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Green Synthesis and Antibacterial Properties of Silver Nanoparticles from *Eugenia uniflora* Fruit Extract

Melissa Moya[1,2], Vanessa Bagnarello[1,3], Julio Mora[2], Idalia Valerio[1,2,4]

[1]Laboratorio de Investigación, Universidad de Ciencias Médicas, San José, Costa Rica
[2]Facultad de Microbiología, Universidad de Ciencias Médicas, San José, Costa Rica
[3]Facultad de Fisioterapia, Universidad de Ciencias Médicas, San José, Costa Rica
[4]Facultad de Medicina, Universidad de Ciencias Médicas, San José, Costa Rica
Email: moyagm@ucimed.com

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Abstract

The synthesis of nanoparticles by biological methods using microorganisms, enzymes, or plant extracts has been suggested as a possible ecofriendly alternative to chemical and physical methods that involve the use of harmful reducing agents. Green synthesis of silver nanoparticles (AgNPs) was achieved using *Eugenia uniflora* ripe fruit extract, which was characterized by phytochemical screening revealing the presence of polyphenols (quinones, flavonoids, and tannins), reducing compounds, and terpenes. These excellent antioxidants reduced silver nitrate to give the AgNPs, which were characterized by transmission electron microscopy (TEM), dynamic light scattering (DLS), and ζ potential analysis. The diameter of the AgNPs ranged from 10.56 ± 1.2 nm to 107.56 ± 5.7 nm. The antibacterial activity of the AgNPs was evaluated using a modification of the Kirby-Bauer technique with *Escherichia coli*, *Pseudomonas aeruginosa,* and *Staphylococcus aureus*. The inhibition halos were 11.12 ± 0.02 mm, 13.96 ± 0.07 mm, and 11.29 ± 0.76 mm, respectively. The synthesis using *E. uniflora* is an ecofriendly and low cost method of obtaining silver nanoparticles that could be used in health sciences because of their activity against bacteria with antibiotic resistance.

Keywords

Eugenia uniflora, Green Synthesis, Silver Nanoparticles, Antibacterial Activity, Polyphenols

1. Introduction

The term nanotechnology is used exclusively to define the science and tech-

niques focused on the study, design, creation, synthesis, control, manipulation, and application of materials at the nanoscale, *i.e.* in the size range 1 - 100 nm [1].

Nanotechnology is a multidisciplinary area that includes nanomedicine, which is defined as the application of nanotechnology for the prevention, diagnosis, and treatment of diseases [2].

Silver nanoparticles (AgNPs) are of great interest in health sciences owing to their antibacterial, healing, optical, and electrical properties and as a result their green synthesis is currently a key area of research [3] [4].

The synthesis of nanoparticles often has a negative effect on the environment because toxic—and often expensive—reagents are required. Green synthesis is more ecofriendly, produces less toxic waste, and avoids the use of toxic substances such as sodium borohydride (NaBH4). Using plant extracts in the green synthesis of silver nanoparticles is therefore an attractive option [2].

Plant extracts contain secondary metabolites such as polyphenols, phenyl propanoids, flavonoids, alkaloids, vitamins, and terpenoids, which can reduce heavy metals to a basal state, such as the reduction of the Ag+ ion to Ag0. These substances are also known to have beneficial health effects, such as reducing the risk of cancer, heart disease, and neurodegenerative disorders, and are abundant in plants, fruits, and vegetables [5].

The objective of this study was to obtain silver nanoparticles as a precursor for applications in health sciences—such as the creation of low-cost antibacterial patches for wound dressing—from *Eugenia uniflora* extract using the abundant antioxidants to replace conventional chemical reducing agents.

2. Materials and Methods

2.1. Collection of the Material and Preparation of the Plant Extract

The equivalent of 600 g of ripe pitanga *E. uniflora* fruit was purchased and then authenticated at the herbarium unit of the Biological Sciences Department of UCIMED.

