing and stabilizing agents for the synthesis of AgNPs (Table 1). Leaf extracts of *Raphanus sativus* L

Table 1: Silver nanoparticles obtained by green synthesis.

Plants	Activity	Shape/size (nm)	Maximum absorbance peak (nm)	Extract compounds	Reference
Cynara scolymus leaves	Anticancer: breast cancer cells MCF7	Spherical 98.47 ± 2.04	434	Aromatic and aliphatic compounds	[3]
Gleichenia pectinata leaves	Antimicrobial: *P. aeruginosa*, *E. coli, Klebsiell pneumoniae*, and *Candida albicans*	Spherical 7.51 ± 2.88	460	Terpenoid, alcohol, and carbonyl group	[4]
Murraya paniculata leaves	Antimicrobial: *Bacillus cereus, Staphylococcus aureus*, *Escherichia coli, Candida albicans* cytotoxicity: human umbilical vein endothelial cell line	Spherical/14 ± 9	438	Alkaloids, phenolic, flavonoids, polysacchari des, and proteins	[5]
Cyperus rotundus complete plant	Antibacterial: *Escherichia coli*, *Bacilus cereus* and *C. haemolyticum*	Spherical 20.5 ± 9.6	446	Essential oils, alkaloids, flavonoids, terpenoids, chromones, phenylpropa noids, phenolic acids, and iridoides	[6]
Selaginella myosurus complete plant	Anti-inflammatory: *in vivo* and *in vitro*	Spherical 58.81	420–480	Flavonoids, saponins, steroids, terpenoids, tannins, and phenols	[7]
Fenogreek seed seeds	Antibacterial: *S. aureus*, *E. coli*	Spherical 40	396	Phenolic compounds and flavonoids (apigenin-7-O-glycoside and luteolin-7-O-glycoside)	[8]
Opuntia ficus-indica fruit	Antimicrobial: microorganisms from wastewater treatment plant. *E. coli* var 1, *Enterobacter aerogenes* var 1, *Citrobacter freudi* var 2, *atypical E. coli*, and aerobic mesophilic microorganisms	Spherical 64.28 ± 11.82	435	Not reported	[9]
Baleria longiflora leaves	Antibacterial: *Enterococcus* sp., *Streptococcus* sp, *B. megaterium, Pseudomonas putida, P. aeruginosa, Staphylococcus aureus*	Spherical 2.4 ± 0.5	443	Amines and flavonoids	[10]
Phoenix dactylifera seeds	Antitoxoplasm a: *in vivo* in mice balb/c	Irregular in shape with some spherical NPs 20	Not reported	Not reported	[11]
Malus domestica fruit	Antibacterial: *K. pneumoniae, E. coli, P. aeruginosa, Enterobacter aerogenes* anticancer: Breast cancer cells MCF7, noncancerous kidney cells HEK29.3	Spherical 50	425	Not reported	[12]
Phyllantus emblica. fruit	Antibacterial: Bacteria that infect rice *Acidovorax oryzae*	Spherical 39.1	430	Amidas, aminas, and alquenos	[13]
Prosopis fracta fruit	Antibacterial: *S. aureus, Streptococcus pneumonia, E. coli, Salmonella typhi*	Spherical 10.26–14.65	425–438	Phenolic compounds and flavonoids	[14]

(radish), *Beta vulgaris* var. cicla (chard), and *Ocimum basilicum* (basil) have compounds that can participate in the synthesis of AgNPs. Leaves of chard contain flavonoids and carotenoids [15], leaves of basil contain monoterpenes, phenylpropanes, alkaloids, glycosides, tannins, saponins, and aromatic compounds [2, 16], and leaves of radish contain tannins and coumarins [17]. *R. sativus* is an annual or biennial plant belonging to the *Brassicaceae* family that is cultivated for its root, and it is destined for human consumption [18]; *Beta vulgaris* var. cicla is a plant of the *Amaranthaceae* family that is cultivated for the consumption of leaves [19]; and *Ocimum basilicum* is an annual plant the *Lamiaceae* family that is used in the kitchen as a spice [20]. Furthermore, these plants are easily grown home or in greenhouses and are available at any time of the year.

In vitro plant tissue culture consists of placing an isolated part of a plant (referred to as an explant) or seeds in a medium with a determined chemical composition and under controlled conditions. This can be carried out with different objectives such as for micropropagation, to obtain pathogen-free plants, for genetic improvement, and for germoplasm conservation [21]. However, these culture conditions favor the development of bacteria and fungi; thus, *in vitro* culture must be performed under aseptic conditions, the material and medium must be sterilized, and the explants must be disinfected [21]. Traditional disinfection uses one or more of the following substances: ethanol, sodium hypochlorite, calcium hypochlorite, mercuric chloride, and surfactants such as Tween 20 and in some cases antibiotics [21]. The selection of disinfecting agents and the procedure depends on the type of explant or seed and must ensure the complete elimination of microorganisms without causing damage to plant tissue. AgNPs could be used as an alternative agent for the disinfection of plant material and its cultivation under axenic conditions.

The objective of this work is to obtain AgNPs by green synthesis from extracts of radish, chard, and basil leaves and to evaluate their effectiveness in the disinfection of seeds for *in vitro* cultivation. In addition, the possible negative impact of this AgNPs on plants was also analyzed.

2. Materials and Methods

2.1. Synthesis of Silver Nanoparticles. Fresh leaves of radish, chard, and basil were obtained from a local market (Aguascalientes, Ags. Mexico). The AgNPs were synthesized following the protocol described by Mahakham et al. [22] with slight modifications: 20 g of clean leaves were crushed in a blender for 20 s with 100 mL of distilled water. This preparation was first incubated in a boiling water bath for 1 h and then left overnight at room temperature. The aqueous extract was separated from the solid residues by centrifugation at 10,000g for 10 min and filtered on Whatman $^{\#}1$ paper. A volume of 10 mL of the aqueous extract was slowly mixed with 90 mL of a 1 mM AgNO3 solution (J. T. Baker™ reactive grade). This mixture was incubated protected from light, at room temperature, in an orbital shaker at 100 rpm (Shaker RK-30) until a color change was observed (after about 7 days). This preparation was centrifuged at 10,000g for 10 min (Sorval ST16 centrifuge), and the supernatant was removed to recover the AgNPs in the pellet. The nanoparticles (NPs) were washed three times with sterile distilled water: the pellet was mixed with 100 mL of sterile distilled water, this mixture was centrifuged at 10,000g for 10 min, the supernatant was removed; once with ethanol, the sediment was mixed with 100 mL of 96% ethanol, the mixture was centrifuged at 10,000g for 10 min, and the supernatant was removed. This pellet was dried at 40°C for 24 h and disintegrated by sonication (Digital ultrasonic, VEVOR).

2.2. Characterization of Silver Nanoparticles. The reduction of Ag^{+} to Ag^{0} was monitored by analyzing the reaction mixture (leaf extract 97 and AgNO3) in the ultraviolet-visible spectrophotometer (Genesys 10S ThermoScientific) in an absorption scan from 300 to 800 nm wavelength. The dry AgNPs were analyzed by scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) in a Joel JSM-5900LV equipment to observe their geometry and to determine their element composition. The SEM and EDS analyzes were performed at the High Resolution Microscopy Laboratory of the Faculty of Biology of the Autonomous University of Aguascalientes (Mexico). The size of the AgNPs was calculated by measuring the images captured by SEM with Image J2 software [23]. One hundred NPs were measured to obtain the size distribution histogram. Their hydrodynamic diameter was determined by the dynamic light scattering technique (DLS) in a Zetasizer ZS90 (Malvem) equipment. The Z potential was obtained by electrophoretic mobility using the Smoluchowski model. The analyses were performed on AgNPs colloidal mixture prepared in ultrapure water (Milli-Q) at a concentration of 100 mg/L. Measurements were taken in triplicate at times of 0, 72, 96, and 120 h.

