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Biosynthesis of silver and gold nanoparticles using *Stevia rebaudiana* extract

Biosíntesis de nanopartículas de plata y oro con extracto de *Stevia rebaudiana*

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Abstract

In the present work, the results of the synthesis of silver and gold nanoparticles prepared through a low-cost and environmentally friendly synthesis strategy are presented, using the aqueous extract of the *Stevia rebaudiana* plant as a reducing agent of the metals. The synthesized nanoparticles were characterized by UV-Vis spectroscopy and Atomic Force Microscopy. The shape and position of the maximum absorption peaks of the UV-vis spectra led us to suppose the presence of particles of preferentially spheroidal morphology and relatively small sizes. This was corroborated by Atomic Force Microscopy studies, since spheroidal shapes and a size distribution close to 100 nm were observed for the case of silver nanoparticles and between 15 and 50 nm for gold nanoparticles. Additionally, the antimicrobial activity of the gold and silver nanoparticles obtained was verified by the well diffusion method on Mueller-Hinton agar. The microbial challenge included *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria).

Metallic nanoparticles, Silver, Gold, Bactericides

Resumen

En el presente trabajo se presentan los resultados de la síntesis de nanopartículas de plata y oro preparadas mediante una estrategia de síntesis de bajo coste y respetuosa con el medio ambiente, utilizando el extracto acuoso de la planta *Stevia rebaudiana* como agente reductor de los metales. Las nanopartículas sintetizadas se caracterizaron mediante espectroscopia UV-Vis y microscopía de fuerza atómica. La forma y la posición de los picos máximos de absorción de los espectros UV-Vis hicieron suponer la presencia de partículas de morfología preferentemente esferoidal y de tamaños relativamente pequeños. Esto fue corroborado por los estudios de Microscopía de Fuerza Atómica, ya que se observaron formas esferoidales y una distribución de tamaños cercana a los 100 nm para el caso de las nanopartículas de plata y entre 15 y 50 nm para las nanopartículas de oro. Además, la actividad antimicrobiana de las nanopartículas de oro y plata obtenidas se verificó mediante el método de difusión en pozo en agar Mueller-Hinton. El desafío microbiano incluyó a *Escherichia coli* (bacteria gramnegativa) y *Staphylococcus aureus* (bacteria grampositiva).

Nanopartículas metálicas, Plata, Oro, Bactericidas

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Introduction

Interest in nano-sized chemicals is one of the most important fields of work in chemical research due to the large number and variety of new properties and applications in different areas of science and technology. This field that has had a vertiginous development in recent years describes the creation and exploitation of materials with controlled structural characteristics with at least one dimension in the nanometric range, specifically structures that are characterized by having sizes between 1 and 100 nm. It should be noted that substances on the nanometric scale significantly alter their physical and chemical properties, which creates a completely new perspective for the design of novel materials where their effects at the nano level are linked to the high relationship between their volume and their area of surface (Duncan, 2009).

Regarding nanostructures, the most used to date are metallic nanoparticles, due to the relative ease with which they are obtained and the possibility of controlling their size and shape. (Monge, 2009). Therefore, one of the objectives of scientists is to obtain nanoparticles of various metallic elements with different shapes and sizes, since they have different optical, electronic, magnetic and catalytic properties which are related to their shape and size (Aguilar, 2017). One of the areas in which nanotechnology has focused is biomedicine, where nanoparticles of silver AgNPs and gold AuNPs have shown an important role due to their strong bactericidal and fungicidal activity, being widely used in medicine for infection control (Geethalakshmi, 2012).

In the preparation of silver and gold nanoparticles of different shape and size, different physical and chemical synthesis methods have been developed (Burda, 2005). However, most of these methods are expensive and aggressive with the environment, for which synthesis with eco-friendly reducing agents has been sought, such as biological methods that make use of microorganisms, plants or plant extracts as reducing agents and stabilizers, methods that have become important due to the advantages they offer over physical or chemical methods.

