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Green synthesis of silver nanoparticles with phytosterols and betalain pigments as reducing agents present in cactus *Myrtillocactus geometrizans*.

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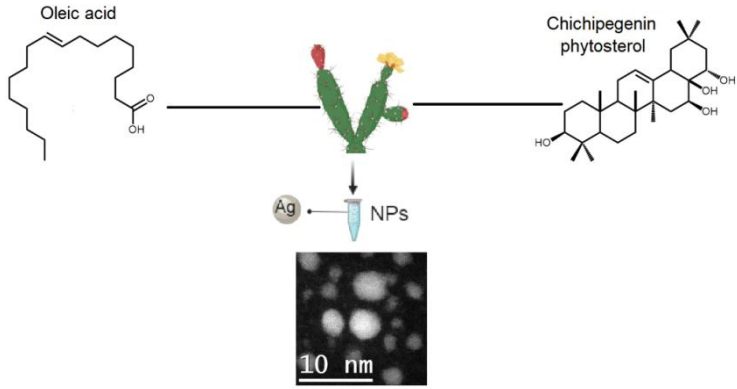
Abstract:

In the current work, we compared the green synthesis of silver nanoparticles (AgNP) using plant extracts, a promising methodology against the use of chemical reducers, such as oleic acid and oleylamine. The advantages of green synthesis are one-step method, economic and ecological while the advantages of classic synthesis methods are high nanoparticle performance, homogeneity in size and smaller average sizes. With this work we want to demonstrate that plant extracts with specific mixtures of chemical compounds can obtain smaller average sizes with greater homogeneity in nanoparticles compared to the use of classical synthesis. Myrtillocactus geometrizans was used as a polar plant extract, which was selected by the chemical components contained in the extract. Phytosterols, oleic acid and betalains contained in Myrtillocactus geometrizans are biomolecules responsible for the reduction and stability of AgNP below 5 nm. TEM analysis of the green synthesis of nanoparticles revealed the formation of spherical particles with an average diameter of 5 nm and with preferential crystallographic directions of the silver plane [111].



Graphical abstract

INTRODUCTION.

Chemical synthesis with conventional methods to produce different nanostructures of Ag, focuses on adjusting the conditions/parameters of synthesis (such as temperature, precursor, and pH) to obtain the desired nanostructures. This means that chemical reducers and stabilizers influence guiding phenomena (thermodynamics, kinetics, and metal reducing-aspects) [1, 2]. Methods of chemical synthesis of nanoparticles using plant extracts have been popularized and these extracts have many chemical compounds qualified for reducing metals as well as economic and environmental advantages [3].

Oleic acid (OA) and oleylamine (OAm) has been selected as the reducer and stabilizer properly described in the literature and as a point of comparison with the plant extract that was examined [2, 4]. The plant selected for obtaining the extract was ***Myrtillocactus geometrizans* (*M. geometrizans*)**, a native cactus from Mexico [5, 6]. The characterization was conducted to determine the major components in the cactus.

OAm / OA favor a facet growth [111], which occurs faster than the rearrangement of the atom on different seed faces, which is attributed to the system reaching equilibrium [1, 2]. In this work, the *M. geometrizans* extract generates a similar phenomenon that favors growth [111].

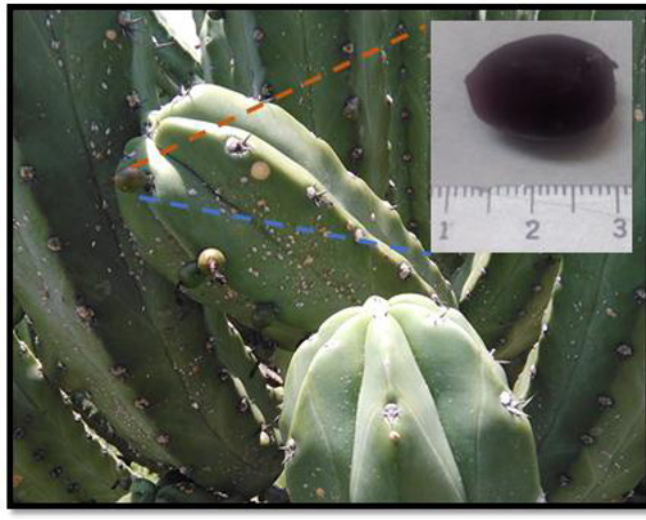


Figure 1. Photograph of *Myrtillocactus geometrizans*.