2.3. Analysis of Anthocyanin in the Plant Extract. Anthocyanin content was calculated in triplicate with the following protocol [24]: 50 mg of 5 mm^2 leaf fragments were incubated in 10 ml of a methanol-HCl solution (99: 1) (J. T Baker), overnight at 4°C. The absorbance of the extract was measured at a wavelength of 530 (A_{530}) and 657 nm (A_{657}) with a Genesys 10 s UV-Vis de thermo-scientific spectrophotometer. The relative anthocyanin units per gram of tissue (RAU/g) were calculated with the following equation: $\text{RAU/g} = A_{530} - (0.25 \times \text{A657})$.

2.4. Phytochemical Screening. Thin-layer chromatography (TLC) was performed on silica gel 60 RP18 F254s aluminum plates (Merk, Darmstadt, Germany) as adsorbent and water-acetonitrile (7 : 3 v/v) as a mobile phase. TLC was sprayed with reagent specific for flavonoid identification; 1% methanolic 2-amino-ethyl diphenyl boric acid (Sigma-Aldrich® Co.); and 5% ethanolic polyethylene glycol 4000 (Sigma-Aldrich® Co.) and then examined by UV-fluorescence (365 nm) [25]. The compounds were detected observing orange, yellow, and blue fluorescence bands,

which are characteristic of phenolic compounds and flavonoids. Finally, the retention factor (Rf) was calculated for each compound, which was determined by dividing the displacement of the selected band by the distance traveled by the solvent. Rutin (Sigma-Aldrich ®, U.S.A) was used as reference standard. The analyzed samples were obtained as follows: 20 g of clean leaves were crushed in a blender for 20 s with 100 mL of methanol (J. T. Baker) and were incubated at 4°C for one day in the dark. Methanol was removed at 50°C on a rotary evaporator.

2.5. Total Phenolic Content Determination. Total phenolic content was determined by the Folin–Ciocalteu method. A standard curve was prepared with gallic acid ($n = 3$) at concentrations of 0, 2, 4, 6, 8, 10, and 12 μg/mL. Next, 2.5 mL of deionized water and 0.1 mL of Folin–Ciocalteu (Sigma-Aldrich) 1 N reagent were added to each tube. After 6 min, 0.25 mL of a 10% sodium carbonate (Baker analyzed) solution was added to each sample and vortexed. The absorbance of each sample was monitored at 760 nm using a spectrophotometer (Jenway, Genova). From these readings, a line equation ($y = 0.0288x - 0.007$) and the correlation coefficient ($R^2 = 0.997$) were calculated to extrapolate samples. The same treatment was carried out with the methanolic extract of each plant to determine its content of phenolic compounds.

2.6. Evaluation of Seed Disinfection with AgNPs. Guava (*P. guajava* cv "media china") seeds were obtained from fresh fruit, washed with distilled water and dried at 25°C for one week. *Arabidopsis thaliana* (Arabidopsis, Columbia ecotype) seeds were collected from plants grown in the Plant Molecular Biology Laboratory of the Autonomous University of Aguascalientes. Dry seeds of guava and Arabidopsis were treated in seven groups with different condition as follows: group 1 were disinfected with a solution of Tween 20 at 0.15% and sodium hypochlorite at 2%; group 2, 3 and 4 were mixed with 0.01 mg/mL each AgNPs in sterile distilled water; and group 5, 6 and 7 were mixed with 1 mg/mL of each AgNPs. For each group, 40 seeds of guava and 10 mg of seeds of Arabidopsis were treated. Seeds were incubated in the dark, at room temperature, with agitation for 24 h. Seeds were sown in MS-agar culture medium [26], in the dark until germination, and afterward in a 16 h-light photoperiod for 2 weeks. Germination, elongation of the seedlings, and the percentage of contamination cultures were measured.

2.7. Analysis of Seed Exposure to AgNPs. Guava and Arabidopsis seeds were disinfected with a solution of 0.15% Tween 20 and 2% sodium hypochlorite and incubated in water at 4°C in the dark for 24 h. Forty seeds were incubated in MS broth culture medium with 0, 0.01, 1, 2, 5, 10, and 20 mg/mL of each of the AgNPs and in MS-broth culture medium with 1 mg/L of benzyladenine as a control. They were incubated at 25°C in 16-h light photoperiods for two weeks.

2.8. Evaluation of Antimicrobial Activity. The antimicrobial activity of the synthesized AgNPs was analyzed following the next protocol: [27] bacterial suspensions with a titer of 1×10^6 colony forming units per milliliter (CFU/mL) in 0.05 M potassium phosphate buffer pH 7 were exposed to 1 mg/mL AgNPs. An aliquot of each suspension was inoculated after 1, 2, 4, 8, and 24 h of exposure onto solid LB-agar plates and incubated for 16 h. Grown colonies were counted to calculate CFU/mL after exposure to AgNPs as well as logarithmic inactivation. The bacteria mentioned in Table 2 was analyzed under the conditions indicated.

2.9. Statistical Analysis. Anthocyanin content and seedling growth were analyzed by comparison of means by one-way ANOVA followed by Tukey's test. The germination of the different treatments was compared using a Chi square test. In both analyzes, statistical significance was set at $p \leq 0.05$. The statistical analysis was carried out with the statistic software StatSoft (version 10), and the graphs were made in Excel.

3. Results and Discussion

3.1. Green Synthesis of Silver Nanoparticles. AgNPs were synthesized using leaf extracts of radish (RsAgNPs), chard (BvAgNPs), and basil (ObAgNPs) as reducing agent. The plant extract mixtures with $AgNO_3$ showed a change in color after 7 days of incubation (Figure 1(a)). The chard extract changed from pale yellow to reddish brown; that of basil extract turned from intense yellow to dark brown; and that of the radish extract changed from pale yellow to metallic gray (Figure 1(a)). The first indication of the formation of AgNPs is the change in color, due to the reduction of Ag^+ ions to Ag^0 [5] and to an excitation of the free electrons of the silver particles [3, 7]. Generally, the AgNPs suspension is yellow or orange if the particles are not aggregated, and gray if they are aggregated [5]. The reaction mixture was analyzed by UV-visible spectrophotometry. This analysis showed a characteristic plasmon and maximum peaks of 430 nm (*Rs*AgNPs), 440 nm (*Bv*AgNPs), and 420 nm (*Ob*AgNPs) (Figure 1(b)), which is in the range reported for other AgNPs (Table 1). AgNPs interact with light through a phenomenon known as surface plasmon resonance; the point of maximum absorbance depends on the size and shape of the NPs, which in turn depend on the synthesis conditions [28].

3.2. Phytochemical Screening. In green synthesis of AgNPs, $AgNO_3$ donates the silver ions and plant extracts provide the reducing and stabilizing agents [29]. Phenolic compounds reduce the silver salt to form NPs, while they themselves are oxidized.6 Flavonoids in the enol form are oxidized to their keto form [6]. Phenolic compounds, proteins, and chlorophyll adhere to the surface of the NPs [30]. These compounds also act as stabilizing agents covering and protecting the formed AgNPs, preventing their agglomeration [6, 30]. Alkaloids, flavonoids, saponins, and sugars are examples of plant extracts components that provide the necessary conditions for the biosynthesis of AgNPs [2]. Organic

Table 2: Bacteria used in the evaluation of the antimicrobial activity of AgNPs.

