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Total Phenolic Composition and Antioxidant Activity of Silver Nanoparticles using Aqueous Extract of Chilca Leaves (*Baccharis Latifolia*)

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Abstract: The importance of studying polyphenols as natural antioxidants has encouraged the search for new methods of rapid, simple analysis. The synthesis of silver Nanoparticles (Ag NPs) using plant extracts has been presented as an alternative to determine the presence of polyphenolic compounds. In this study, aqueous extract of chilca (*Baccharis latifolia*), an endemic plant species from South America known for its medicinal properties, was used. This extract, because of its composition, can convert Ag^{+} ions to Ag^{0} in a chemical reduction process. To determine the optimal conditions for microwave-assisted nanoparticle synthesis, factorial experimental designs were used and analyzed with Statgraphics software. Ag NPs characterization was carried out with transmission electron microscopy; synthesized nanoparticles measured 4.86±2.44 nm on average. For the extract and Ag NPs, total polyphenolic content and antioxidant capacity were determined using oxygen radical absorbance capacity and cyclic voltammetry analysis. The method used to prepare both plant extracts and Ag NPs was determined to be fast and reliable. In addition to being a green and economical, this method allows the direct measurement of the plant extract's total polyphenolic content and antioxidant capacity using analytical techniques that may be potentially applicable in the pharmaceutical industry.

Keywords: Silver Nanoparticles, Chilca, *Baccharis latifolia*, Polyphenols, Antioxidant Activity

Introduction

Nanomaterials are presently studied with great interest because of their novel properties (Roco and Bainbridge, 2005; Whitesides, 2005; Zaman *et al*., 2014). Nanoparticles of noble metals such as gold, silver and platinum have generated greater attention for their electrical, optical, thermal and catalytic properties, among others (Jemilugba *et al*., 2019). Silver nanoparticles (Ag NPs) also have antibacterial properties and can be used as raw material for the preparation of drugs, surgical material and food containers (Morones *et al*., 2005; Kim *et al*., 2007; Michna *et al*., 2019). Ag NPs are also used for other applications, such as in textiles, cosmetics, instrument analysis and water treatment (Jini and Sharmila, 2020). This versatility is the result of its high surface ratio with respect to the volume presented by the material (Zheng and Wang, 2001; Sotiriou *et al*., 2011; Zaman *et al*., 2014).

Ag NPs can be prepared using chemical, electrochemical, physical and biological methods (Abou El-Nour *et al*., 2010; Iravani *et al*., 2014; Wei *et al*., 2015). The current chemical method has some disadvantages, including the use of expensive, toxic and dangerous reducing reagents, which negatively affect both the profitability of the process and the environment (Tripathi *et al*., 2019). An alternative to this method is green synthesis, a technique that consists in taking advantage of a plant's phytochemical composition, given that several of its compounds have reducing and stabilizing properties that can be used to produce Ag NPs

(Iravani, 2011). In general, plants' are composed of polyphenols, polysaccharides and polyoxometalates, among other molecules, which facilitate the reduction of the metal precursor (Cowan, 1999; Mittal *et al*., 2013; Rauwel *et al*., 2015). The advantage of this technique is the use of a non-polluting reducing agent, which is also economical, readily available and easy to handle as compared to others (Devaraj *et al*., 2013; Tripathi *et al*., 2019).

Polyphenols are among the most common secondary metabolites and have antioxidant characteristics (Ribeiro *et al*., 2010). Antioxidants are defined as compounds that prevent oxidation reactions, which means they can prevent free radical formation, thus avoiding aging and degradation processes in plants and humans, respectively (Prior *et al*., 1998; Kähkönen *et al*., 1999). The importance in determining antioxidant capacity using Ag NPs relies in the fact that it is the same reaction mechanism in which polyphenols inactivate free radicals (Avello and Suwalsky, 2006).

The plant *Baccharis latifolia* (Fig. 1), commonly known as chilca, has antioxidant properties (Loayza *et al*., 1995). This endemic plant of Ecuador belongs to the *Asteraceae* family and is distributed throughout the Ecuadorian Andean region between 1000 to 4000 masl (Shafi *et al*., 2004; Jadhav *et al*., 2009; Palá-Paúl *et al*., 2019). It can reach 2 m high and 3 m wide and its leaves have a maximum length of 20 cm (Abad *et al*., 2006). *B. latifolia* is considered a medicinal plant thanks to its anti-inflammatory effect (Abad and Bermejo, 2007). It is used frequently in traditional medicine for treating stomach pains, fractures and kidney problems, among other ailments (Loayza *et al*., 1995; Abad *et al*., 2006). Several studies have shown that its aqueous extract has a bactericidal effect on different microorganisms such as *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Proteus subtilis* (Fogliano *et al*., 1999; Mantena *et al*., 2003; Vijayakumar *et al*., 2013; Zambrano-Moreno *et al*., 2015).

