Universidad de Guayaquil

Facultad de Ingeniería Química

Ingeniería Química y Desarrollo

https://revistas.ug.edu.ec/index.php/iqd

Obtaining AGNPS (silver nanoparticles) via green synthesis with marcella (achyrocline satureioides) extract, catalysis with microbial and antioxidant activity

Obtención de AGNPS (nanopartículas de plata) vía síntesis verde con extracto de marcela (achyrocline satureioides), catálisis con actividad microbiana y antioxidante

Luis Felipe Zalamea Molina [1] * ; Jenniffer Andreina Chila Álvarez [2]; Irina Suley Menéndez Realpe [3]

Recibido: 02/07/2020 – Recibido en forma revisada: 29/08/2020 -- Aceptado: 26/11/2020
*Autor para la correspondencia.

Abstract
Green synthesis of NPs of silver, gold, copper, are a subject of investigative study that are currently carried out in India, Indonesia and Ecuador as is the case of this work from extract of Marcela (Achyrocline satureioides) and silver nitrate. The methodology used in obtaining NPsAg is via green synthesis that was performed with ionic reduction methods of the chemical components polyphenols and flavonoids such as: caffeic acid, galangin-3-methyl ether esters, quercetin; luteolin; 3-methoxy-quercetin and a new chalcone: achyrobichalcone. These methods of identification and characterization of the ionic organic components, UV-Vis spectrophotometry and SEM (electron scanning microscopy) were performed. The sizes of NPs that were found in their spherical shape, with a diameter of 10-15nm. The result obtained from the antimicrobial activity with the strains E. coli and S. auerus resulting in an inhibition halo of 3.00 to 3.098 and 3.12 to 2.05 respectively.

Keywords
Achyrocline satureioides, Silver nanoparticles, Reducing agent, Flavonoid.

Resumen
Síntesis verde de NPs de plata, oro, cobre, son motivo de estudio investigativo que se realizan en la actualidad en la India, Indonesia y Ecuador como es el caso del presente trabajo a partir de extracto de Marcela (*Achyrocline satureioides*) y nitrato de plata. La metodología utilizada en la obtención de NPsAg es vía síntesis verde que se realizó con métodos de reducción iónica de los componentes químicos polifenoles y flavonoides como: ácido cafeico, ésteres de galangin-3-metil éter, quercetina; luteolina; 3-metoxi-quercetina y un nuevo chalcone: achyrobichalcone. Estos métodos de identificación y caracterización de los componentes orgánicos iónicos, se realizaron espectrofotometría UV-Vis y SEM (microscopia de barrido electrónico). Los tamaños de NPs que se encontraron en su forma esférica, con un diámetro de 10-15nm. El resultado obtenido de la actividad antimicrobiana con las cepas *E. coli y S. auerus* dando como resultado un halo de inhibición de 3.00 a 3.098 y 3.12 a 2.05 respectivamente.

Palabras claves
Achyrocline satureioides, Nanopartículas de plata, Agente reductor, Flavonoide.

1. Introduction

The National Institute of Health in the United States defines nanotechnology as the creation of functional materials such as systems and devices at a scale of 1 to 100 nanometers (1x10^-9 meters) along with the use of new properties of chemical elements at the same scale. Richard Feynman, winner of the Nobel Prize in Physics in 1965, being the first to mention the scientific advancement of nanotechnology in his speech *There's Plenty of Room at the Bottom*, and data reveal advances in products designed with nanotechnology in the years 1999-2000 [1].

Its applications are broad; in energy, it improves renewable energy generation systems such as solar panels. In medicine, it increases the development of drug nano-carriers, nanobots for the minimization of malignant cells. In the environment, water treatments, non-polluting processes, desalination, etc. In constructions, the obtaining of reinforced, flexible materials. In agriculture, it is related to herbicides, pesticides, foliar sprays, among others. [1].

The application of nanotechnology in the field of medicine has aimed to prevent, diagnose, and treat diseases caused by bacteria that have developed resistance to conventional drugs. Metallic nanoparticles, in this case, silver ones, are

[1] Universidad de Guayaquil; https://orcid.org/0000-0002-1730-118X ; luis.zalameam@ug.edu.ec .
[2] Universidad de Guayaquil; jenniffer.chilaal@ug.edu.ec .
[3] Universidad de Guayaquil; irina.menendezr@ug.edu.ec .

of great medical importance due to their antibacterial properties which, compared to silver macroparticles, this has led to the research and development of new synthesis methods for silver nanoparticles (physical, chemical, and organic). [1].