Bacteria/plasmid	Antibiotic resistance	Incubatin	Interest
Escherichia coli DH5α/pUC18	Ampicilin	37°C	Clinical
Klebsiella oxytoca	No	37°C	Clinical
Agrobacterium tumefaciens EHA 105/pBI121	kanamycin, rifampicin, streptomycin	28°C	Agronomic

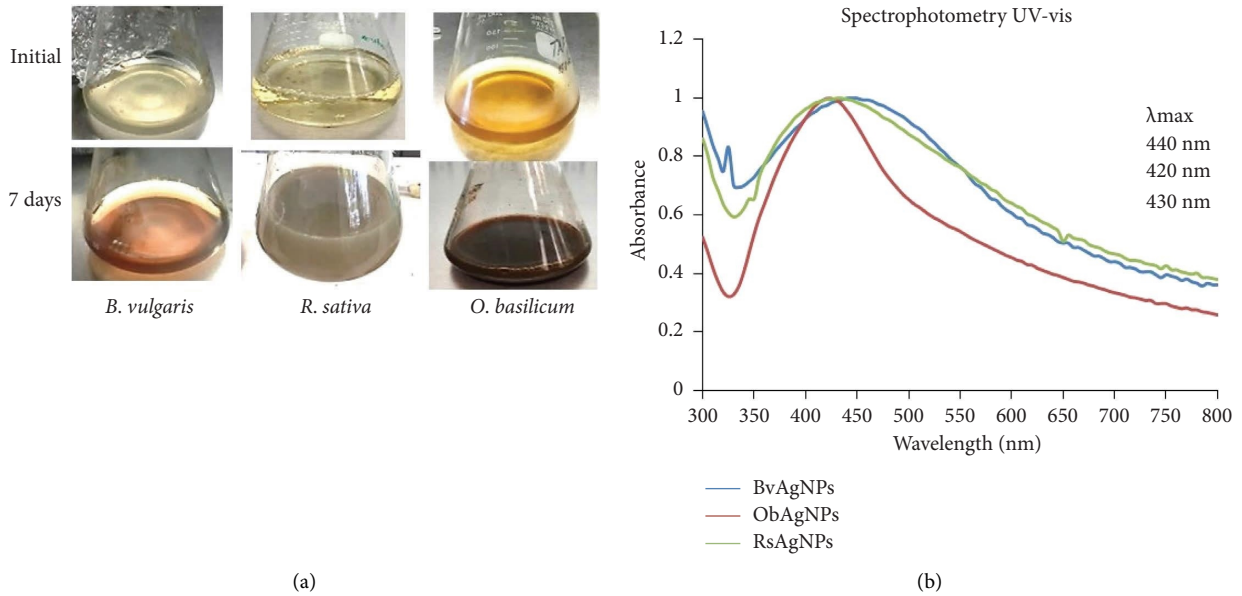


Figure 1: Analysis of biosynthesized AgNPs from plant extracts of *B. vulgaris*, *O. basilicum*, and *R. sativa*. (a) AgNPs synthesis reaction mixture ($AgNO_3$ + plant extract), a color change was observed after 7 days of incubation. (b) UV-visible spectrophotometry, the plasmon of each AgNPs showed a maximum peak at a different wavelength.

compounds bind to the metallic component of AgNPs through the functional groups -C=O-,-NH_2, and -SH- [29]. The plant extracts fulfill a double function, both for the reduction and to stabilize AgNP [30]. We analyzed the content of phenolic compounds in the plant extracts (Table 3). The results showed that basil extracts have a higher content of phenolic compounds, approximately 10 times more than that found in radish and chard (Table 3). Coumarins, lignins, lignans, tannins, phenolic acids, and flavonoids are phenolic compounds that are produced by plants as defense mechanisms against environmental conditions [30]. Anthocyanins are phenolic compounds from the flavonoids group [31], so it was decided to quantify them (Table 3). They protect the plant from the excess of reactive oxygen species and have antioxidant, antimicrobial, and medicinal properties [32]. Anthocyanins were detected in the three plant extracts analyzed, though in different amounts (Table 3). Anthocyanin content was similar in basil and radish leaves (0.14 RAU/g), while in the chard leaves a smaller amount was found, approximately half (0.07 RAU/g) (Table 3). Plant extract from each plant was analyzed by TLC for flavonoids. These compounds are observed as an orange, yellow, or blue fluorescence band (Figure 2). Rutin is another flavonoid found in many plants. This flavonoid has antioxidant, anti-inflammatory, cardiovascular, neuroprotective, antidiabetic, and anticancer properties [31]. Results of TLC indicate that the chard and radish extracts contain rutin (Figure 2). The phenolic compounds of the extracts showed quantitative (Table 3), and qualitative differences (Figure 2), which leads us to suppose that the composition of the extracts determines the final characteristics of the AgNPs.

Table 3: Components of plant extracts.

Plants	Total phenolic content (mg GAE/g)	Anthocyanin (RAU/g)
B. vulgaris (chard)	26.773 ± 4.289	0.07 ± 0.008
R. sativus (radish)	33.192 ± 9.199	0.149 ± 0.037
O. bacilucum (basil)	240.83 ± 20.301	0.148 ± 0.053

mg GAE/*G* = mg of gallic acid equivalents/gram of dry material; RAU/g = relative anthocyanin units per gram of tissue.

3.3. Characterization of Silver Nanoparticles. SEM analysis allowed to observe the morphology and size of biosynthesized AgNPs (Figure 3). The *Bv*AgNPs had lamellar structures with a thickness on the nanometric scale and aggregates of NPs with an irregularly shaped, almost spherical (Figure 3(a)), with a diameter of 78 ± 17 nm (Figure 3(b)). *Ob*AgNPs were obtained as nanometer-thick sheets and cubes (Figure 3(c)) of approximately 99 nm per edge (Figure 3(d)). The *Rs*AgNPs produced were spherical (Figure 3(e)) with an approximate

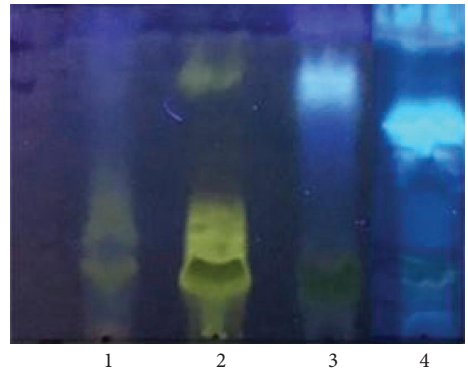


Figure 2: Separation by thin layer chromatography: (1) chard extract, (2) rutin, (3) radish extract, and (4) basil extract. Stationary phase: silica gel 60 RP18 F254s and mobile phase: water/acetonitrile (70 : 40 v/v).