Studies have shown that *B. latifolia* consists of sterane derivatives (Hoyos Vargas and Yep Chu, 2008). The most relevant compounds are α-phellandrene (Robledo *et al*., 2019), caryophyllene oxide (Meeran *et al*., 2019), camphene (Ceborska, 2017), terpinen-4-ol (Luo *et al*., 2019) and γ-gurjunene (Harman-Ware *et al*., 2017). It also contains terpenes such as α- pinene and limonene, as well as various flavonoids (da Fonseca and de Carvalho, 2006; Barrón-Yánez *et al*., 2011). These compounds are responsible for the plant having a high resistance to UV radiation and allow it to generate a photo protective layer that prevents the formation of free radicals that may degrade its physical structure (Bennett and Walls grove, 1994; Bourgaud *et al*., 2001). Dichloromethane extract of *B. latifolia* leaves promotes anti-inflammatory activity at doses of 300 $mg \cdot kg^{-1}$, while acetone extract inhibits 89% of cancer cells in epithelial tissue (Prada *et al*., 2016; Calle *et al*., 2017). Derivatives of thymol (Kłeczek *et al*., 2019) and sesquiterpenes (Wang *et al*., 2019) have been found in its roots, while diterpenes from the labdane and germacrene nucleus (Zdero and Bohlmann, 1989) have been reported in the plant's upper part. Regarding its biological activity, its cytotoxic, antiproliferative, antifungal and anti-inflammatory effects have been studied (Abad *et al*., 2006; Sequeda-Castañeda *et al*., 2015; Prada *et al*., 2016).

Fig. 1: *Baccharis latifolia*

A number of investigations related to the synthesis of Ag NPs using plant extracts have been conducted (Jha *et al*., 2009; Song and Kim, 2009; Tippayawat *et al*., 2016); however, the use of *B. latifolia* in the preparation of Ag NPs has not been reported. Similar plants from the *Asteraceae* family (*Calendula Officinalis L, Some Launaea* and *Tagetes erecta*) have been studied and even larvicidal application has been found (Scampicchio *et al*., 2006; Özyürek *et al*., 2012; Akkoc *et al*., 2019). This study determined total polyphenolic content and antioxidant activity of the *B. latifolia* extract and the Ag NPs prepared with it to relate the reducing potential of the phenolic compounds contained in the extract with the nanoparticle formation as a method of chemical analysis.

Materials and Methods

Collection of B. latifolia Leaves

B. latifolia leaves were obtained from San Miguel de Arcángel in the city of Ibarra, Ecuador (geographical coordinates 0.353060, -78.101828). The collection was carried out with a Wildlife Management Patent N°06-2019-ICFAU-FLO DPAI/MAE from the Herbarium ECAA-PUCESI and authorization for Scientific Research N°006-2019-IC-FAU-FLO-DPAI/MAE from the Ministry of the Environment of Ecuador. A botanical voucher was previously deposited for reference at Herbaria of the PUCESI. The plant material was dried for 4 h at 30°C. Dehydration was performed in a Termokool oven for 20 min. Finally, a Retsch Grindomix GM 200 knife mill was used for four min at 4500 rpm.

The optimal conditions for extract preparation were selected with Statgraphics Centurion XVI software through a completely randomized 2^3 factorial type screening design (degrees of freedom: 35) and by following the extract preparation protocol reported by (Jaiswal *et al*., 2010). A total of 36 replicates were performed, varying parameters of plant mass, stirring time and reaction temperature. For each parameter, the following levels were used: Vegetable mass of 0.5 and 1.0 g; temperature of 20, 45 and 70°C; and heating time of 20, 40 and 60 min. The optimal plant extract was prepared with 0.63 g of plant sample with a stirring time of 60 min using a Boeco plate at room temperature (20°C). Finally, the extract was filtered through vacuum filtration and stored at 4°C.

Synthesis of Ag NPs

Synthesis was performed with a microwave-assisted procedure (Indurama MWI 28 BL) as suggested by Oukarroum *et al*. (2012). For the selection of optimal conditions, a fractional factorial type 2^4 screening design with two blocks and two central points per block (24 degrees of freedom) was applied. A total of 36 experimental tests were performed, varying the parameters of the concentration of $AgNO_3$ as the metallic precursor, volume of the extract, pH and heating time. The levels for each factor were: $AgNO_3$ concentration of 0.5 and 2.0 mm; extract volume of 0.5 and 2.0 mL; pH of 7 and 10; heating time 30 and 120 s. The response variables were absorbance and maximum wavelength, both obtained by visible spectrophotometry; target values were 1.000 UA and 410 nm, respectively. For optimal Ag NPs synthesis, a 1.54 mm silver solution was prepared using a high purity reagent (Merck 99.9%); 1.29 mL of aqueous extract at a pH of 8 was used for 49 s at a power of 800 W. The colloidal solution was cooled in an ice bath and stored in an amber bottle at 4°C.

Characterization of Ag NPs

Ag NPs were characterized using an FEI Tecnai G2 Twin Transmission Electron Microscope (TEM) operating at 80 kV, which allowed us to determine the size of the nanoparticles. To calculate average size, 412 nanoparticles were measured using Fiji software (Borase *et al*., 2014). Additionally, an X-Ray Diffraction (XRD) analysis was performed. Diffractograms were obtained using an Empyrean PANanalytical diffractometer in a Bragg-Brentano configuration of θ-2θ (generator-detector) equipped with a nickel filter, Cu K-alpha (λ = 1.541Å) and an X'Celerator detector. Prior to the analysis, the liquid sample was dried at 30°C on a microscope slide, which generates a thin layer while avoiding organic degradation. The average of 6 XRD patterns from 5° a 90° (configuration 2θ) was taken to obtain the final diffractogram.