Such advantages include the elimination of organic solvents, and other reducing and stabilizing agents, high energy consumption or sophisticated equipment are not needed to produce the nanoparticles, in addition to the fact that the reactions are carried out under environmental conditions of pressure and temperature (Mitla, 2013). Biosynthesis with plant extracts as reducing agents has been among the most used for the production of nanoparticles of silver AgNPs and gold AuNPs since these extracts contain among their components natural antioxidant agents that are generally made up of mixtures of compounds with high molecular diversity and functionality. biological, bio-synthesized by plants in their fruits, leaves, stems, roots, seeds or other plant parts, among the most important compounds are polyphenolic compounds, which are a group of chemical substances found in plants characterized by the presence of more of a phenol group attached to one or more benzene rings, which are responsible for their activity as antioxidants, by being attached to a benzene ring, the hydroxyl groups confer on the polyphenol the ability to act, either as a donor of a hydrogen atom or as donor of an electron to a free radical (Pietta, 2000). The low redox potentials of these antioxidants make the reduction of metal ions to metal atoms thermodynamically favorable (Han, 2012). Silver (AgNPs) and gold (AuNPs) nanoparticles have been the particular focus of plant-based syntheses, extracts from a diverse range of plant species have been used successfully in the manufacture of these nanoparticles.

In this sense, the objective of this work is the study of silver AgNPs and gold AuNPs nanoparticles prepared at room temperature from $AgNO_3$ and $HAuCl_4$ solutions with the aqueous extract of the *Stevia rebaudiana* plant as a reducing agent. The choice of this plant was motivated by its availability and because studies carried out on the chemical composition of its leaves reveal that they contain a large amount of polyphenols, which show good antioxidant activity in in vitro systems (Shruti et al., 2012). Its economic importance lies in its content of substances with great sweetening power, steviosides and rabaudiosides, compounds 400 times sweeter than sucrose, it also contains carbohydrates, proteins, vitamins and minerals, and has no calories (Bravo et al., 2009).

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This plant is used as a natural sweetener, and from bibliographic references it is known that it has many beneficial properties, among which is its antioxidant property. Figure 1 shows the chemical structure of stevioside (Bravo et al., 2009).

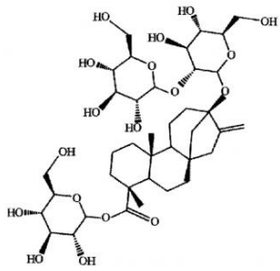

Figure 1 Chemical structure of stevioside
Source: Bravo et al., 2009

The silver AgNPs and gold AuNPs nanoparticles prepared by this method were characterized by the color of their solutions, a characteristic property of them. In addition, the stability that these solutions presented with the passage of time was determined through the UV-Visible spectroscopic technique, the formation of the metallic nanoparticles was determined by the appearance of the absorption band called resonance plasmon, characteristic of the silver AgNPs and gold AuNPs nanoparticles. Through the scanning of the nanoparticles by means of Atomic Force Microscopy (AFM), the hemispherical structure and the size of some of the nanoparticles were determined. Finally, the antimicrobial activity of the nanoparticles was tested against *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram-positive bacteria) bacteria.

Materials and methods

The chemical reagents used and their concentrations for the preparation of the AgNPs and AuNPs nanoparticles were: $AgNO_3$ silver nitrate $1x10^{-3}$ M (Sigma-Aldrich), $HAuCl_4 \cdot 3H_2O$ $1x10^{-3}$ M (Sigma-Aldrich), deionized water (18 MΩ cm^{-1}) and driy crushed *Stevia rebaudiana* leaves. To adjust the pH, drops of a sodium hydroxide (NaOH) solution 0.5 M (J. T. Baker) in water were added to the plant extract until the desired value was adjusted.

Preparation of the Stevia plant extract.

Dry crushed *Stevia rebaudiana* plant leaves were used and the extract was prepared using a traditional methodology called infusion, which consists of a simple solid-liquid extraction. 1.0 g of the ground plant material was put in contact with 100 mL of deionized water at 80 ° C until a volume of 70 mL was obtained, and then filtering was carried out.

Synthesis of silver AgNPs and gold AuNps nanoparticles

Dilutions of 30 mL of the aqueous extract of *Stevia rebaudiana* were prepared, using 0.5 mL of the extract obtained from the infusion with 29.5 mL of water. To adjust the pH = 8 drops of a sodium hydroxide (NaOH) solution 0.5 M (J. T. Baker) in water were added to the extract solution until the desired pH value was adjusted. The synthesis of AgNPs was carried out at room temperature, through the reduction of Ag^{1+} ions to silver Ag^0 with the aqueous extract of *Stevia rebaudiana*. We proceeded as follows: to the 30 mL solution prepared containing the aqueous extract of *Stevia rebaudiana* at pH = 8 and under constant stirring, 10 mL of $AgNO_3$ silver nitrate solution $1x10^{-3}$ M were slowly added, showing almost of an immediate color change from to amber yellow.