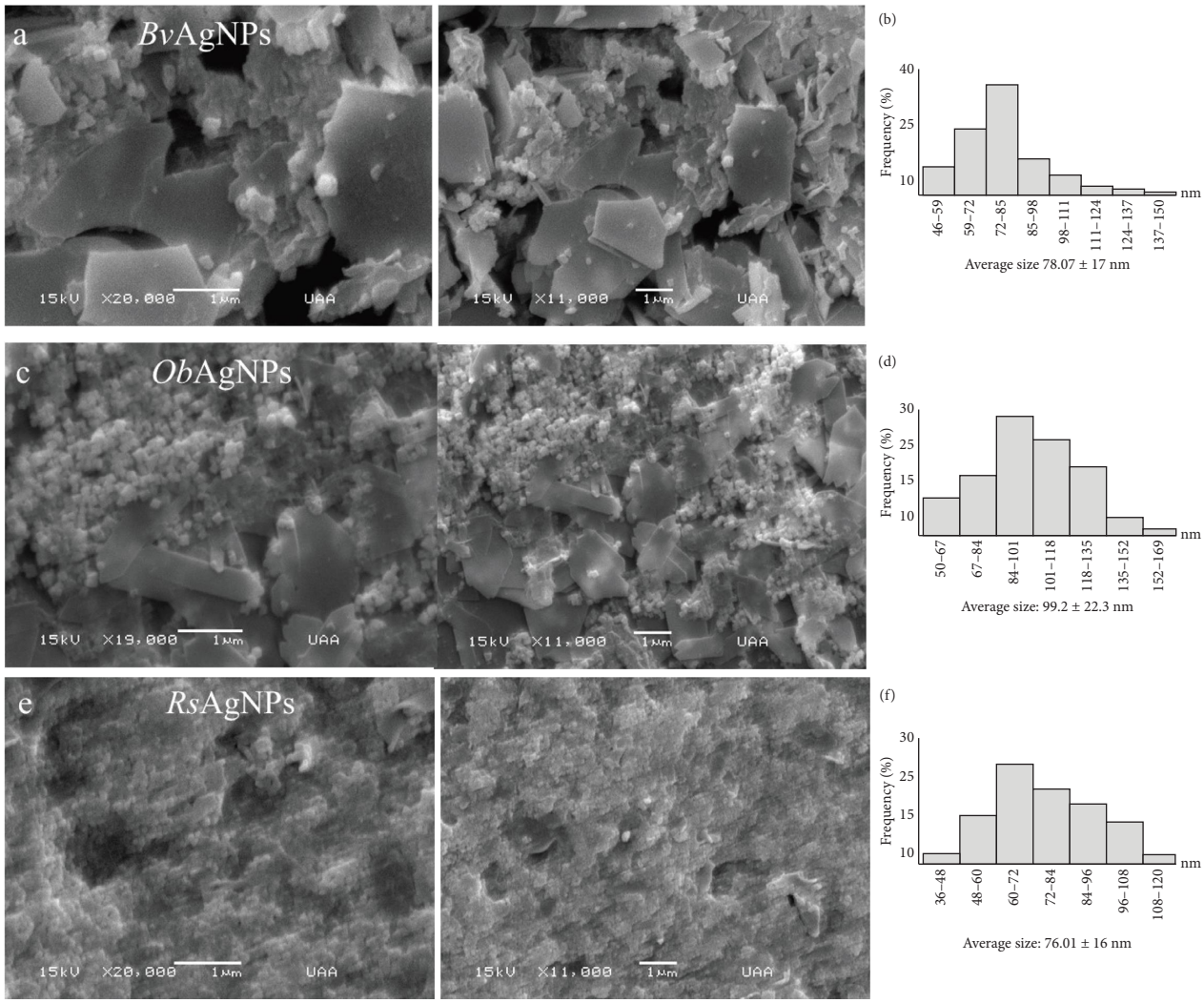


Figure 3: Analysis of morphology and size of AgNPs. Scanning electron microscopy photographs and size distribution histograms of AgNPs synthesized from (a, b) *B. vulgaris* (*Bv*AgNPs), (c, d) *O. basilicum* (*Ob*AgNPs), and (e, f) *R. sativa* (*Rs*AgNPs).

diameter of 76 ± 16 nm (Figure 3(f)). These differences highlight the influence of the components of the plant extracts on the size and shape of the AgNPs, since other factors that also affect these characteristics, such as temperature, pH, AgNO3 concentration, and synthesis method, [33] were kept constant. There are many reports that AgNPs obtained by biosynthesis are spherical (Table 1), such as *Rs*AgNPs. However, AgNPs with other morphology, like irregular,

triangular, hexagonal, polyhedral, scaly, floral, rod, and pentagonal shapes have also been produced [28], such as those we observed in *Bv*AgNPs and *Ob*AgNPs.

The EDS analysis showed that silver is the main component of the synthesized AgNPs, with making up the content of 39.76% for *Bv*AgNPs (Figure 4(a)), 69.74% for *Ob*AgNPs (Figure 4(b)), and 54.1% for *Rs*AgNPs (Figure 4(c)). Other elements such as carbon, oxygen, chlorine, and calcium were found in the AgNPs obtained. Furthermore, iron was found in *Bv*AgNPs, magnesium and sulfur in *Ob*AgNPs, and boron and copper in *Rs*AgNPs. In green synthesis, the NPs are generated as a metal core covered by organic matter from the plant extract. These elements are part of the molecules that cover the biosynthesized NPs and that are provided from the plant extract, which give them stability and increase their dimensions. [3, 4, 8] In other AgNPs, in addition to silver, other elements from the plant extract have been found, such as carbon [3, 8], oxygen [3, 8], nickel [8], silicon [4, 8], calcium [8], potassium [3, 4, 8], sodium [3, 8], and aluminum [4].

The stability of the AgNPs was analyzed by determining the potential *Z*. The found *Z* potential values were −23 (*Ob*AgNPs), −24 (*Bv*AgNPs), and −32 mV, (*Rs*AgNPs) (Table 4). A negative *Z* potential represents the electrical charge of the AgNPs surface and is indicative of stability [3]. The electrical charge is provided by the organic matter from the plant extract and that covers the AgNPs [33]. With these *Z* potential values, it can be affirmed that the AgNPs are stable, since they are between −18 and −31 mV, like those reported in AgNPs synthesized from *P. guajava* leaves [34]. The hydrodynamic diameter was calculated to determine the dimensions of the AgNPs. The hydrodynamic diameter values were 158.7 nm (*Bv*AgNPs), 104.1 nm (*Ob*AgNPs), and 118.8 nm (*Rs*AgNPs) (Table 4). These values were determined in phosphate buffer, where AgNPs acquire a dipolar electrical layer on their surface that increases their dimensions. Therefore, these values were higher than those calculated by SEM, as has been reported in other works (Figure 3). For example, in AgNPs synthesized from *Opuntia ficus-indica* peel extract, an average size of 64 nm was determined by SEM and 220 nm by hydrodynamic diameter [9].

3.4. Seed Disinfection for In Vitro Cultivation. The disinfection capacity of AgNPs in Arabidopsis and guava seeds was evaluated. The seeds were treated with the AgNPs and compared with a traditional disinfection treatment. The percentage of disinfection of *P. guajava* seeds was 90 (*Bv*AgNPs), 89 (*Ob*AgNPs), 95 (*Rs*AgNPs), and 85% (traditional disinfection treatment). Arabidopsis seeds showed 100% disinfection with all the different treatments. With both seeds, no significant differences were observed between the treatments with the AgNPs and the traditional disinfection treatment. In another work, similar results were obtained, a 100% disinfection of Lamiacea seeds with AgNPs synthesized from extracts of *Alkanna tinctorum* rhizomes roots and *Syzygium aromaticum* flowers was achieved [35]. Our results support the idea that bisynthesized AgNPs could be used to disinfect seeds for *in vitro* cultivation.