Total Polyphenol Content

Total polyphenols were determined by the Folin-Ciocalteu spectrophotometric method (Folin and Ciocalteu, 1927).

Oxygen Radical Absorbance Capacity Fluorescence Antioxidant Capacity

Oxygen Radical Absorbance Capacity Fluorescence (ORAC-FL) analysis was carried out on a PerkinElmer EnSpire multimode plate reader using 96-well plates made of Nunc white polystyrene (Copenhagen, Denmark) by means of a Trolox calibration curve (3.0 to 20 μm). All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample. The area under the fluorescence decay curve (ABC) was calculated by integrating the decrease in fluorescence. Data processing was performed with Origin Pro 8.5 SR2 software (Origin Lab Corporation, Washington, USA).

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Results

Synthesis and Characterization of Ag NPs

The experimental design was applied to determine the optimal conditions for the preparation of the aqueous extract of *B. latifolia* leaves. With this methodology, it is possible to assess the effect of each one of the variables in the experimental result, as well as their interactions, while considering statistical significance ($p \leq 0.05$). Pareto diagrams (Fig. 2) show significant effects from the following factors: Plant mass, temperature and, to a lesser extent, the interaction between these two.

Experimental design results for Ag NPs synthesis (Fig. 3) show that silver nitrate concentration is the only common factor with a significant effect ($p \leq 0.05$) on the targeted wavelength and absorbance.

In Fig. 4, UV-Vis spectrum of the Ag NPs obtained with all parameters is shown.

Figure 5 shows the micrograph of the Ag NPs analyzed using TEM and the frequency histogram.

The XRD patterns of the silver nanoparticles synthesized according to the protocol described in section 3.2 are shown in Fig. 6.

Total Polyphenol Content

The results of total polyphenol content for *B. latifolia* and Ag NPs are shown in Table 1.

Table 1. Total polyphenols present in *B. latifolia* and Ag NPs.

Antioxidant Activity

The results of antioxidant activity for *B. latifolia* and Ag NPs are shown in Table 2.

Electrochemical Behavior

Results concerning the electrochemical behavior of the *B. latifolia extract* and Ag NPs are shown in Figs. 7 to 9.

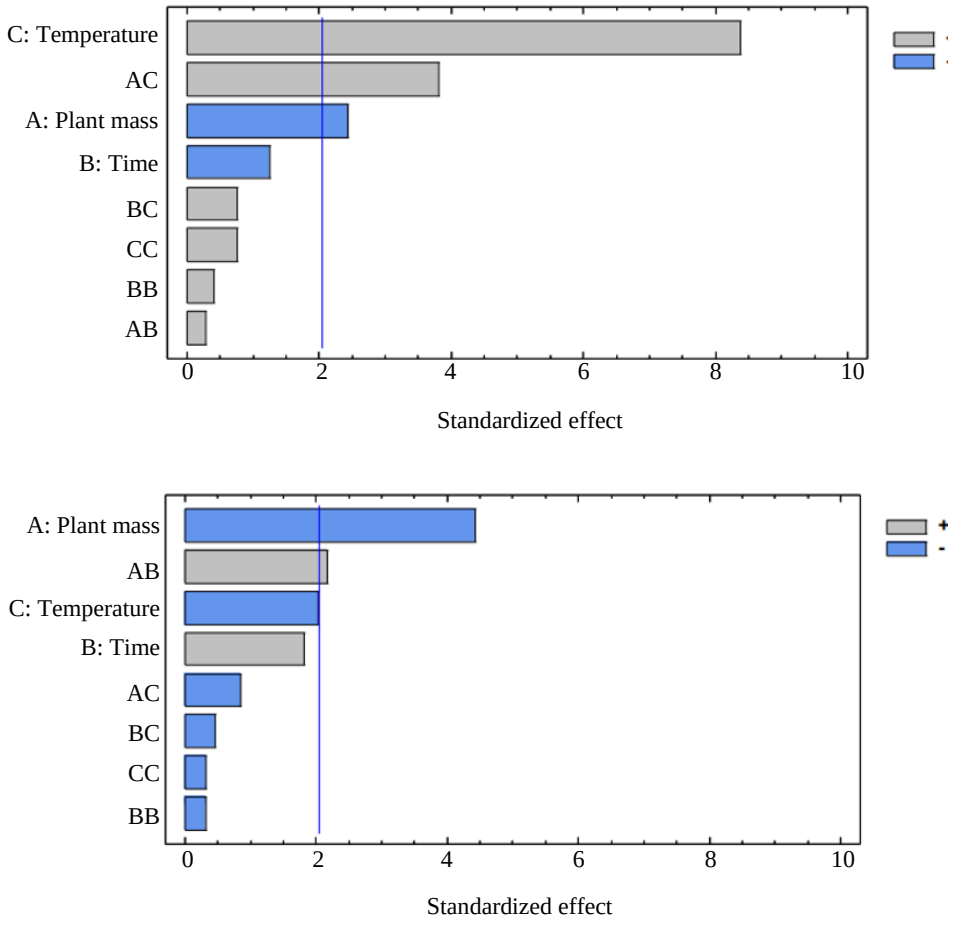

Fig. 2: Pareto diagram for optimization of maximum wavelength (above) and absorbance (below) in the selection of conditions for preparing *B. latifolia* leaf extract