The material was washed with distilled water and fruits with no sign of damage were air dried under shade at room temperature.

Ethanolic fruit extract of *E. uniflora* was prepared by soaking 0.5 kg of fruit in 2 L of 70% ethanol for 7 days.

The liquid portion was then separated from the fruit using sartorius grade 1288 paper and an amber extract of pitanga fruit was obtained with a total volume of 2088 mL, which was kept refrigerated at 5˚C until further use. The final concentration of the extract was determined using a dry weight method.

2.2. Phytochemical Study

The method described by Sharapin *et al.* [6] was used with the following modifications: liquid-liquid extractions were carried out with 40 mL of crude extract and 15 mL of ethyl ether (in triplicate) to obtain an aqueous phase (AQ1) and an

ether extract. The obtained ether extract was concentrated to dryness to give fraction (E). Half of the volume of the aqueous phase (AQ1) was hydrolyzed with 15 mL of 3 mol/L HCl and subsequently extracted with ethyl ether to give an ether phase (AQ2) and a hydrolyzed aqueous phase. Each extract was subjected to qualitative tests, as described by Sharapin, to determine the secondary metabolites present. Different chemical analysis techniques indicated the presence of terpenes (vanillin), alkaloids (Dragendorff), flavonoids (Shinoda), coumarins (KOH), triterpenes and sterols (Liebermann-Burchard), and quinones (Bornträger-Kraus) in the ether phases (E and AQ2). Phenols and tannins ($FeCl_3$), polysaccharides (Lugol), reducing sugars (Fehling), saponins (foam), and alkaloids (Dragendorff) were determined to be present in the aqueous sample (AQ1). The hydrolyzed aqueous sample also contained anthocyanins, as determined by an acid-base test.

2.3. Determination of Polyphenol Content

The total phenolics content was determined using the Folin-Ciocalteu assay with some modifications [7]. The crude extract was diluted 1:10 with methanol. Then 100 µL of sample was added to 1000 µL of Folin-Ciocalteu reagent diluted 1:10 v/v and 800 µL of Na_2CO_3 (7.5% m/v). The mixture was vortexed for 15 s and incubated in dark room for 30 min.

Methanol was used as the blank. The absorbance was determined at 765 nm against a blank using a BioTek EPOCH microplate spectrophotometer. Gallic acid was used as a standard for the calibration curve. The total amount of phenolic compounds was calculated and expressed as mg gallic acid g-1 sample. Samples were measured in triplicate [8].

2.4. Determination of Antioxidant Activity

The antioxidant capacity of the pitanga alcoholic extract and the green synthesized AgNPs was determined using the oxygen radical reduction capacity (ORAC) method. The analysis was performed using the fluorescence method described by Rodriguez Bonilla *et al.* [9] Fluorescence was read with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. Measurements were made using 96-well polystyrene microplates. The antioxidant capacity of the samples was expressed as µmol/L Trolox equivalents. The analysis was performed in triplicate [10].

2.5. Synthesis of Silver Nanoparticles

Several AgNP syntheses were carried out with varying proportions of silver nitrate and volumes of the extract until a final protocol was standardized.

After several attempts, we proceeded with the proportions given in **Table 1**.

The reactions were carried out at room temperature (70˚C ± 2˚C) for 4 h in a dark room with continuous stirring. Silver nitrate was added at a rate of 1 mL/min with continuous stirring. The formation of silver nanoparticles was monitored through visual observation of the change in color [11].

Table 1. Proportions of AgNO3 and pitanga extract used for the synthesis of AgNPs.