3.5. Germination of Seeds Treated with AgNPs. The effect of AgNPs on seed germination was evaluated. *A. thaliana* and *P. guajava* seeds were exposed to different concentrations of the three types of AgNPs, and their germination was compared with those of the control group. *A. thaliana* germination was 94 ± 1.7% on average and remanded similar with all treatments. While with the *P. guajava* seeds treated with 1 mg/mL of AgNPs, a germination of 60% (*Rs*AgNPs), 45% (*Bv*AgNPs), 55% (*Ob*AgNPs), and 37% (control) was obtained. (Figure 5). Statistical analysis indicates a significantly higher germination in the seeds treated with the *Rs*AgNPs. Guava seeds have a very hard coat that limits the passage of water, so the percentage of germination under natural conditions is around 7%. [36] Therefore, we suppose that the *Rs*AgNPs could be facilitating the access of water to the seeds of *P. guajava*. Something similar was observed in rice seeds treated with AgNPs synthesized from kaffir lime extracts where a higher germination was obtained because the NPs increased the enzymatic activity that favors the entry of water into the seed [22]. However, germination inhibition has been observed in the germination of lettuce [37] and *Mimusops laurifolia* [38] seeds due to exposure to AgNPs. These contradictory effects are related to the dimensions and concentration of the AgNPs applied to the seeds. [35, 36, 39] The *Rs*AgNPs presented a more homogeneous spherical shape and a smaller size compared to the *Bv*AgNPs and *Ob*AgNPs. (Table 4), which may have caused the lower observed germination results.

3.6. Effect of AgNPs on Seedling Growth. Guava and *A. thaliana* seedlings obtained after seed exposure to AgNPs showed similar growth compared to controls (Figure 6(a)). AgNPs remain attached to the surface of the seed, so they did not produce effects on the growth or morphology of the seedling. This coincides with what was observed in *Lamiaceae* seeds exposed to biosynthesized AgNPs, which did not show effects on development [35]. In seeds of *A. thaliana* germinated in medium with 0.01 mg/mL of *Bv*AgNPs and *Ob*AgNPs, the seedlings showed greater elongation than the control, by 60% and 39%, respectively (Figures 6(b) and 6(c)), while with the *Rs*AgNPs no effect was observed at that concentration. The *Bv*AgNPs and *Ob*AgNPs were observed to be less homogeneous than those of *Rs*AgNPs, which together with others such as size and silver content (Table 4) could have contributed to the results obtained in plant elongation. (Table 4). It was also observed that the seedlings exposed to 1 mg/mL grew the same as the control, while those exposed to 10 mg/mL were 25% (*Bv*AgNPs), 31% (*Ob*AgNPs), and 45% (*Rs*AgNPs) smaller in size than those of the control. (Figures 6(b) and 6(c)). The hormetic effect of AgNPs was previously observed in sugarcane explants grown in medium supplemented with AgNPs [40]. The authors reported an increase in the number and length of the shoots at concentrations lower than 0.1 mg/mL, and toxicological effects with 0.2 mg/mL of AgNPs [40]. At low concentrations, AgNPs provide N, Mg, and Fe as nutrients for the plant, without being harmed by silver, while at high concentrations of AgNPs, the plant is unable to counteract the oxidative stress induced by silver. [40] In addition, high

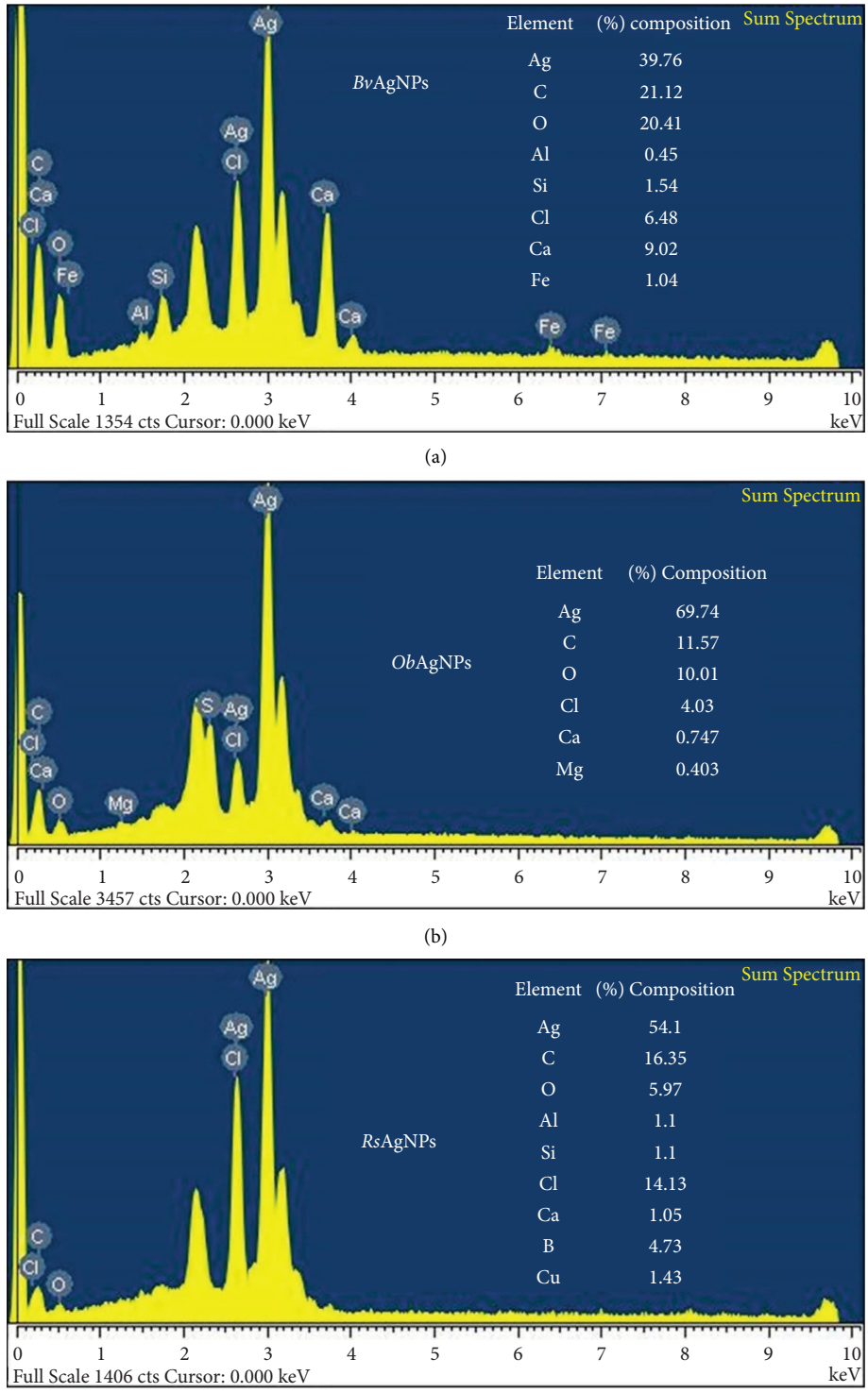


Figure 4: Dispersive X-ray energy spectroscopy and elemental composition of (a) *Bv*AgNPs, (b) *Ob*AgNPs y, and (c) *Rs*AgNPs.

concentrations of AgNPs affect plant metabolism [41]. Furthermore, AgNPs could affect the action of some phytohormones like indole acetic acid (IAA) and ethylene. IAA participates in the formation and elongation of stems, and ethylene is involved in the maturation of organs, at the beginning of flowering, and in the appearance of fruits [36]. The overproduction of ethylene causes seedlings to have short hypocotyls and roots [36]. Ethylene acts on receptors that use copper as cofactor, but Ag^+ occupies the copper binding site, inhibiting the ethylene response [42]. The amount of Ag^+ coming from a low concentration of AgNPs could cause an increase in the elongation of the seedlings by

Table 4: Characteristics of biosynthesized AgNPs.