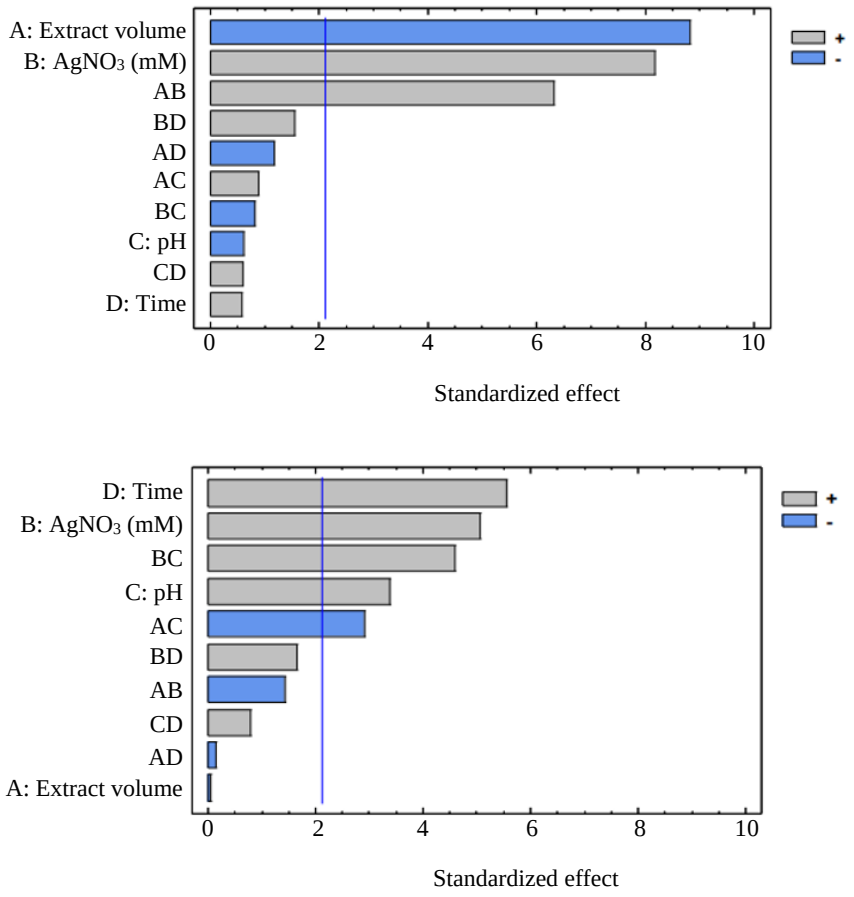

Fig. 3: Pareto diagram for optimization of the maximum wavelength (above) and absorbance (below) in the selection of Ag NPs synthesis conditions with *B. latifolia* aqueous extract