ID	AgNO3 volume (mL)	AgNO3 concentration (mol/L)	Pitanga extract volume (mL)	Pitanga extract concentration (mg/mL)
A	35	0.001	35	15
B	35	0.01	35	15

Finally, the AgNPs of each reaction mixture were purified following the standard procedure INTE/ISO TR 20489:2021 INTE standard "preparation of samples for the characterization of metallic nanoobjects and metallic oxides in water samples" [12], which establishes the centrifugation conditions for the sample in different cycles, varying the speed to finally obtain a solution rich in nanoparticles smaller than 200 nm. In this process, two fractions were obtained for each synthesis; A1 and A2 from synthesis (A) and B1 and B2 from synthesis (B).

2.6. Antibacterial Activity

The antibacterial activity was evaluated using the Kirby-Bauer method as a reference. Mueller-Hinton agar plates were scratched with *Pseudomonas aeruginosa, Escherichia coli,* and *Staphylococcus aureus* previously prepared at McFarland standard 0.5. For the antibiogram, Whatman AA discs were impregnated with 20 µL of the samples, placed on the plates and allowed to incubate for 18 - 24 h at 37˚C [13] [14].

2.7. Transmission Electron Microscopy and Dynamic Light Scattering Characterization

After evaluating the antibacterial activity of each synthesis, the best performing sample was characterized by TEM, JEOL instrument, model JEM 2010 and DLS, HORIBA instrument, model NanoPartica SZ-100V2 to analyze size, agglomeration, and morphology of the particles.

For characterization by transmission electron microscopy, a 5-µL aliquot was taken from the samples, previously diluted (1:10 v/v) with Milli-Q water, placed on copper grids coated with carbon and then dried in a desiccator with silica for 16 h. The measurements were made at an acceleration voltage of 120 kV.

For size assessment by dynamic light scattering, samples were diluted (1:10 v/v) with Milli-Q water and subjected to a 15-min ultrasound cycle. The analysis was performed with a dispersion angle of 90˚ at a temperature of 25˚C.

2.8. ζ Potential

ζ potential is related to the stability of colloidal dispersions and indicates the degree of repulsion between adjacent particles.

For molecules and particles that are small enough, a high ζ potential gives them stability, that is, the solution or dispersion will resist aggregation maintaining in this case the nanometric scale and related properties. These measure-

ments were made using Zetasizer equipment and the samples were subjected to vortex dispersion and ultrasonication for 10 min before reading.

3. Results and Discussion

3.1. Synthesis of Silver Nanoparticles and Phytochemical Analysis of the Extract

The silver nanoparticle synthesis was enabled by plant extracts that function as an effective reducing agent, allowing the nanoparticles to be formed from a metallic silver precursor such as silver nitrate in the appropriate size and desired conditions. A review of different plant extracts in the literature was carried out, and as a result *E. uniflora*, popularly known as "pitanga", was selected as a precursor of the reducing agent [13].

Table 2 shows the results obtained from the phytochemical screening of the pitanga fruit extract, which confirms the presence of several polyphenols. These substances are known to be powerful antioxidants that act by various mechanisms, such as electron donation and the interruption of oxidation reaction chains [8].

The presence of quinones, flavonoids, and tannins was verified among the main polyphenols. Additionally, the ether extract showed terpenes were present among the metabolites [8] [15].

For the determination of the phenolic content, gallic acid was reduced as a calibration standard and the total phenolic content in the alcoholic extract was 152.2 ± 8.5 mg EAG/g of extract. From the results obtained it can be said that the extract has a high polyphenol content, which indicates that the alcoholic extract of *E. uniflora* is a good source of natural antioxidants. However, studies carried out by Baguetti *et al.* [16] and Rodrigues *et al.* [15] indicate that variations in the

Table 2. Phytochemical screening of the crude extract of ripe *E. uniflora* fruit.