AgNPs	Shape/size	% Ag	Size/hydrodynamic diameter (nm)	Zeta potential (mV)
*Bv*AgNPs	Sheets and almost spherical shape	39.76	78 ± 17 nm/158.683 ± 3.384	−23.383 ± 1.514
*Ob*AgNPs	Sheets and cubes	69.74	99 nm ± 22.3/104.083 ± 1.685	−24.783 ± 0.354
*Rs*AgNPs	Spherical	54.1	76 ± 16 nm/118.8 ± 2.244	−32.3 ± 0.565

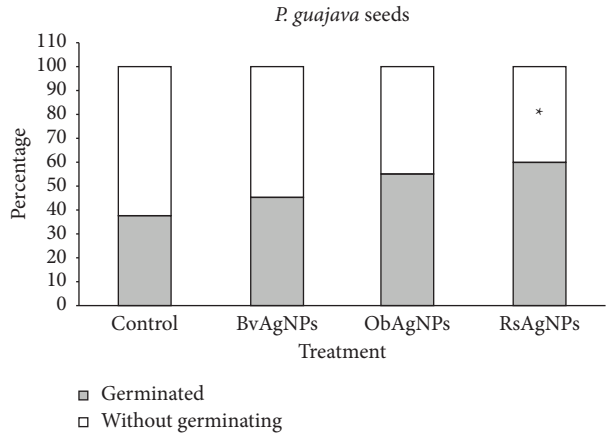
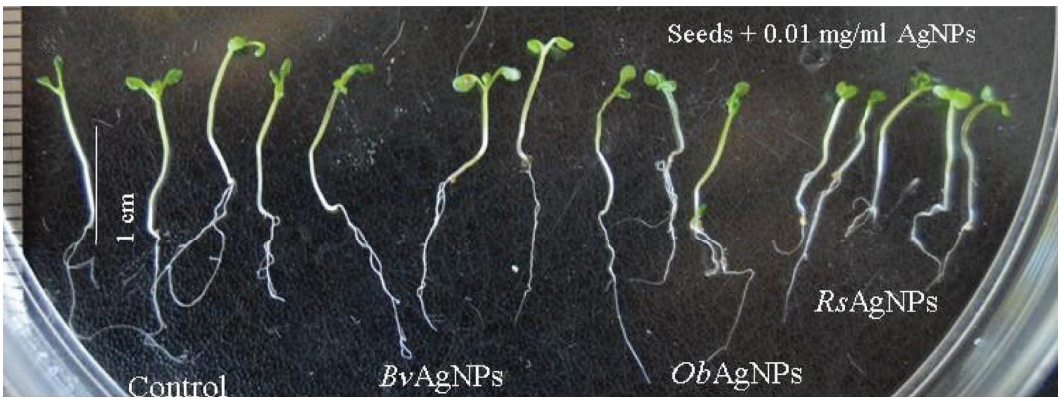
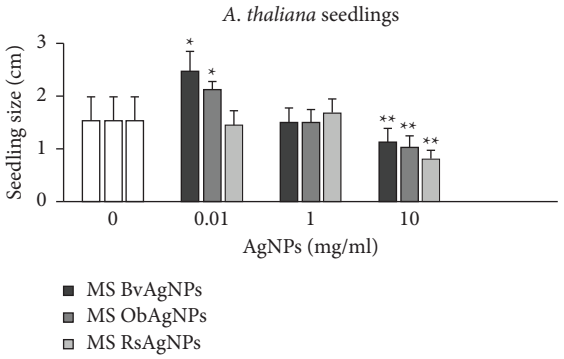


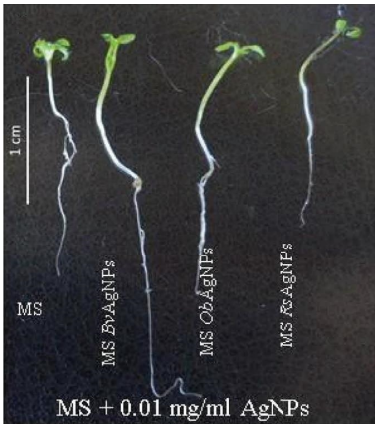
Figure 5: Germination of *P. guajava*. Comparison of the germination rate of guava seeds treated with sodium hypochlorite as control and with *Bv*AgNPs, *Ob*AgNPs, and *Rs*AgNPs. The reported treatment (*) showed a higher percentage of germination ($\alpha < 0.05$).



(a)



(b)



(c)

Figure 6: Growth of *A. thaliana*. (a) Seedlings from seeds exposed to 0.01 mg/mL of 357 AgNPs. (b) Average size of seedlings grown at different concentrations of AgNPs; those indicated showed higher (*) or lower (**) growth than the control ($\alpha < 0.05$). (c) Seedling from seeds sown in medium with 0.01 mg/mL of AgNPs.

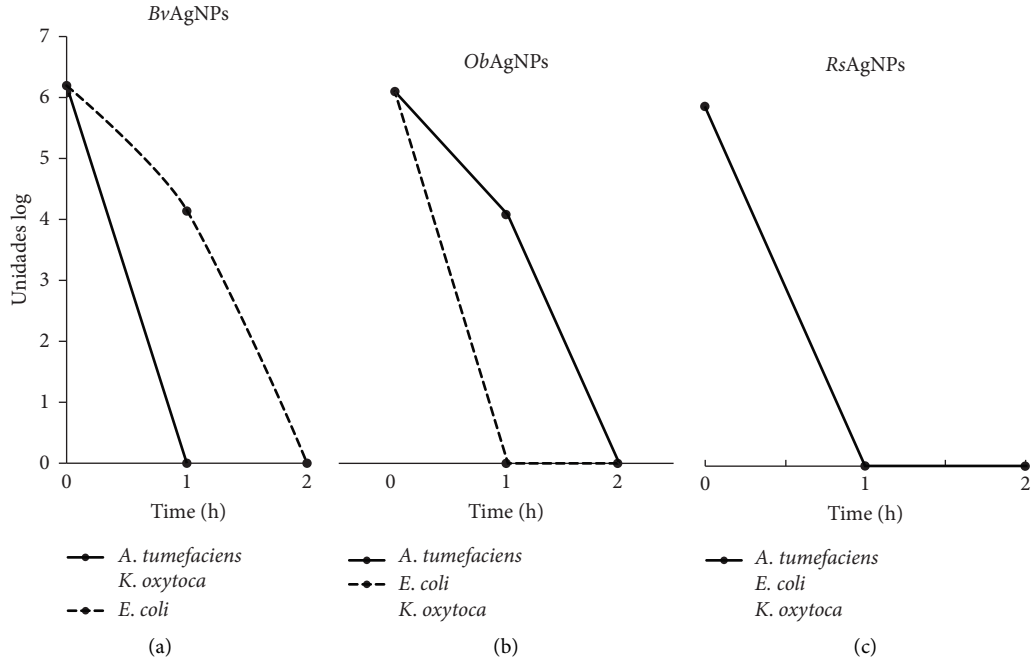


Figure 7: Antimicrobial activity. Logarithmic inactivation of (a) *Bv*AgNPs, (b) *Ob*AgNPs, and (c) *Rs*AgNPs against *A. tumefaciens*, *E. coli* and *K. oxytoca*.

inhibiting the action of ethylene. The decrease in seedling growth at higher concentrations of AgNPs could be related to the induction of the efflux of IAA that facilitates Ag^+ [42].