Taking into account that synthesizing silver nanoparticles through physical and chemical methods generates a significant environmental and economic impact, based on the innovation of studies in organic methods, environmentally friendly, taking the name of green synthesis, generating a minimal amount of polluting waste compared to the aforementioned methods. [2].

New studies on the synthesis of silver nanoparticles from plant extracts of aloe vera, rosemary, garlic, pomegranate, and marcela, which possess antioxidant components capable of reducing silver and synthesizing nanoparticles [3]. Plant extracts such as that of marcela *(Achyrocline satureioides)* en varios estudios han demostrado que en su composición tienes propiedades antibacterianas, antiinflamatorias y anticancerígenas que han sido utilizadas en la medicina natural y ancestral [3].

1.1. Antioxidant activities of Marcela (*Achyrocline satureioides*)

PhD. Felicia Rivera Megret conducted a study titled "*Achyrocline satureioides (Lam.) DC. (marcela) reduces brain damage in permanent focal ischemia in rats"* here she mentions the analysis of marcela, noting that due to its significant antioxidant and anti-inflammatory activity attributed to the presence of flavonoids (quercetin), it is the subject of study in cerebrovascular accidents (strokes), which are the second most frequent cause of death worldwide. Ischemic stroke results from a transient or permanent reduction in blood flow affecting the territory of a cerebral artery and accounts for approximately 80% of all strokes. These molecular mechanisms occurring during the ischemic cascade have promoted the search for antioxidant and anti-inflammatory molecules that could interfere with oxidative stress, reducing neuronal damage. Due to its high antioxidant and anti-inflammatory actions, early evidence pointed to plants, fruits, beverages like wine and tea, and their main compounds, such as flavonoids, as significant candidates in the search for neuroprotective agents. The relentless search for pharmacological actions uses the intervention of medicinal plants, supported by studies on their value in preventing nervous system diseases. In particular, Achyrocline satureioides (Lam.) DC. (marcela) is a plant of popular use in this region, whose decoctions or infusions have traditionally been used for gastrointestinal disorders, as a sedative, and as an antispasmodic. [3] [4].

1.2 Antimicrobial activity of silver nanoparticles

The company NanoComposix researched the properties of silver nanoparticles, among which the antibacterial activity was reconfirmed. Through analysis using electron microscopy and UV-Visible light spectrophotometry, it was determined that the efficacy of silver nanoparticles is achieved when the particles are as small as 10nm in granularity [5].

The University of New León conducted an investigative study, demonstrating the antimicrobial activity both in HIV viruses (some strains) and in bacteria, which is attributed to silver nanoparticles. When these nanoparticles came into contact with Gram-Positive bacteria *(S. aureus)* and Gram-Negative bacteria *(E. coli)*, growth inhibition was observed on the plates containing the solution, indicating that silver nanoparticles at nanometric dimensions prevent bacterial growth [6].

1.3. Importance of the use of metallic nanoparticles

Nanoparticles offer advantages compared to the same material at macrometric scale; some are highlighted [2]:

- The surface helps carry drug molecules so that they can be structurally modified in cells and easily absorbed, due to their size [2].
- They are made of a crystalline, strong, flexible, and lightweight material that, at macrometric scales, is less abrasive to cells and the human body [2].
- Nanoparticles, especially silver ones, have created the need for their development due to their antibacterial activity and have been used in treatments against viruses and bacteria [2].

1.4. Reaction mechanism of silver nanoparticles

The reaction between silver cations and the reducing agent of higher concentration in the plant leaves is the flavonoid quercetin. [7].