Proceeding in the same way, the synthesis of AuNPs was carried out at room temperature, through the reduction of Au^{3+} ions to Au^0 gold with the aqueous extract of Stevia rebaudiana. To the 30 mL prepared solution containing the aqueous Stevia extract at pH = 8 and under constant stirring, 10 mL of $HAuCl_4 \cdot 3H_2O$ chloroauric acid solution $1x10^{-3}$ M were slowly added.

Characterization of AgNPs and AuNPs nanoparticles

The samples obtained from silver and gold nanoparticles were measured by UV-Visible spectroscopy (Perkin Elmer Lambda 35 Spectrophotometer), a technique used to characterize the optical properties of metallic nanoparticles since it allows obtaining the surface plasmon band, band that is considered as evidence of the formation of nanoparticles. In the case of silver nanoparticles, the plasmon band appears between 350 and 400 nm (Murray et al., 2007).

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And for gold, this band appears between 500 and 600 nm, according to studies carried out for its characterization (Cornejo, 2015). Furthermore, from the information provided by the UV-Vis spectra, it is possible to estimate the concentration and size of the nanoparticles formed. To confirm the size and distribution of the metallic nanoparticles obtained, they were characterized by AFM Atomic Force Microscopy (JSPM-5200 Equipment). The topographic study carried out on the samples allows from images to determine the presence of the nanoparticles in the form of aggregates, as well as their morphology and their size distribution intervals (García, 2020).

The antimicrobial activity of the colloidal solutions of the prepared nanoparticles was carried out by the well diffusion method on Mueller-Hinton agar. The microbial challenge included *Escherichia coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria).

Results

The results obtained in the preparation and characterization of the AgNPs silver nanoparticles and AuNPs gold nanoparticles with the aqueous extract of *Stevia rebaudiana* are shown below.

Silver AgNPs nanoparticles

The synthesis of AgNPs was visually detected by color, the aqueous solution of Ag^{1+} ions after being in contact with the *Stevia rebaudiana* plant extract at pH = 8 immediately showed an amber yellow coloration, characteristic of silver nanoparticles. which refers to the formation of silver nanoparticles (Cruz et al., 2012). This coloration occurs a few minutes after adding silver nitrate ($AgNO_3$) to the aqueous extract of *Stevia rebaudiana*. Figure 2 shows the color of the solution of the silver nanoparticles obtained at different reaction times: 30 minutes, 60 minutes and 90 minutes.

Figure 2 Color of the solutions of the AgNPs at different times from the beginning of their formation reaction: a) 30 min, b) 60 min, c) 90 minutes
Source: author's own

The reaction process was simple and was monitored by ultraviolet-visible (UV-vis) spectroscopy, spectra of the sample obtained were determined at 30 minute intervals until color stabilization at 90 minutes. In figure 3 graphs of the spectral sweeps are shown, where the plasmon band is observed, which appears between 350 and 400 nm, due to the oscillations of the density of the free electron atmosphere existing on the surface of the AgNPs (plasmons), as has been reported in other silver nanoparticle synthesis processes (Murray, 2007).

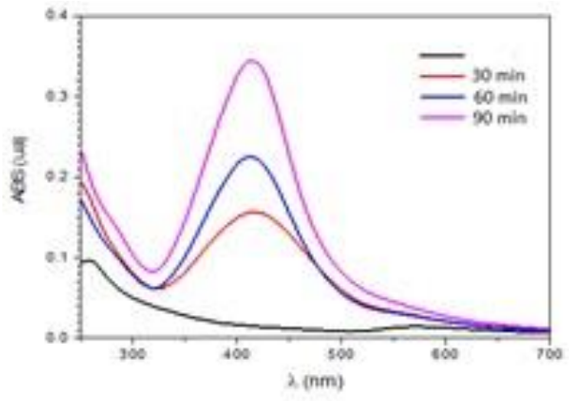

Figure 3 UV-Vis absorbance spectra of the solution of AgNPs prepared with the aqueous extract of *Stevia rebaudiana* at different times from the start of its formation reaction
Source: author's own

The peak of the spectral band at a wavelength of 430 nm that can be seen at 90 minutes confirmed the formation of the nanoparticles in higher concentration. On the other hand, the shape and width of the bands reveal that the silver nanoparticles obtained by reduction with the *Stevia rebaudiana* extract are polydisperse and most likely spherical in all cases (Majles, 2009; Frank, 2010).