Metabolite	Ether extract (E)	Aqueous extract (AQ1)	Hydrolyzed aqueous extract (AQ2)
Alkaloids	-	-	-
Flavonoids	+	-	+
Coumarins	-	-	-
Triterpenes and sterols	-	-	-
Quinones	+	N/A	-
Tannins	+	+	+
Reducing Compounds	N/A	+	-
Terpenes	+	N/A	-
Saponins	N/A	-	-

composition of phenolic compounds should be considered in studies of pitanga because the differences may be associated with intrinsic (species, crop, and maturity) and extrinsic (cultivation conditions, seasonality, transport, and storage) characteristics, as well as the extraction method or the solvents used for the extraction of these compounds. It should also be noted that the phenolic content may vary between samples owing to the lack of specificity of the Folin-Ciocalteu method since the reagent detects other reducing agents, including ascorbic acid [16].

3.2. Antioxidant Activity

The antioxidant activity of the alcoholic extract of pitanga was evaluated using the ORAC assay and found to contain 263,042 ± 3341 μmol/L equivalent of Trolox for pitanga extract and 44,133 ± 1141 μmol/L for the A1 synthesis nanoparticles.

The reduction of Ag+ ions to Ag° uses the antioxidant components of the plant extracts as green reducing agents.

Studies carried out by Bagetti *et al.* [16] have shown a directly proportional relationship between antioxidant activity and phenolic content, which suggests that phenolic compounds are primarily responsible for the antioxidant activity of the fruit, and not the ascorbic acid content of the extract [3] [17].

After completing the different syntheses, and based on macroscopic results—discarding those that showed significant precipitation—solution A1 was selected to characterize the nanoparticles and evaluate their antibacterial activity.

3.3. Transmission Electron Microscopy and Dynamic Light Scattering Characterization of the Nanoparticles

The syntheses A1 was characterized by transmission electron microscopy and DLS, which showed the formation of nanoparticles smaller than 100 nm in diameter. As shown in **Figure 1**, mostly spherical particles were obtained and individual particles can be observed, reducing the amount of clusters. In **Figure 2**, a layer the covers the particles can be observes, and this is because the great stabilizing action of the fruit extract.

The size of the nanoparticles was also evaluated using dynamic light scattering technique (**Table 3** and **Figure 3**), which demonstrated two populations based on the Brownian motion of the particles in the suspension analyzed. For the synthesis denoted A1, the smallest particles had an average size of 10.56 ± 1.2 nm and the largest had an average size of 107.56 ± 5.7 nm [18].

3.4. ζ Potential

The ζ potential was determined using a Zeta Sizer to investigate the stability of the nanoparticles obtained. The ζ potential gives us an indication of the possibility of particle agglomerates forming, which can affect the properties. In this case, the values obtained indicated good stability, as shown in **Table 4** [14].