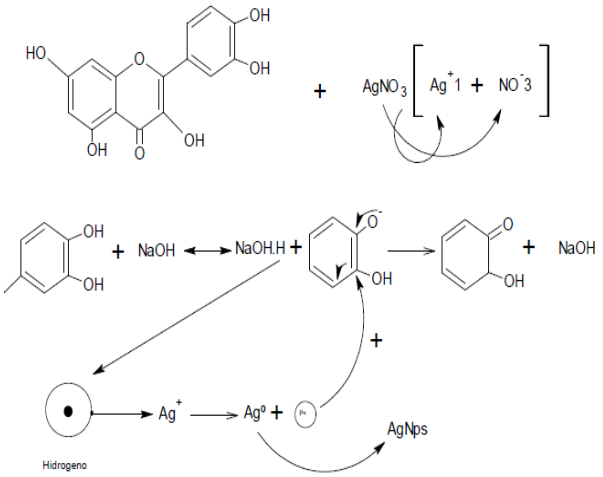

Fig. 1. Specific reaction mechanism of nanoparticle synthesis from the hydrogen of a hydroxyl group of the phenyl radical in quercetin
Sources: [8] [9]

Quercetin is a polyprotic acid polyphenol, with 5 hydroxyl groups with hydrogens acting as acids in a REDOX reaction. The spontaneous reaction occurs when acidic hydrogens are present. Quercetin has two hydrogens (2) that are strong acids, and when they dissociate, they form an enolate ion *(O−conjugate base)* that requires an accelerating catalyst (strong base). Previously, the oxidation of the hydroxyl group continues reducing the Ag ions. [8] [9] [10]

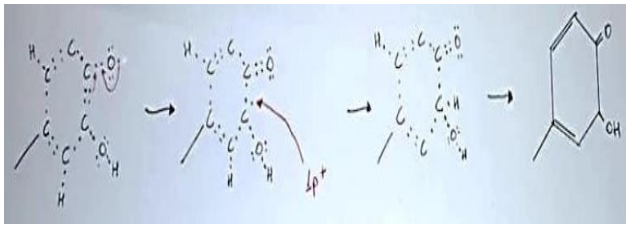

Fig. 2. Stability of the conjugate base of the hydroxyl group in the phenyl radical of quercetin
Sources: [8] [9] [10]

The weak conjugate acid stabilizes as a strong base, and the hydrogen that was taken is individualized in the solution and reacts with the silver. The hydrogen donates the electron to the silver to reduce it, making it stable with a 0 charge. The positive charge (P+) is free in the solution and bonds with the carbon electron to fulfill the octet rule. The hydrogen that bonds with the carbon stabilizes the ketonic tautomer formed in quercetin. [8] [9] [10]

The silver ion with a 0 charge, in contact with the stable silver atoms, modifies its single electron (valence), generating a layer of electrons that align constantly across all the silver atoms to fulfill the octet rule, forming a metallic bond that causes the aggregation of silver atoms, resulting in stabilized nanoparticles. [8] [9] [10]

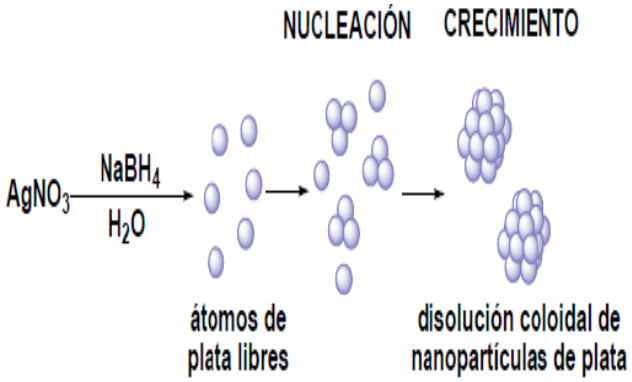

Fig. 3. Reaction mechanism for the formation of nanoparticles from silver ions.
Sources: [11]

Since there are two electrons that can be donated by quercetin according to Table 1 and its explanation, and because the oxidation state of ionized silver is +1, the chemical reaction uses one atom of quercetin and two monovalent silver cations.