MATERIALS AND METHODS.

Reagents and chemical substances:

Silver nitrate (reactive ACS), oleic acid, oleylamine, methanol, and ethanol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Tri-distilled water (18 Ω, Milli-Q) was used.

Preparation of cranberry cactus extract (Garambullo):

The cactus berry (*Myrtillocactus geometrizans*) was collected in Hidalgo State, México. The fruit was selected (Berries of good quality: ripe, bright, colorful, not contaminated), cleaned with water, and ground by hand to obtain a berry paste. The resulting paste was soaked with ethanol (1:1 volume/volume at 25 ° C) to produce the extraction by maceration that took one day. The mixture was filtered, and ethanol was added again to the remaining paste. The ethanol extracts were collected and dried using a rotary evaporator at 70 °C under reduced pressure conditions [7-9]. The final dry extract was solubilized in water.

Synthesis of silver nanoparticles:

The synthesis methodology used is similar to the hot-injection synthesis route, in the first experiment a mixture of 60% OA / 40% OAm by volume was used, the second

experiment used a polar extract of *Myrtillocactus geometrizans* [10, 11]. The first experiment: 5-ml of the mixture (60% OA / 40% OAm) was poured into a flask and heated to a temperature of 100 ° C. Subsequently, 1-ml of 0.1 M aqueous solution of $AgNO_3$ was added. The total reaction time was 30 min. The mixture was named reaction solution A. Second experiment: 5-ml of the polar extract diluted in water (2.2% mass/mass) was poured into a flask and heated to a temperature of 85 ° C. Subsequently, 1-ml of 0.1 M aqueous solution $AgNO_3$ solution was added. The total reaction time was 30 min. The mixture was called reaction solution B. Solid residues from both reactions were separated by centrifugation at 10,000 rpm for 12 minutes and the colloidal solution was preserved.

UV - visible spectroscopy (UV-vis):

Spectra were collected in a quartz cuvette with a path length of 1 cm, requiring approximately 1-ml of the solution to fill the cuvette. Spectra were collected at room temperature (25 ° C). The spectra were collected from 200 to 800 nm. The equipment used was a Shimadzu UV-1800 spectrometer.

High-resolution Liquid Chromatography analysis with Mass Spectrometry (HPLC/MS) of Myrtillocactus geometrizans extract:

HPLC was performed on a Thermo Scientific UltiMate 3000 device with a fraction collector equipped with Zorbax SB C18 column (150 x 4.6 mm, 3µm). The established measurement parameters were flow: 0.7 mL /min, column temperature: 40 ° C Sample: 200 µg / µL (3:7 methanol: Water), Autosampler temperature: 30 ° C, Injection volume: 50 µL, detection: 280 nm, MeOH / H2O 9: 1 Volume/Volume, mobile phase. The mass spectrometer was a quadrupole analyzer and linear ion trap QTRAP 3200, ABSciex with Turbo Spray source. Injection: By direct infusion, flow: 10 µL / min, a type of scan: Q1 polarity: positive and negative. Range m / z: 100-1500.

Scanning transmission electron microscopy (STEM):

High-resolution scanning transmission electron micrographs were obtained in a JEM-ARM200F microscope operating at a working voltage of 200 kV. The samples for electron microscopy observation were prepared from an aqueous solution. Subsequently, 40 µL of the solution was deposited on a carbon film supported on a copper grid (Cu) and allowed to evaporate at ambient conditions. All size analyzes were performed using the Gatan Microscopy Suite software version 2.01 (Gatan Inc. CA United States).

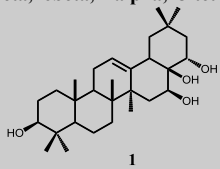
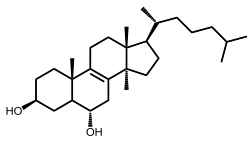
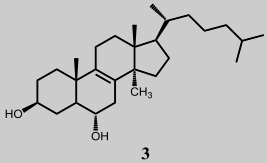
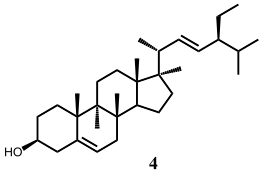
RESULTS AND DISCUSSION.