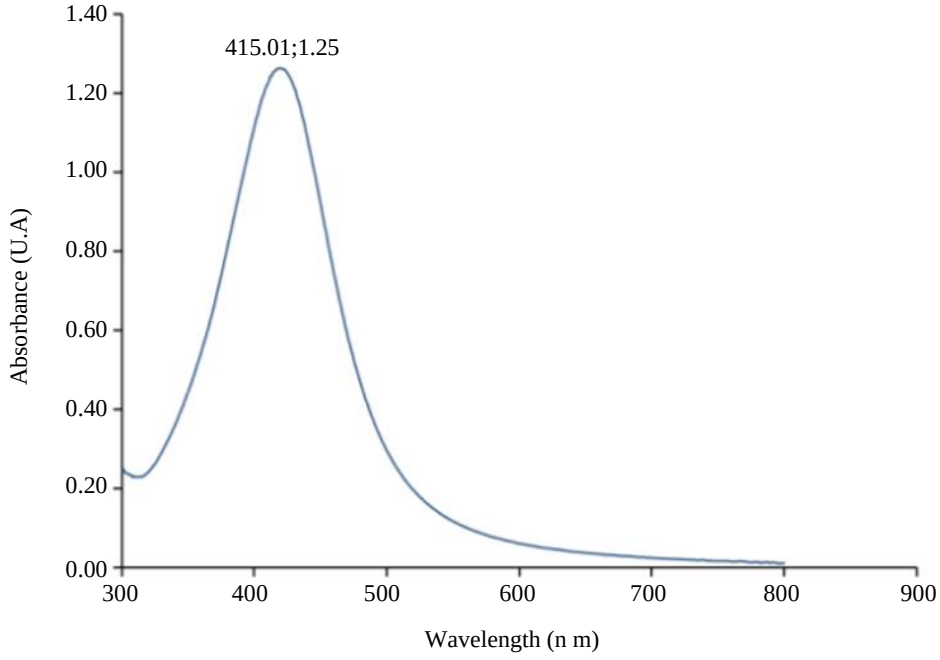

Fig. 4: UV-Vis spectrum of Ag NPs synthesized with *B. latifolia* extract

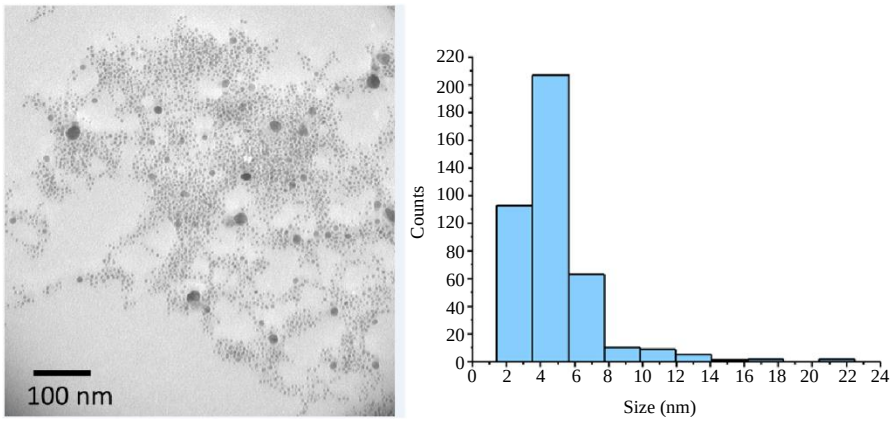

Fig. 5: TEM micrograph of Ag NPs (a) and size frequency histogram (b)

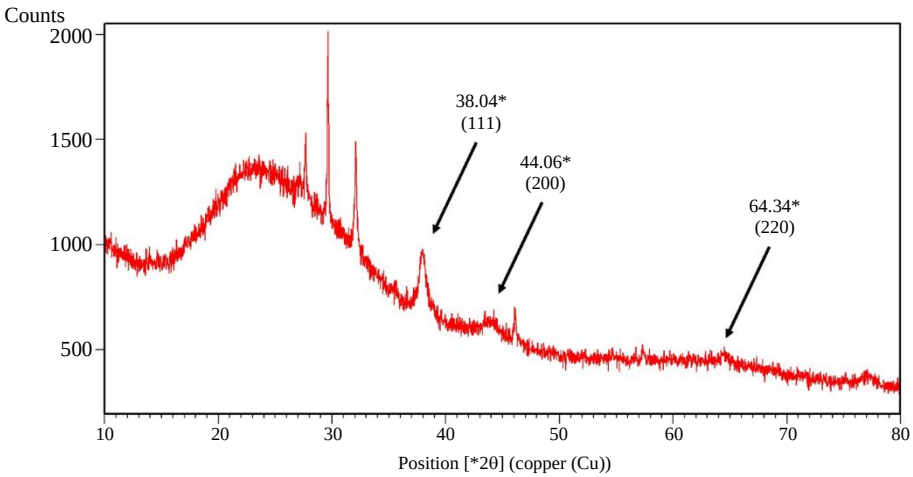

Fig. 6: XRD diffractometer for Ag NPs

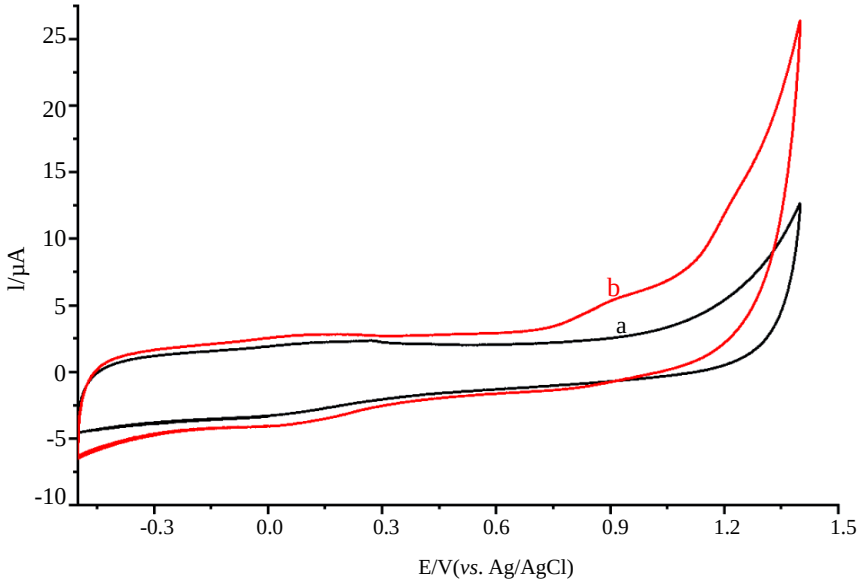

Fig. 7: Cyclic voltammograms of: (a) *B. latifolia* extract in 0.10 mol L^{-1} sodium acetate solution and (b) blank acetate solution, using a GC electrode. Scanning rate 50 mVs^{-1} Vs Ag/AgCl at 25°C