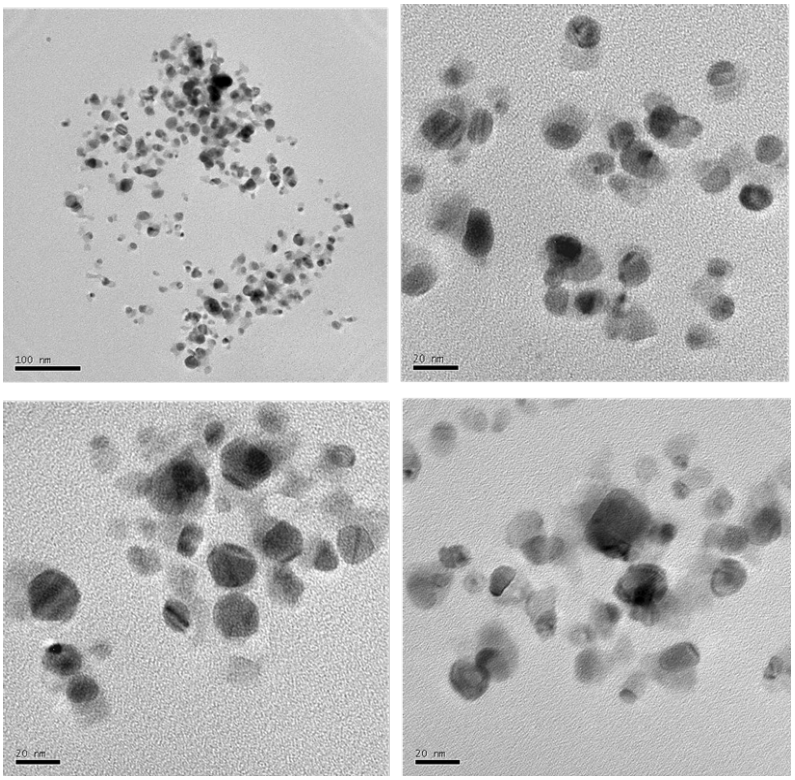


Figure 1. Micrographs of the synthesized silver nanoparticles (A1) acquired using transmission electron microscopy.

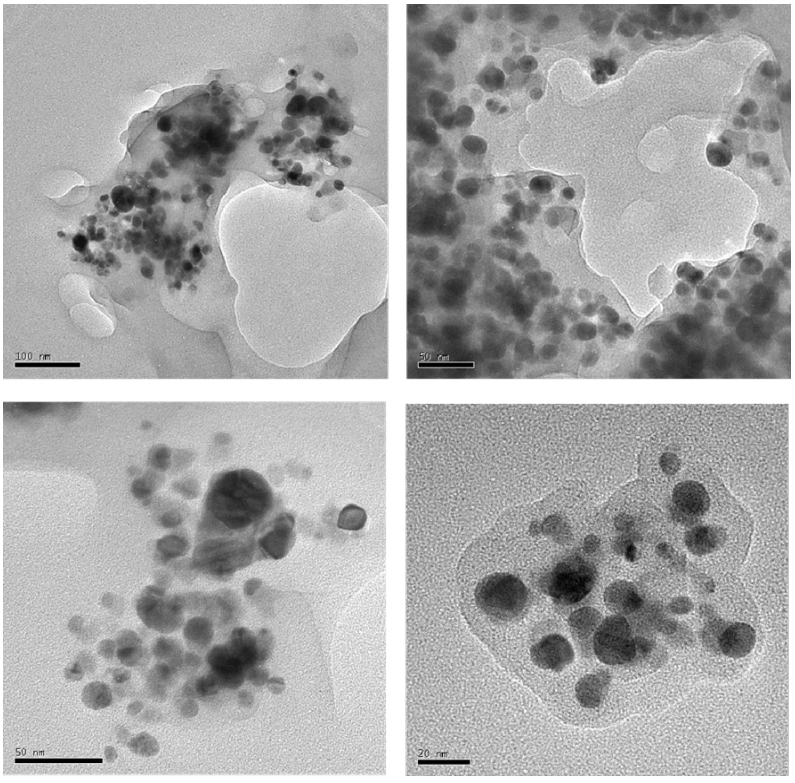


Figure 2. Micrographs of the particles present in the analyzed sample. The layer covering the particles is attributed to the extract used.