3.7. Antimicrobial Activity of AgNPs. The antimicrobial activity of AgNPs against the bacterium *A. tumefaciens*, which is used for the genetic transformation of plants, was evaluated. The results showed a 6 U log inactivation (99.9999% removal) after 1 h exposure with the *Bv*AgNPs (Figure 7(a)) and *Rs*AgNPs (Figure 7(b)). The genetic transformation of plants mediated by *A. tumefaciens* allows the insertion of DNA to provide advantages and greater agricultural productivity [43]. This bacterium must be eliminated for the explant to develop in a healthy way. Antibiotics are traditionally used; however, they can also damage the explant [44]. AgNPs could be an alternative for the elimination of this bacterium. The antimicrobial mechanism of action of AgNPs is related to the damage they cause to the cell wall and membrane [10] and applies to various species. However, it is the first time that its use is proposed in this area, since most of the investigations of AgNPs reported are against bacteria of clinical interest (Table 1). In addition, in this work, the activity of AgNPs against bacteria of clinical interest was also demonstrated (Table 2). A log inactivation of 6 U was observed with *Ob*AgNP and *Rs*AgNP against *E. coli*, and with *Bv*AgNP and *Rs*AgNP against *K. oxytoca*, after 1 h of exposure (Figures 7(b) and 7(c)). The *Rs*AgNPs were the only ones of the biosynthesized NPs that had activity against all three evaluated bacteria after 1 h of exposure. This is probably due to the shape of the *Rs*AgNPs since it has been seen that NPs with a spherical shape have increased antimicrobial properties.

4. Conclusions

An AgNPs production protocol was effectively established by means of green synthesis, from extracts of leaves of easily available plants: radish (*Rs*AgNPs), chard (*Bv*AgNPs), and basil (*Om*AgNPs). Obtained AgNPs showed different characteristics which may be related to the different chemical composition of plant extracts. These AgNPs had seed disinfection capacity as efficient as reagents traditionally used in disinfection for *in vitro* plant tissue culture. They were active against various bacteria their activity against *A. tumefaciens* is highlighted. This bacterium is used as a vector in the genetic plant transformation; however, it is difficult to eliminate it later. Therefore, treatment with AgNPs could be an option to keep transgenic plants free of *A. tumefaciens*. This work opens the way to use biosynthesized AgNPs for the benefit of *in vitro* plant cultivation.

Data Availability

The data that support the findings of this study are available from the corresponding author (JFMD), upon reasonable request.

Conflicts of Interest

The authors declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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References

[1] X. F. Zhang, Z. G. Liu, W. Shen, and S. Gurunathan, "Silver Nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches," *International Journal of Molecular Sciences*, vol. 17, no. 9, p. 1534, 2016.

[2] S. Ahmad, S. Munir, N. Zeb et al., "Green nanotechnology: a review on green synthesis of silver nanoparticles — an ecofriendly approach&lt," *International Journal of Nanomedicine*, vol. 14, pp. 5087–5107, 2019.

[3] O. Erdogan, M. Abbak, G. M. Demirbolat et al., "Green synthesis of silver nanoparticles via *Cynara scolymus* leaf extracts: the characterization, anticancer potential with photodynamic therapy in MCF7 cells," *PLoS One*, vol. 14, no. 6, Article ID e0216496, 2019.

[4] A. G. Femi-Adepoju, A. O. Dada, K. O. Otun, A. O. Adepoju, and O. P. Fatoba, "Green synthesis of silver nanoparticles using terrestrial fern (*Gleichenia Pectinata* (Willd.) C. Presl.): characterization and antimicrobial studies," *Heliyon*, vol. 5, no. 4, Article ID e01543, 2019.

[5] P. Rama, A. Baldelli, A. Vignesh et al., "Antimicrobial, antioxidant, and angiogenic bioactive silver nanoparticles produced using *Murraya paniculata* (L.) jack leaves," *Nanomaterials and Nanotechnology*, vol. 12, pp. 184798042110561–10, 2022.

[6] A. Syafiuddin, T. Salmiati Hidibarata, and A. B. Hong Kueh, "Novel weed-extracted silver nanoparticles and their antibacterial appraisal against a rare bacterium from river sewage treatment plan," *Nanomaterials*, vol. 8, no. 9, pp. 1–17, 2018.

[7] P. B. E. Kedi, F. E. Meva, L. Kotsedi et al., "Eco-friendly synthesis, characterization, in vitro and *in vivo*anti-inflammatory activity of silver nanoparticle-mediated*Selaginella myosurus* aqueous extract," *International Journal of Nanomedicine*, vol. 13, pp. 8537–8548, 2018.

[8] A. R. Deshmukh, A. Gupta, and B. S. Kim, "Ultrasound assisted green synthesis of silver and iron oxide nanoparticles using fenugreek seed extract and their enhanced antibacterial and antioxidant activities," *BioMed Research International*, vol. 2019, Article ID 1714358, 14 pages, 2019.

[9] M. G. Muñoz-Carrillo, C. Garcidueñas-Piña, R. C. Valerio-García, J. L. Carrazco-Rosales, and J. F. Morales-Domínguez, "Green synthesis of silver nanoparticles from the *Opuntia ficus-indica* fruit and its activity against treated wastewater microorganisms," *Journal of Nanomaterials*, vol. 2020, Article ID 6908290, 10 pages, 2020.

[10] V. Cittrarasu, B. Balasubramanian, D. Kaliannan et al., "Biological mediated Ag nanoparticles from *Barleria longiflora* for antimicrobial activity and photocatalytic degradation using methylene blue," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 47, no. 1, pp. 2424–2430, 2019.

[11] R. A. Alajmi, W. A. Al-Megrin, D. Metwally et al., "Anti-*Toxoplasma* activity of silver nanoparticles green synthesized with *Phoenix dactylifera* and *Ziziphus spina-christi* extracts which inhibits inflammation through liver regulation of cytokines in Balb/c mice," *Bioscience Reports*, vol. 39, no. 5, Article ID BSR20190379, 2019.

[12] A. V. A. Mariadoss, V. Ramachandran, V. Shalini et al., "Green synthesis, characterization and antibacterial activity of silver nanoparticles by *Malus domestica* and its cytotoxic effect on (MCF-7) cell line," *Microbial Pathogenesis*, vol. 135, Article ID 103609, 2019.

[13] M. M. I. Masum, M. M. Siddiqa, K. A. Ali et al., "Biogenic synthesis of silver nanoparticles using *Phyllanthus emblica* fruit extract and its inhibitory action against the pathogen *Acidovorax oryzae* strain RS-2 of rice bacterial brown stripe," *Frontiers in Microbiology*, vol. 10, no. 10, p. 820, 2019.