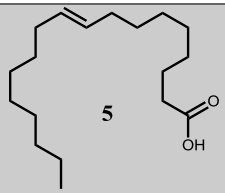
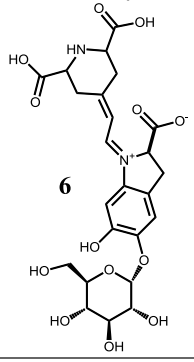
HPLC / MS analysis of the fruit extract of M. Geometrizans:

Starting with 0.1 g of dry extract, a 200 µg / ml sample was prepared for duplicate HPLC (reverse phase) / MS analysis, the mass analysis was performed by direct flow. According to conditions described in the methodology section in the analysis, the highest intensity peaks detected in the chromatogram were identified and collected (experiment time 60 min); these fractions (mass> 100 ng) were analyzed by MS. The

partial information available on the chemical characterization of *Myrtillocactus geometrizans* [6-9, 12], excludes the use of internal standards in HPLC. The principal compounds in Table 1 were identified by their characteristic molecular weight and their polarity-retention time relationship. As shown in Table 1, the retention times (RT) of compounds 1-4 are close to each other, between a medium-high polarity, making it difficult to separate them by conventional techniques as described in the literature [13]. Oleic acid is detectable in a wide range of RT, indicating that there are significant amounts. The betalains are found in low RT values according to their high polarity.

Table 1 Molecular mass, data retention time (RT) and experimental mass ($[M+H]^+$ $[M-H]^-$).

Compound	Molecular mass* (g/mol)	[M+ H]+ (m/z) polarity -	[M-H]- (m/z) polarity +	RT (min)
(1) chichipegenin Olean-12-ene-3beta,16beta,22alpha,28-tetrol  **1**	474.7	475.2	473.4	17.8-18.43 24.6 - 25.5 37.44 - 44.14
(2) peniocerol (3beta,5alpha,6alpha)-Cholest-8-ene-3,6-diol  **2**	402.7	403.4	401	17.8 - 22.6 y 44.14
(3) macdougallin 14-Methylcholest-8-ene-3,6-diol  **3**	416.7	417.4	415.3	15.0 5 - 18.43 21.43 - 25.5 37.44 - 39.94
(4) stigmasterol (24S)-5,22-Stigmastadien-3β-ol  **4**	412.7	413.3	-	24.6 y 37.4 -38.1
(5) oleic acid (9z,12z)-hexadecadienoic acid	282.3	283.4	-	18.13 - 41.9

				
(6) betalains (1E,2R)-1-[(2E)-2-(2,6-Dicarboxy-2,3-dihydro-4(1H)-pyridinylidene)ethylidene]-5-(α-D-glucopyranosyloxy)-6-hydroxy-2,3-dihydro-1H-indolium-2-carboxylate 	550.5	551.2	549.1	8.4 -17.8

* Molecular mass reported in the literature [14-16].

The chemical mixture 1-6 favors the crystallographic orientation [111] in AgNP, which is similar to the orientation found in nanoparticles synthesized with oleic acid and oleylamine (Fig 3). Consequently, the extract has components that favor growth [111] [17], in particular, chemical 6 which has a secondary amine (R_1-NH-R_2) similar to the primary amine (R_1 NH_2) present in OAm [2], increasing the presence of similar amines would increase the yield of green synthesis.

UV-Vis monitoring of M. Geometrizans extract decline:

The synthesis of silver nanoparticles was examined using a UV-vis spectrophotometer. The absorbance of the extract of the fruit of ***M. geometrizans*** is in a range of 200-450 nm since this is the characteristic range of compounds **1-5,** phytosterol 205-254 nm [18], pure oleic acid 230 -290 nm, and oleic acid with impurities 230-330 nm [19] in UV-vis.

Figure 2 shows the UV-vis spectra of the aqueous solution of the extract with 1 ml of 0.1 M $AgNO_3$. The nanoparticles form in the first 30 minutes of reaction. Smaller sizes are obtained at this time. The resonance of surface plasmons (SP) characteristic of silver nanoparticles (380-420 nm) is marginal due to minimal performance in synthesis with plant extracts (55-90% [3]) and a wide range of absorbance of M. extract residues geometrize that cover the surface of nanoparticles [17, 20].