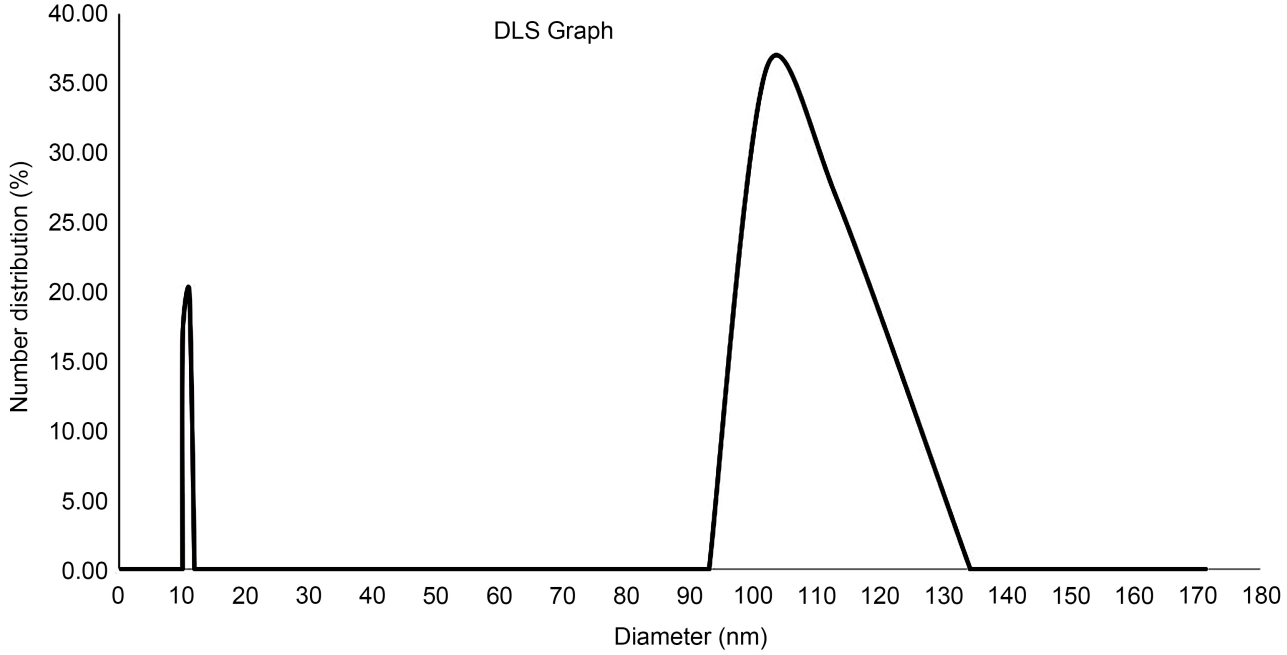


Figure 3. Dynamic light scattering (DLS) graph depiciting the size of the silver nanoparticles (A1).

Table 3. Hydrodynamic diameter in nanometers.

Population of synthesis A1	Hydrodyamic diameter (nm)
1	10.56 ± 1.25
2	107.56 ± 5.7

Table 4. Evaluation of potential Z.

Population	ζ potential (mV)
Synthesis A1	29.80 ± 0.20
Synthesis A2	28.90 ± 2.03

Since a ζ potential greater than ±25 mV provides an indication of stable and well dispersed particle suspensions, it can be assumed that the synthesized AgNPs have a high degree of repulsion between adjacent particles and that the colloidal dissolution or dispersion will resist aggregation or flocculation [19] [20].

3.5. Antibacterial Activity

Assessment of the antibacterial activity of the particles using *Pseudomonas aeruginosa*, *E. coli*, and *S. aureus* on Mueller Hinton agar plates, showed the growth inhibition.

As shown in **Table 5** and **Figure 4**, there was inhibition of bacterial growth for the three bacteria used, suggesting effective nanoparticle synthesis. The

greatest inhibition was for *P. aeruginosa*, which had an inhibition halo of 13.96 ± 0.07 mm.

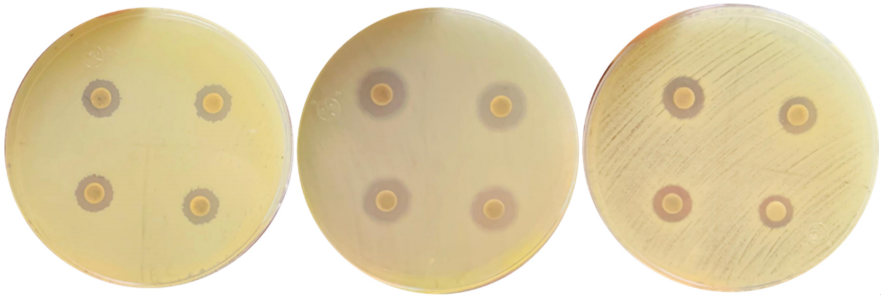
An additional finding to highlight was the increase in production of pyoverdine by *P. aeruginosa*, with respect to the control, when it was in contact with the nanoparticles as shown in **Figure 5** [3] [20].