1.5. Organic components of the reducing agent quercetin

Table 1.
Most important organic components identified in marcela leave

Acidity constants of the –OH groups in quercetin	
PKa	Location of the hydroxyl group
7.17	Hydroxyl group located on the third carbon of the main structure (1,4-benzopyran or 4-chromone)
8.26	ydroxyl group located on the fourth carbon of the phenyl radical
10.13	Hydroxyl group located on the third carbon of the phenyl radical

12.3	Hydroxyl group located on the fifth carbon of the main structure
13.11	Hydroxyl group located on the seventh carbon of the main structure

Sources: *[12]*

The acid constants of hydroxyl groups contained in the molecular composition of quercetin, the lowest are located:

- In the hydroxyl group bonded to the carbon 4 of the phenyl radical (PH).
- In the hydroxyl group bonded to the carbon 3 of the main structure of the molecule, the 4-chromone or 1,4-benzopyran.

The importance of these low acidity constants lies in the greater possibility for the structure of quercetin to donate an electron for each hydrogen it possesses with a low pKa, resulting in a total of two electrons donated by the hydroxyl groups of quercetin.

These electrons are used to reduce silver found in an aqueous medium in the form of silver ion Ag+1. Since there are two electrons that can be donated by the flavonoid and because the Ag+1 ion is monovalent, meaning it can only accept one electron, the chemical reaction uses one atom of quercetin and two monovalent silver cations.

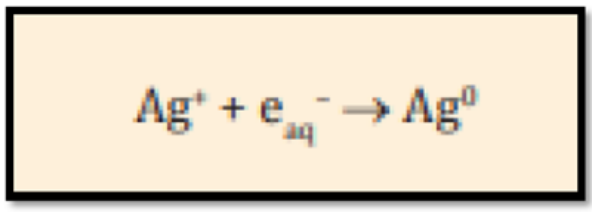

Fig. 4. Reduction of Ag+1 cation through a single electron in the médium.
Sources: *[12]*

1.6. Reduction of an electron

The reduction of silver ions due to the single electron donated by the reducing agent (quercetin) is called radiochemical reduction (electrons are donated by the most acidic hydrogens of the –OH groups in quercetin, see Figure 1) [12].

The electron moves to the orbital of the silver cation, generating the reduction to silver 0, which leaves it as a free element due to its oxidation state shifting to 0.

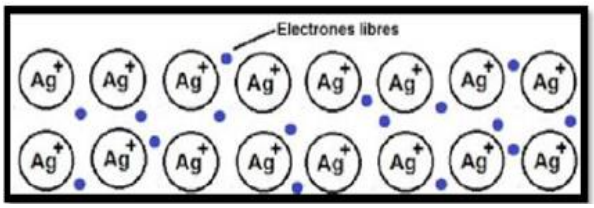

Fig. 5. Metallic bonding of silver nanoparticles in the electron cloud.
Sources: *[12]*

1.7. Nanoparticle Size

The size of nanoparticles is defined by the surface plasmon resonance energy, which is linked to the density of free electrons interacting with photons and results in the low electrical conductivity of nanoparticles.

These energies are not proportional to the diameter of nanoparticles; that is, if the diameter is smaller, the wavelength increases, measured in nm.

Metallic nanoparticles dispersed in a colloidal medium with a size less than 30nm, the SPR absorbs visible light in the blue-green spectrum (λ=450 nm) and produces reflected light of red color, giving the colloidal medium an intense red color (λ=700 nm).

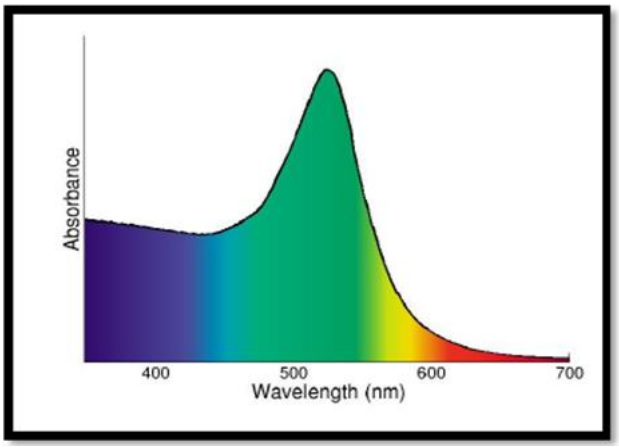

Fig. 6. Absorption spectrum of NPs with a diameter smaller than 30 nm, with primary absorption of green and blue photons, transmitting red color to the colloid
Sources: [13]

Large metallic nanoparticles shift the absorbed wavelength towards lower energy waves (red), meaning they are absorbed and project a faint blue to the mixture.