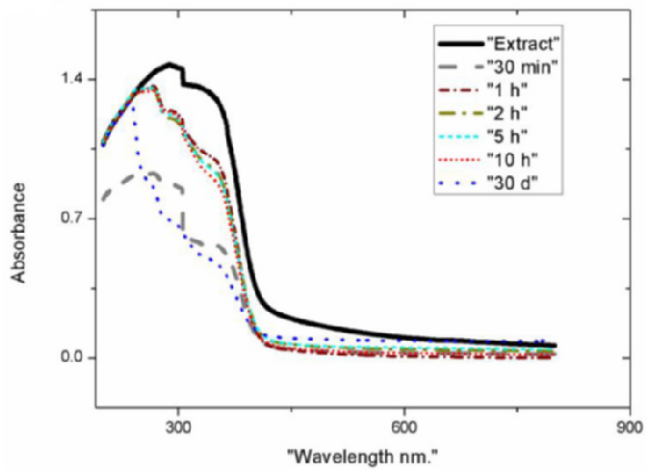


Figure 2. UV-visible synthesis spectrum using M. geometrizans fruit extract. UV-vis spectra were recorded from the aqueous reaction of the extract.

Analysis of scanning transmission electron microscopy (STEM):

Transmission electron microscopy experiments are shown in figure 3. Figure 3a) corresponds to the synthesis with OAm / OA and 0.1 M $AgNO_3$, figure 3b) corresponds to *M. geometrizans* extract with 0.1 M $AgNO_3$. The nanoparticles in both experiments adopted a semi-spherical morphology with a slight tendency to form aggregates. For the synthesis with OAm / OA average diameter of 6.33 nm; for the reaction with extract, an average diameter of 4.71 nm. Figure3b)4, the Fast Fourier Transform (FFT) shows that the preferential crystallographic directions correspond to the planes [111] characteristic of a cubic silver face-centered structure (FCC) [21].

The evidence of the crystallographic planes [111], confirms that there is a preferential orientation in both syntheses, in the first experiment it is due to the presence of OAm / OA that favor structures [111], and in the reaction of the extract is due to the presence of compounds 5-6. This is because OAm / OA has chemical similarities with the components identified in the *M. geometrizans* extract.

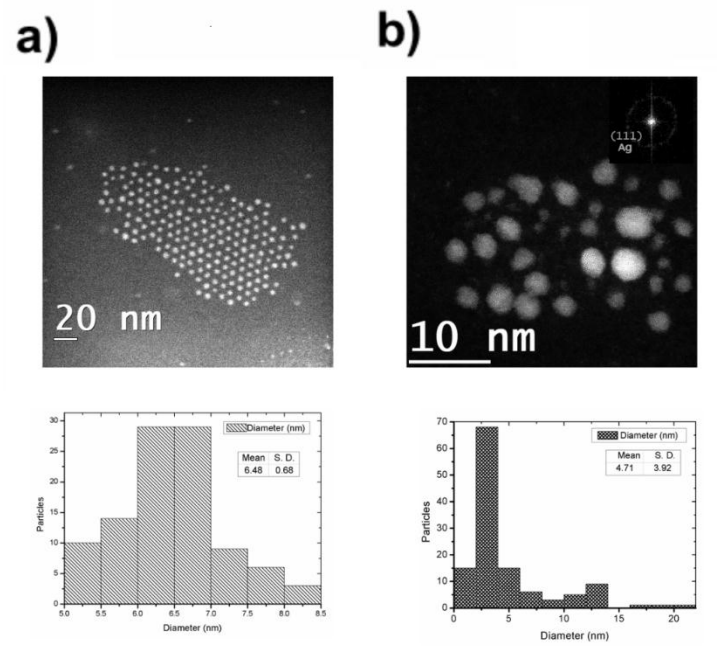


Figure 3. STEM micrograph and size histograms of the synthesized AgNPs. (a) using OAm 40% / OA 60 %, (b) using *M. geometrizans* fruit extract.

CONCLUSIONS.

The extract of *M. geometrizans* can generate semi-spherical AgNPs with crystallographic directions [111], similar to traditional reducers, such as OA and OAm but with a smaller diameter for green synthesis AgNPs. The specific orientation [111] by OA and OAm is for a lower surface binding energy of Ag and Pt atoms with amines and a carboxylic acid, this is demonstrated with DFT calculations and experimentally [2]. By analogy, compound 6 with amine is the first cause of orientation [111] in the green synthesis.

Purification of extract compounds 5 and 6 by HPLC would increase yield and homogeneity of size for AgNPs by green synthesis, this project for future work.

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