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It should be mentioned that the black band observed in the spectrum of Figure 3 corresponds to the absorbance band of the plant extract.

To confirm the size, morphology and distribution of the silver nanoparticles obtained, they were characterized by AFM Atomic Force Microscopy (Jeol-JSPM-5200 Equipment). The topographic study carried out on the samples made it possible to determine the presence of the nanoparticles in the form of aggregates, as well as their morphology and their size distribution intervals. Figure 4 shows the micrographs of the scan performed on the sample of silver nanoparticles obtained with the aqueous extract of *Stevia rebaudiana* at pH = 8 at 90 minutes of reaction. The topographic AFM image (2.06 nm x 2.06 nm x 69.5 nm) is observed in "shaded" mode of the silver nanoparticles obtained with the extract, particles of spherical morphology can be seen, and a conglomerate can be seen in the lower central part of the image of these spherical particles, the conglomerate is of the order of about 250 nm. In the rest of the surface, spherical nanoparticles with a size smaller than 100 nm are seen dispersed in a dispersed manner.

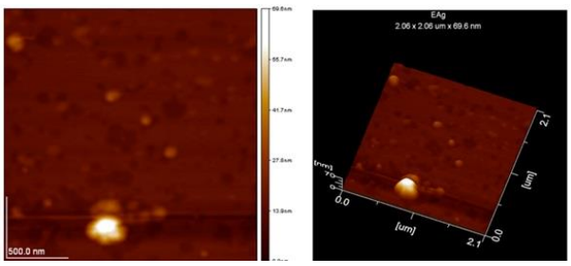

Figure 4 Images of the scan at 2.06 x 2.06 x 69.5 nm, "shaded" mode of the silver nanoparticles prepared with the aqueous extract of *Stevia rebaudiana*
Source: author's own

Figure 5 shows an amplification of the same area (500.7 x 500.7 x 27.0 nm), in which the hemispherical morphology of the particles is observed in greater detail, it is confirmed that the diameter of one of these particles is 100 nm. The image allows to detect the hemispherical shape of the particles consistent with the results of the electron absorption spectra in the visible region.

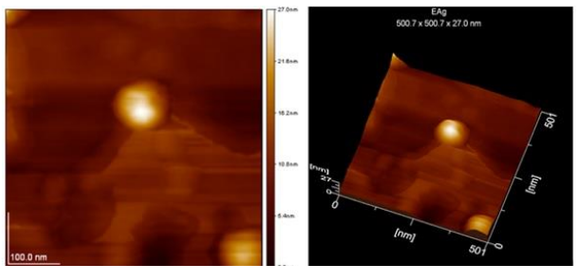

Figure 5 Images of the scan at 500.7 nm x 500.7 nm x 27.0 nm, shaded mode of the silver nanoparticles prepared with the aqueous extract of *Stevia rebaudiana*.
Source: author's own

AuNPs gold nanoparticles

The synthesis of AuNPs was carried out at room temperature, through the reduction of Au^{3+} ions with the aqueous extract of Stevia rebaudiana, their formation was detected visually by the change of the typical yellow color of $HAuCl_4$ to violet blue, the latter characteristic of AuNPs (Cornejo, 2015).

The resulting aqueous solutions have a characteristic coloration, which refers to the formation of gold nanoparticles. This coloration appears a few minutes after adding the *Stevia rebaudiana* extract, and it changes as time passes until it stabilizes. Likewise, the effect that reaction time exerts on the synthesis of gold nanoparticles can be observed, since over time the color of the solution changes from pink to intense pink until it reaches blue-violet. In figure 6 the color changes can be seen.