The mechanism of the antibacterial activity of silver nanoparticles is a topic of debate and is not completely clear, but has been demonstrated that the nanoparticles accumulate around the cell wall or inside the cell, and can affect the DNA replication which can lead tha production of ROS (reactive oxygen species), that cause bacterial death [3] [11].

If we compare the results obtained by this green synthesis with nanoparticles synthesized by wet chemical routes, for example with research made by Raza *et al.* [14], both methods were done using $AgNO_3$ 1 mm as precursor, but they used tri-sodium citrate ($Na_3C_6H_5O_7$) as reducing agent, and we used the green synthesis by *Eugenia uniflora*. The AgNPs obtained by green synthesis

Table 5. Diameter of zone of inhibition of bacterial growth.

Bacteria	Zone of inhibition (mm)
Escherichia coli	11.12 ± 0.02
Pseudomonas aeruginosa	13.96 ± 0.07
Staphylococcus aureus	11.29 ± 0.76



(a) (b) (c)

Figure 4. Antibacterial activity test, zone of inhibition. (a) *Escherichia coli*, (b) *Pseudomonas aeruginosa*, (c) *Staphylococcus aureus*.

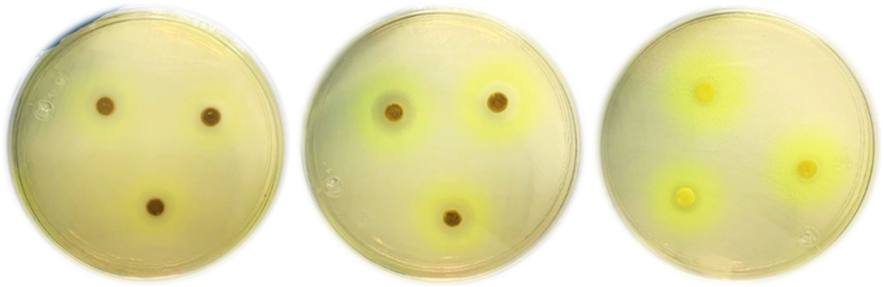


Figure 5. Production of pyoverdine in *Pseudomonas aeruginosa*.

shown better antimicrobial activity, for example with *Escherichia coli* there was an inhibition of 11.12 ± 0.02 mm and *Pseudomonas aeruginosa* 13.96 ± 0.07 mm, and Raza *et al*., reported inhibition zone of 7.2 ± 0.1 mm and 7.0 ± 0.1 mm respectively. About the size, they reported nanoparticles between 30-80 nm, and we obtained AgNPs between 10.56 - 107.56 nm. By this way it is demonstrated that the green synthesis with *E. uniflora* can improve the synthesis and antibacterial activity in a cheaper and less toxic way than the chemical synthesis.

4. Conclusion

A protocol for preparing highly dispersed and stable silver nanoparticles by green synthesis using the ripe fruit extract of *E. uniflora* was established. Obtaining silver nanoparticles was attributed to the high antioxidant content of the *E. uniflora* extract based on polyphenols. Antibacterial activity was demonstrated at a concentration of 17 mg/mL for nanoparticles ranging between 10.56 ± 1.2 nm and 107.56 ± 5.7 nm in size, obtained from the synthesis A1. In addition, AgNPs increased the production of pyoverdine in *P. aeruginosa*. AgNPs are useful in medicine, biochemistry, and nanomaterials owing to their various applications; therefore, further studies are recommended for the development of biodegradable dressings with antioxidant and antimicrobial activity using this nanoparticles. The green synthesis of nanoparticles using fruit extracts has advantages over other methods because it can be applied to large scale synthesis to reduce cost and improve characteristics of nanoparticles obtained by wet chemical routes.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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