[14] S. Salari, S. Esmaeilzadeh Bahabadi, A. Samzadeh-Kermani, and F. Yosefzaei, "*In vitro* evaluation of antioxidant and antibacterial potential of green synthesized silver nanoparticles using *Prosopis farcta* fruit extract," *Iranian Journal of Pharmaceutical Research*, vol. 18, no. 1, pp. 430–455, 2019.

[15] J. Amaro, "Influencia de la betarraga (*Beta vulgaris* var. cruenta) en el aumento de leucocitos, en ratones," *Anales de la Facultad de Medicina*, vol. 75, no. 1, pp. 9–12, 2014.

[16] J. A. Reyes, J. G. Patiño, J. R. Martínez, and E. E. Stashenko, "Caracterización de los metabolitos secundarios de dos especies de *Ocimum* (Fam. Labiatae), en función del método de extracción," *Scientia et Technica*, vol. 13, 2007.

[17] J. Huamán-Malla, M. Guerrero-Aquino, and G. Tomás-Chota, "Estudio químico y nutricional de las hojas del rabanito, *Raphanus sativus* L., como alimento para el consumo humano," *Rev Peru Quim Ing Quim*, vol. 6, no. 2, pp. 45–49, 2003.

[18] Britannica, "The editors of encyclopedia. "radish". encyclopedia britannica," 2019, https://www.britannica.com/plant/radish.

[19] Britannica, "The editors of encyclopedia. "chard". encyclopedia britannica," 2022, https://www.britannica.com/plant/chard-plant.

[20] Britannica, "The editors of encyclopedia. "basil". encyclopedia britannica," 2021, https://www.britannica.com/plant/basil.

[21] W. M. Roca and L. A. Mroginski, *Cultivo de tejidos en la agricultura: fundamentos y aplicaciones*, Cent Int Agric Tropical, Rome, Italy, 1991.

[22] W. Mahakham, A. K. Sarmah, S. Maensiri, and P. Theerakulpisut, "Nanopriming technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles," *Scientific Reports*, vol. 7, no. 1, p. 8263, 2017.

[23] C. T. Rueden, J. Schindelin, M. C. Hiner et al., "ImageJ2: ImageJ for the next generation of scientific image data," *BMC Bioinformatics*, vol. 18, no. 1, p. 529, 2017.

[24] I. Rabino and A. L. Mancinelli, "Light, temperature, and anthocyanin production," *Plant Physiology*, vol. 81, no. 3, pp. 922–4, 1986.

[25] H. Wagner and S. Bladt, *Plant Drug Analysis: A Thin Layer Chromatography Atlas (2nd ed.)*, Springer, 1996, https://link.springer.com/book/10.1007/978-3-642-00574-9?page=1&oscar-books=true#toc.

[26] T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiologia Plantarum*, vol. 15, no. 3, pp. 473–497, 1962.

[27] C. Garcidueñas-Piña, I. E. Medina-Ramírez, P. Guzmán, R. Rico-Martínez, J. F. Morales-Domínguez, and I. Rubio-Franchini, "Evaluation of the antimicrobial activity of nanostructured materials of titanium dioxide doped with silver and/or copper and their effects on *Arabidopsis thaliana*," *International Journal of Photoenergy*, vol. 2016, Article ID 8060847, 14 pages, 2016.

[28] A. S. Jain, P. S. Pawar, A. Sarkar, V. Junnuthula, and S. Dyawanapelly, "Bionanofactories for green synthesis of silver nanoparticles: toward antimicrobial applications," *International Journal of Molecular Sciences*, vol. 22, no. 21, pp. 1–48, 2021.

[29] E. Y. Ahn, H. Jin, and Y. Park, "Assessing the antioxidant, cytotoxic, apoptotic and wound healing properties of silver nanoparticles green-synthesized by plant extracts," *Materials Science and Engineering: C*, vol. 101, pp. 204–216, 2019.

[30] A. Khoddami, M. A. Wilkes, and T. H. Roberts, "Techniques for analysis of plant phenolic compounds," *Molecules*, vol. 18, no. 2, pp. 2328–75, 2013.

[31] A. B. Enogieru, W. Haylett, D. C. Hiss, S. Bardien, and O. E. Ekpo, "Rutin as a potent antioxidant: implications for neurodegenerative disorders," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 6241017, 17 pages, 2018.

[32] H. E. Khoo, A. Azlan, S. T. Tang, and S. M. Lim, "Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits," *Food & Nutrition Research*, vol. 61, no. 1, Article ID 1361779, 2017.

[33] Z. A. Ratan, M. F. Haidere, M. Nurunnabi et al., "Green chemistry synthesis of silver nanoparticles and their potential anticancer effects," *Cancers*, vol. 12, no. 4, p. 855, 2020.

[34] T. Suwan, S. Khongkhunthian, and S. Okonogi, "Antifungal activity of polymeric micelles of silver nanoparticles prepared from *Psidium guajava* aqueous extract," *Drug Discoveries & Therapeutics*, vol. 13, no. 2, pp. 62–69, 2019.

[35] P. Nartop, "Effects of surface sterilisation with green synthesised silver nanoparticles on Lamiaceae seeds," *IET Nanobiotechnology*, vol. 12, no. 5, pp. 663–668, 2018.

[36] F. B. Salisbury and C. W. Ross, *Fisiología vegetal*, Grupo Editorial Iberoamérica, Madrid, Iberoamericana, 1994.

[37] C. Wang, K. Jiang, B. Wu, J. Zhou, and Y. Lv, "Silver nanoparticles with different particle sizes enhance the allelopathic effects of *Canada goldenrod* on the seed germination and seedling development of lettuce," *Ecotoxicology*, vol. 27, no. 8, pp. 1116–1125, 2018.

[38] L. Abdulla Abdulaziz Alshehddi and N. Bokhari, "Influence of gold and silver nanoparticles on the germination and growth of *Mimusops laurifolia* seeds in the South-Western regions in Saudi Arabia," *Saudi Journal of Biological Sciences*, vol. 27, no. 1, pp. 574–580, 2020.

[39] P. Thuesombat, S. Hannongbua, S. Akasit, and S. Chadchawan, "Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth," *Ecotoxicology and Environmental Safety*, vol. 104, pp. 302–9, 2014.

[40] J. J. Bello-Bello, R. A. Chavez-Santoscoy, C. A. Lecona-Guzmán et al., "Hormetic response by silver nanoparticles on *in vitro* multiplication of sugarcane (*Saccharum* spp. Cv. Mex 69-290) using a temporary immersion system," *Dose-Response*, vol. 15, no. 4, Article ID 155932581774494, 2017.

[41] S. Budhani, N. P. Egboluche, Z. Arslan, H. Yu, and H. Deng, "Phytotoxic effect of silver nanoparticles on seed germination and growth of terrestrial plants," *Journal of Environmental Science and Health, Part C*, vol. 37, no. 4, pp. 330–355, 2019.

[42] L. C. Strader, E. R. Beisner, and B. Bartel, "Silver ions increase auxin efflux independently of effects on ethylene response," *The Plant Cell*, vol. 21, no. 11, pp. 3585–3590, 2009.

[43] S. B. Gelvin, "Agrobacterium-mediated plant transformation: the biology behind the "gene-jockeying" tool," *Microbiology and Molecular Biology Reviews*, vol. 67, no. 1, pp. 16–37, 2003.

[44] C. Díaz-Granados, M. Denis, and S. Plotkin, "Seasonal influenza vaccine efficacy and its determinants in children and non-elderly adults: a systematic review with meta-analyses of controlled trials," *Vaccine*, vol. 31, no. 1, pp. 49–57, 2012.