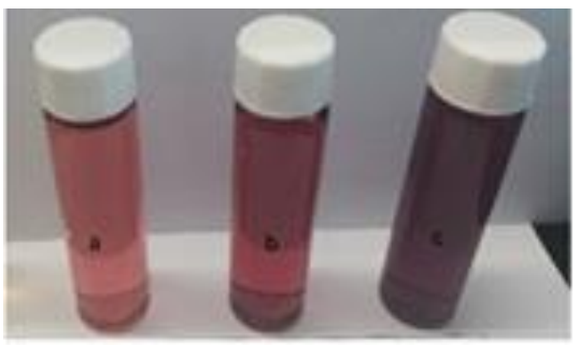

Figure 6 Color of the AuNPs solutions prepared with the aqueous extract of *Stevia rebaudiana* at different reaction times, a) 30 min, b) 2 h, c) 4 h.
Source: author's own

In the same way as in the case of silver nanoparticles, the reaction of the formation of gold nanoparticles was monitored by ultraviolet-visible spectroscopy (UV-vis), absorbance spectra of the sample obtained were determined at different intervals of time to color stabilization at 4 hours. The absorbance spectra as a function of wavelength are shown in Figure 7.

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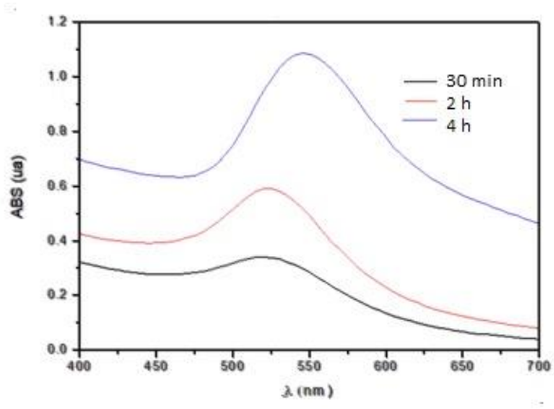

Figure 7 UV-Vis absorbance spectra of the AuNPs solution at different reaction times

From the UV-Vis spectra in Figure 7, the surface resonance plasmon band can be seen, which occurs between 500 and 600 nm, a characteristic position of gold nanoparticles, the intensity of plasmon absorption increases as a function of time, until reaching a stable value at 525 nm after 4 hours of reaction. Monitoring the absorbances of the solution for 4 hours showed that as the reaction time increased, the solution took a more bluish-violet color, consequently, the plasmon band shifted to higher wavelengths, 525 nm. On the other hand, the effect that reaction time exerts during biosynthesis can be observed in the displacement presented by the maximum of the absorption band associated with the plasmonic resonance of the gold nanoparticles (figure 7), the result indicates that the nanoparticle size was increased. The shape and width of the bands reveal that the solutions of the gold nanoparticles obtained are polydisperse and the shape of the nanoparticles is most likely spherical in all cases (Lien et al, 2011).

In the same way as for the silver nanoparticles, to confirm the size, morphology and distribution of the gold nanoparticles obtained, they were characterized by AFM Atomic Force Microscopy (JEOL JSPM-5200 Equipment).

Figure 8 shows the topographic AFM image (510.9 nm x 510.9 nm x 125.2 nm) in "shaded" mode of the gold nanoparticles obtained at pH = 8. In this figure, hemispherical particles can be seen, scattered on the surface of the silicon wafer.

Areas with nanoparticle aggregates are observed that cover the central part of the image, although there are also areas where there is greater dispersion of the aggregates, with sizes of hemispherical nanoparticles of the order of 50 to 15 nm. Polydispersity analysis showed that the particles obtained with said solution showed high dispersion.

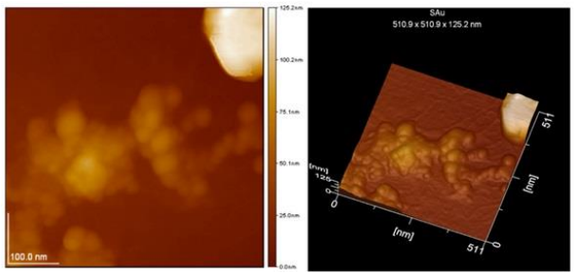

Figure 8 Images of the scan at 510.9 x 510.9 x 125.2 nm, "shaded" mode of the gold nanoparticles prepared with the extract of *Stevia rebaudiana*

Figure 9 shows an amplification of the same area (235.9 x 235.7 x 64.8 nm), in which the hemispherical morphology of the particles is observed in greater detail, consistent with the results of the electronic absorption spectra in the visible region. On the other hand, it is confirmed that they are polydisperse, that is, with different sizes, which can be seen in the image, and it is confirmed that there are diameters from 15 nm to 50 nm, all these particles are in a conglomerate.