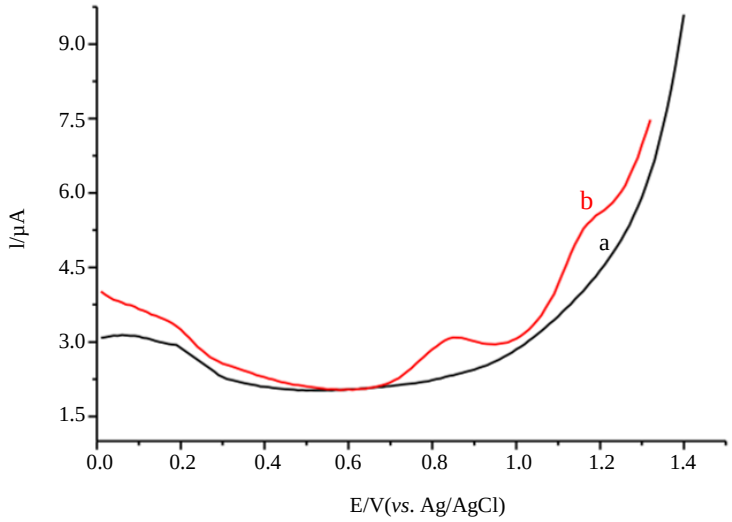

Fig. 8: Differential pulse voltammetry analysis of: (a) *B. latifolia* extract electrode in 0.10 mol L^{-1} sodium acetate solution and (b) blank acetate solution, using a GC. Scanning rate 50 mV. s^{-1} Vs Ag/AgCl at 25°C

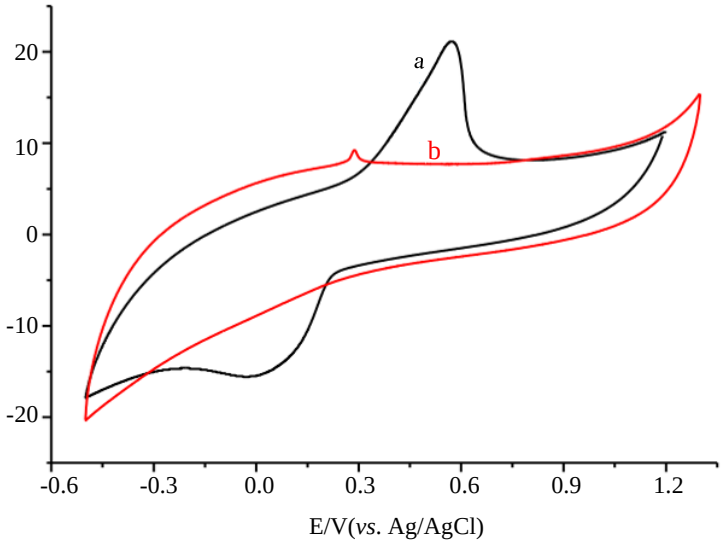

Fig. 9: Cyclic voltammograms of: (a) 5 mmol L^{-1} $AgNO_3$ + 0.10 mol L^{-1} sodium acetate solution and (b) Ag NPs- *B. latifolia* in 0.10 mol L^{-1} sodium acetate solution, using a GC electrode. Scanning rate 50 mV. s^{-1} Vs Ag/AgCl at 25°C

Table 1: Total polyphenols present in *B. latifolia* and Ag NPs

Sample	Total polyphenols (mg EAG/100 g dry mass)
B. latifolia	598.5±16.7
Ag NPs	146.1±3.2

Table 2: Antioxidant capacity of *B. latifolia* and Ag NPs

Sample	ORAC-FL (μm Trolox equivalent/l g extract)
B. latifolia	27183±3183
Ag NPs	3497±742

Discussion

Synthesis and Characterization of Ag NPs

The optimization analysis indicated the following experimental parameters of Ag NPs synthesis with *B. latifolia* leaves as optimal: 0.63 g of vegetable matter, 20 mL of distilled water, 60 min of stirring and a temperature of 20°C. Since the maximum absorption wavelength (λ) is related to the size of the nanoparticles, the experimental design response was optimized to obtain a lambda as close to 410 nm as possible. The obtained Pareto diagram (Fig. 2)

indicates that temperature and vegetable mass, as well as their interaction, are the factors that significantly affect obtaining the desired wavelength, therefore the NPs size. On the other hand, we considered that absorbance should have a high value as it is related to Ag NPs concentration, based on which the response optimization was performed to obtain a value close to 1000 UA. In this case, it was observed that vegetal mass and the interaction of vegetal mass with time are the two factors with significant effects, the former having greater magnitude. The temperature had a positive effect on lambda, meaning that at a higher temperature, the synthesized Ag NPs' wavelength was greater, which is not desirable. Conversely, the plant mass had a negative effect on both wavelength and absorbance, indicating that a low proportion of the aqueous extract of *B. latifolia* leaves should be maintained to maximize absorbance; however, this would affect the size of the Ag NPs synthesized with the extract. To balance these factors, an optimization of multiple responses was performed through the desirability function created with the aforementioned wavelength and absorbance values. With this, it was established that the best extraction conditions to reach a maximum absorption at 410 nm and an absorbance of 1000 UA for nanoparticle synthesis were: 0.63 g of *B. latifolia* leaves and a temperature of 20°C. Other factors, although being non-significant, were optimized as follows: 20 mL of distilled water, 60 min of agitation.