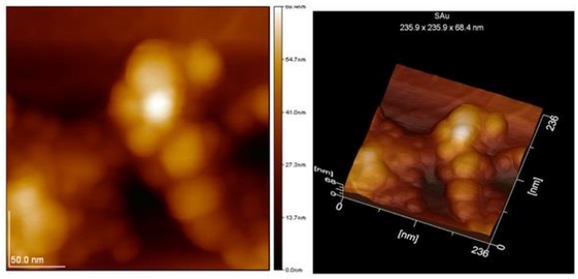

Figure 9 Images of the scan at 235.9 x 235.9 x 68.4 nm, "shaded" mode of the gold nanoparticles prepared with the *Stevia rebaudiana* extract

Bactericidal tests

The antimicrobial activity of the silver AgNPs and gold AgNPs nanoparticles was carried out by the well diffusion method on Mueller-Hinton agar. The microbial challenge included Escherichia coli (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria).

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Figure 10 shows the image of the plate with *Escherichia coli* where the results obtained from the application of the silver nanoparticle solution (well 1), the gold nanoparticle solution (well 2) and the extract solution can be appreciated. of *Stevia rebaudiana* (well 3). You can see the inhibition halo of 0.5 cm in diameter in well 1 where the solution of the silver nanoparticles was applied, as well as in well 2 an inhibition halo of 0.7 cm in diameter corresponding to the application of the dissolution of gold nanoparticles, inhibition halos considered as evidence of their bactericidal effect against E. coli bacteria. In the case of well 3, a weak presence of the inhibition halo is observed.

Figure 10 Image of the plate with *E. coli*. Test with silver nanoparticles (well 1), with gold nanoparticles (well 2) and aqueous extract of *Stevia rebaudiana* (well 3)

In the same way, figure 11, plate with the bacterium Staphylococcus aureus, shows the image of the results obtained from the application of the solutions of silver nanoparticles (well1), gold nanoparticles (well 2) and Stevia rebaudiana extract. (well 3). The inhibition halo of 0.6 cm in diameter can be seen in well 1 where the solution of the silver nanoparticles was applied, as well as in well 2 corresponding to the application of the solution of the gold nanoparticles, inhibition halos 0.8 cm in diameter, considered as evidence of its bactericidal effect against Staphylococcus aureus bacteria (gram-positive bacteria) and well 3 shows a weak inhibition halo of 0.3 cm in diameter.

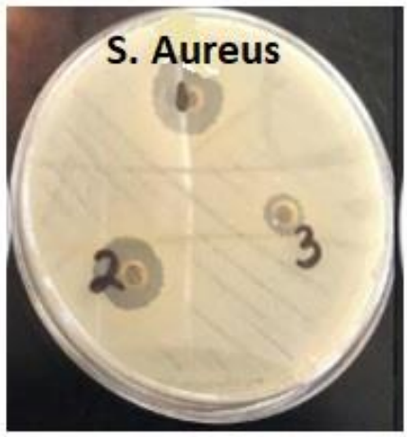

Figure 11 Image of the plaque with *Staphylococcus aureus*. Test with silver nanoparticles (well 1), with gold nanoparticles (well 2) and with aqueous extract of *Stevia rebaudiana* (well 3)

Table 1 summarizes the observations related to the inhibition halos.

Samples applied on the discs.	Inhibition diameter in *Escherichia coli*	Inhibition diameter in *Staphylococcus aureus*
Silver nanoparticles (well1)	0.5 cm	0.7 cm
Gold nanoparticles (well 2)	0.6 cm	0.8 cm
Stevia rebaudiana aqueous extract (well 3)	0.2 cm	0.2 cm

Table 1 Inhibition diameters presented

Conclusions

Spherical and polydisperse nanoparticles of silver AgNPs and gold AuNPs were obtained. Its formation was carried out directly under environmental conditions by reducing the Ag + and Au3 + ions with the aqueous extract of Stevia rebaudiana at pH = 8 as a reducing agent, with time being a factor that decisively influences the formation of the synthesized nanoparticles. Its formation was corroborated with the results of UV-vis spectroscopy and Atomic Force Microscopy.

Antimicrobial activity tests of AgNPs and AuNPs nanoparticle solutions against Escherichia coli (gram-negative bacteria) and Staphylococcus aureus (gram-positive bacteria) showed inhibition halos, proof of their bactericidal activity.