As in the previously described optimization, the target values for colloidal solutions of Ag NPs were a maximum wavelength near 410 nm and an absorbance close to 1000 UA. The performed experimental design shows that the concentration of silver nitrate directly influences both (maximum lambda and absorbance), so its balance is critical to obtain the desired characteristics, since a high concentration achieves a higher lambda (undesirable) but also a greater absorbance (desirable). It is key to note the reaction stoichiometry's influence, since changes in any of the reagents' concentrations affect the size of the obtained NPs, as evidenced by the effect of the AB interaction: Extract volume and silver nitrate concentration (Fig. 3). The inversely proportional relationship between extract volume and the wavelength of maximum absorption could be explained by the organic coating that remained on the Ag NPs, thus increasing their size. On the other hand, regarding absorbance, several factors and interactions had positive effects, time and pH being the most predominant. The interaction of H^+ ions with nitrate concentration and with extract volume is an important aspect related to the reduction reaction necessary to synthesize nanoparticles, since the pH influences the chemical form in which molecules from the extract will be in its active form to participate as reducing agents.

When performing the optimization based on the desirability parameter created from the absorbance and lambda target values, the following conditions were obtained: $AgNO_3$ 1.54 mM, 1.29 mL of plant extract, a pH of 8.2 and a time of 49 s. When applied to the synthesis procedure, Ag NPs formation was evidenced by the color change in the solution. The color of the aqueous extract of *B. latifolia* leaves is normally brown and it changed to yellowish-brown, which implies the presence of Ag NPs in the solution. The above is corroborated in Fig. 4, where it is also observed that the maximum absorption is 415 nm and the band has an absorbance of 1.25 UA, denoting compliance with the optimization performed through the experimental design. Similar studies using plant extracts from the *Asteraceae* family to prepare Ag NPs indicate that the maximum absorption band is between 414 and 430 nm. Morejón *et al.* (2018) found a maximum absorbance of 414 nm using *Ambrosia arborescens* extract, while (Padalia *et al*., 2015; Mousavi *et al*., 2018) obtained maximum absorbance at 430 nm with *Artemisia turcomanica* and *Calendula officinalis*, respectively.

The average size of the nanoparticles was 4.86±2.44 nm. As can be seen in the TEM image (Fig. 5a) and size frequency histogram (5b), the organic layer that covers the silver nanoparticles measures 1 nm. Previously reported TEM analysis for Ag NPs obtained with extracts of *Ambrosia arborescens*, *Artemisia turcomanica* and *Caléndula officinalis* indicate an average size of 14, 21.22 and 46.11 nm, respectively. The size of the Ag NPs obtained in this study was significantly smaller, thanks to the experimental design approach we employed.

In Figure 6 the peaks observed at the 2θ angle for the silver nanoparticles are the following: 38.04, 44.06 and 64.34°, which correspond to the (110), (200) and (220) reflection planes, respectively, of the FCC silver lattice (ICSD No. 98-018-0878) (Kumar *et al*., 2016). The most intense peak in the diffractogram, corresponding to the predominant orientation of Ag nanocrystals, is along the (111) plane and the Debye-Scherrer equation predicts an Ag NPs average size of around 15 nm, which is consistent with the value obtained by TEM. Other peaks correspond to the formation of AgCl and the presence of a bio-organic phase on the particles' surface (Kumar *et al*., 2017); this indicates that the reaction is competitive and not just Ag NPs nanocrystals are produced (Gorji *et al*., 2019).

Total Polyphenol Content

Prior to beginning this study, a phytochemical screening was performed on the *B. latifolia* aqueous extract to confirm the presence of polyphenolic compounds. Recent studies on *Asteraceae* plants have reported that total polyphenol content can be between 265 and 1334 mg EAG/100 g of dry mass; *Helianthus annuus L*. is the plant with the lowest phenolic content, while

Echinacea purpurea L. Moench has the highest content (Güneş *et al*., 2019). As can be seen in the results for *B. latifolia* (Table 1), the concentration of polyphenolic compounds is within the range reported by (Güneş *et al*., 2019). However, the characteristics of the solvent used to extract the phenolic compounds suggest that the quantified polyphenols' chemical nature (mainly organic acids of low molecular weight or glycosylated polyphenols) differs from that described above. In addition, it is important to keep in mind that not only can polyphenolic compounds reduce Folin's reagent (the solvent used in the extraction), but other compounds with reducing capacity, such as carbohydrates with terminal reducing ends, could cause a false positive in this type of assay. Therefore, the results must be complemented with other techniques to accurately assess polyphenolic compounds.

The decrease in the amount of phenolic compounds in Ag NPs when compared to the extract is because part of the concentration of polyphenols extracted from *B. latifolia* transforms Ag^+ to Ag^0. Therefore, the difference between the two values could be the result of some polyphenols being used to form Ag NPs. This suggests that with the solvent used, most of the reducing compounds were polyphenols or organic acids that have the necessary reducing power to form Ag^0.

Antioxidant Activity

The antioxidant capacity of the *B. latifolia* aqueous extract was determined by the reaction of the phenolic compounds present in the extract with oxygen-centered radicals (RO/ROO$^\bullet$) generated by the thermolysis of the ABAP azo compound. The extract showed greater antioxidant capacity than the Ag NPs because to convert Ag^+ to Ag^0, the antioxidant compounds were oxidized, thus decreasing their concentration to quench the free radicals formed through this methodology. Therefore, some of the *B. latifolia* extract's antioxidant capacity was used to create Ag NPs, which accounts for the difference in antioxidant capacity between the extract and the Ag NPs.