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This method is an excellent route for the synthesis of gold and silver nanoparticles giving good results avoiding the use of toxic and high-cost substances in their preparation, it also opens the way for their use in various applications and studies that are currently being carried out by our group of work.

References

Aguilar, T. A., Zanella, R. (2017). Las nanopartículas bimetálicas y algunas de sus aplicaciones. *Mundo nano,* vol.10 no.19.

Bravo, A. M., Ale, B. N., Rivera, C. D., Huamán, M. J., Delmás, R. D., Rodríguez, B. M., Polo, S. M y Bautista, C. M. (2009) Caracterización química de la *Stevia rebaudiana*. *Rev. Per. Quím. Ing. Quím.,* Vol. 12 N.º 2. Págs. 5-8.

Burda, C**.**, Chen, X., Narayanan, R., El-Sayed, M. A. (2005). Chemistry and Properties of Nanocrystals of Different Shapes. *Chem. Rev.*, 105, 1025–1102.

Cornejo, L. (2015). Resonancia del plasmón de la superficie (RPS). 2015b [Internet]. [Consultado el 30 enero 2019]. Disponible en: https://nuevastecnologiasymateriales.com/resonancia-del-plasmon-de-la-superficie-rps-propiedades-optoelectronicas/Oro.

Cruz, D. A., Rodríguez, M., López, J., Herrera, V. (2012). Nanopartículas metálicas y plasmones de superficie: una relación profunda. *Avances en Ciencias e Ingeniería*., 3(2), 67-78.

Duncan, T. (2011). Aplications of nanotechnology in food packaging an food safety: Barrier materials, antimicrobials and sensors. *Journal of colloid and Interface Science.*, Nov 1; 363(1): 1–24.

Frank, A. J., Cathcart, N., Maly, K. E. y Kitaev V. (2010) "Synthesis of silver nanoprisms with variable size and investigation of their optical properties: a first-year undergradua-te experiment exploring plasmonic nanoparticles". *Journal of Chemical Education,* 87(10), 1098–1101. doi:10.1021/ed100166g.

García, A., Kikut, K. (2020). Microscopía de fuerza atómica como herramienta en la investigación de asfaltos. *Revista Infraestructura Vial, LanammeUCR ISSN electrónico: 2215-3705*., Volumen 22, Número 40, p.p. 20 – 27.

Geethalakshmi, R. y Sarada, D. V. L. (2012). Gold and silver nanoparticles from Trianthema decandra: synthesis, characterization, and antimicrobial properties. International Journal of *Nanomedicine.*, 7, 5375-5384.

Han, R. M., Zhang, J.P., Skibsted, L. H. (2012). Reaction Dynamics of Flavonoids and Carotenoids as Antioxidants. *Molecules.*, 17: 2140-2160.

Lien, N. T. H., Hoa, V. X., Duong, V. T. T., Tinh, N. V., y Nhung, T. H. (2011). Synthesis and optical properties of colloidal gold nanoparticles for biomedical applications. *Communications in Physics.,* 21(1): 63–69.

Majles, M. H., Dehghani, Z., Sahraei, R., Nabiyouni, G. (2010). Non-linear optical properties of silver nanoparticles prepared by hydrogen reduction method M.H. *Optics Communications.*, 283. 1650–1653.

Mittal, A. K., Chisti, Y., Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances.*, 31, 346–356.

Monge, M. (2009). Nanopartículas de plata: métodos de síntesis en disolución y propiedades bactericidas. Real Sociedad Española de Química. *An. Quím*., 105(1), pp. 33−41.

Murray, W.A. & Barnes W.L. (2007). Plasmonic materials. *Adv. Mater*., 19, 3771-3782.

Pietta, P.G. (2000). Flavonoids as Antioxidants. *J. Nat. Prod., 63, 1035–1042.*

Bravo, A. M., Ale B. N., Rivera C.D., Huamán M. J., Delmás R. D., Rodríguez B.M., Polo S.M., Bautista C. M. (2009). Caracterización química de la *Stevia Rebaudiana*. *Rev. Per. Quím. Ing. Quím*., Vol. 12 N.º 2, Págs. 5-8

Shruti, S., Archana, M., Pradeep, M., Vivek K. B. (2012). Antioxidant ability and total phenolic content of aqueous leaf extract of Stevia rebaudiana Bert. *Experimental and Toxicologic Pathology*., 64 (2012) 807–811.

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