The antioxidant capacity of the *B. latifolia* extract was found to be greater than that described for several other plants from the *Asteraceae* family (Ledoux *et al*., 2018). This could possibly be because of the solvent used to extract phenolic compounds, which could consist of mainly glycosylated compounds or low molecular weight organic acids such as gallic acid.

Electrochemical Behavior

Oxidation potential is a physicochemical parameter that determines the energy necessary for a compound to yield electrons. The current study's oxidation potential values are linked to antioxidant capacity such that low oxidation potential implies a greater antioxidant capacity. By means of Cyclic Voltammetry (CV), the electrochemical behavior of the *B. latifolia* aqueous extract was evaluated. Figure 7a shows the cyclic voltamperogram of the extract using 0.1 mol L^{-1} sodium acetate as a supporting electrolyte; here, two oxidation waves at potentials between (-0.1 V; 0.2 V) and (0.71 V; 1.0 V) suggest the presence of at least two types of reducing species or a reducing species that can be oxidized by two stable intermediates. The voltamperogram shows waves toward a reduction potential between (+0.75 and +0.8 V) and (-0.2 and +0.2 V), signals apparently coupled with the anodic processes in direct scanning, suggesting a certain degree of electrochemical reversibility. If this is the case, it could be attributed to quinone groups formed in the oxidation scan from $^-$OH groups present in the structures of oxidants. This is consistent with the presence of species of phenolic character in the extract.

To corroborate the results obtained by CV, Differential Pulse Voltammetry (DPV) was used. This technique results in current-potential signals with greater sensitivity (Bar *et al*., 2009) because it can eliminate the contribution of the background current obtained in cyclic voltamperograms, which does not allow the signals per CV to be precisely defined and can also deconvolve the current signals in order to demonstrate the existence of other additional waves in the system. Figure 8b shows the presence of two oxidation waves at the same intervals of potentials obtained for the CV signals and, in addition, a third wave at +1.16 V, which confirms the existence of three types of species with antioxidant capacity in the extract. Current values for the first two waves have the same order of magnitude, indicating that both types of species could be present in similar amounts in the extract, while the third wave at +1.16 V, which is a greater current value, suggests that this species has a higher concentration in the extract than the first two. On the other hand, if we consider that each of the signals corresponds to a chemical species with individual characteristics and behavior, the extracted compounds may have different antioxidant capacity, that is one with less capacity (+1.16 V) and the other two with greater capacity (+0.2 and +0.9 V).

The electrochemical behavior of the Ag NPs and *B. latifolia* extract was evaluated using CV (Fig. 9b), which was compared to that of an aqueous solution of five mmol L^{-1} of silver nitrate (Fig. 9a). The latter showed a reduction signal at +0.027 V and an oxidation signal at +0.559 V Vs. Ag/AgCl; the reduction peak is associated with the electrodeposition of silver ions on the surface of the vitreous carbon electrode, while the oxidation peak corresponds to the redissolution signal of the silver deposited on the electrode in the reduction scan. The voltamperogram obtained for the Ag NPs and *B. latifolia* extract does not show reduction waves but an oxidation signal at +0.30 V. The absence of cathodic signal suggests the non-existence or low presence of Ag^+ ions in said extract, which shows that it comprises mostly Ag^0

nanoparticles, products of the extract's reducing action. The oxidation signal at +0.30 V is associated with the oxidation of Ag particles present in the extract. These results also show the ability of *B. latifolia* extract to stabilize Ag^0 nanoparticles.

Conclusion

The proposed method of Ag NPs synthesis using aqueous extract of *B. latifolia* leaves was reliable, easy, fast, economical and environmentally friendly. Antioxidant capacity tests are reliable as similar results are obtained by different analytical and electrochemical techniques. Regarding total polyphenolic content and antioxidant capacity as analyzed by ORAC-FL and CV/DPV, the results in all cases indicate a significant decrease in Ag NPs compared to the extract, suggesting that the polyphenolic compounds present in the plant sample intervene in the synthesis process as reducing agents. It is a simple and fast Ag NPs formation technique, since the color change is evident in the formation of the surface plasmon, in addition to being a readily available and non-toxic plant reducer. The reducing effect of the aqueous extract from *B. latifolia* leaves demonstrated in this study could be extrapolated to antioxidants' biological effect on free radicals within an organism. Phenolic compounds react in the presence of reactive chemical species, such as chemical monitors. This effect could potentially be applied in the phytochemical, pharmaceutical and food industries, among others, for the detection of antioxidant substances. In addition, the optimization by experimental design allowed the best parameters for both the preparation of the aqueous extract of *B. latifolia* leaves and the Ag NPs synthesis to be determined, resulting in a reduced nanoparticle size (mean diameter <5 nm).

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Author's Contributions

Fernanda Pilaquinga: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Camila Granja and Josué Pozo: Performed the experiments.

Eliza Jara, Mauricio Moncada-Basualto, Lenys Fernández, Patricio Espinoza-Montero, Alexis Debut and Fernanda López: Analyzed and interpreted the data. Wrote the paper.

Claudio Olea-Azar: Analyzed and interpreted the data.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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