As nanoparticles increase in size nanometrically, the wavelength moves into the infrared zone of the visible spectrum; it is reflected but not visible, making the mixture translucent.

Table 2.
Wavelength, absorbed and reflected color of nanoparticles according to their size and shape

Wavelength (nm)	Absorbed Color	Reflected Color in Colloidal Solution
780-650	Red	Blue-green
650-595	Orange	Blue-green
595-560	Yellow-green	Purple
560-500	Green	Red-purple
500-490	Green-blue	Red
490-480	Blue-green	Orange
480-435	Blue	Yellow
435-380	Violet	Yellow-green

Sources: Autores

2. Materials and Methods

In this research project, sources of information such as doctoral theses, books, master's theses, and scientific papers were used, with a selection period from 2010 to 2018.

The following is a list of laboratory instruments and equipment used for the development of this project, with the central place of operation being the Microbiology Laboratory of the Faculty of Chemical Engineering, University of Guayaquil.

- Drying oven
- Digital scale
- Analytical balance
- Ball mill
- Mechanical mill
- Electric stove
- Pot
- Laboratory refrigerator
- UV light machine
- Test tubes
- Watch glass
- Pipettes

2.1. Microbial Activity Tests

For the development of the microbial inhibition assay, it was necessary to culture the strains of E. coli and S. aureus, which were provided by the Microbiology Laboratory of the Faculty of Chemical Engineering, University of Guayaquil. The E. coli and S. aureus strains were in a concentrated broth. 100 microliters of concentrated solutions were used for each inoculation. The solution was placed in a quadrant of the Petri dish and spread over the entire agar surface using a Drigalsky loop. Duplicates of each microbial culture were performed to obtain an average of the data. To obtain the inhibition halo, 0.5 cm diameter blank discs for antibiograms were used, on which 15 microliters of each synthesized AgNPs solution and standard solutions were placed to verify antimicrobial activity. The Petri dishes were divided into four quadrants as follows:

- Ag: 1mM silver nitrate
- M: 4% extract of Marcela leaf powder
- Np6: Silver nanoparticles synthesized on the sixth day of extract preparation.

Each synthesized AgNPs solution had a resting time of 6 days to ensure that stable AgNPs samples, rather than agglomerated particles that could reduce the inhibition effect, were used in the analysis, according to a report by Erna Susanti [14]. Each Petri dish was divided into four parts: in the first quadrant, 1mM AgNO3 was placed; in the second quadrant, 4% w/v extract was used; in the third quadrant, AgNPs formed on day zero of extract resting; and in the last quadrant, AgNPs synthesized on the sixth day of resting. After the respective resting time for the AgNPs synthesized on day 0 and day 6, the bacterial strains of E. coli and S. aureus were incubated at 38ºC for 24 hours after the proper inoculation was done. After the described resting time for the cultures, the dishes were removed from the incubator, and the inhibition halos produced by each of the substances were measured using a Vernier caliper..

2.3. Preparation of Silver Nitrate

Three different concentrations of silver nitrate were prepared:

- 0.05 M AgNO3: Dissolve 0.85 grams in 100 ml of alcoholic extract
- 0.1 M AgNO3: Dissolve 1.69 grams in 100 ml of alcoholic extract
- 0.15 M AgNO3: Dissolve 2.55 grams in 100 ml of alcoholic extract

Seal the container tightly and refrigerate to take samples at intervals over the course of days.

2.4. Characterization of Quercetin in Marcela Powder

The main flavonoid contained in Marcela powder is quercetin, which acts as a reducing agent [15]. The analyte will be quantitatively determined using HPLC chromatography, following the methodology used for the determination of quercetin in green tea leaf powder [16].

In the HPLC determination methodology, two phases are identified; methanol was used as the polar solvent (mobile phase) with a flow rate of 1 ml/min. When it comes into contact with the analyte (stationary phase), it will dissolve in the methanol, allowing the quercetin to be quantified at 370 nM [16].

Table 3.
Phytochemical Screening of Ethanolic Extract of A. satureioides Leaves

Resin Test	Negative
Catechin Test	Positive
Phenolic Compounds. Ferric Chloride Test	Green (tanino pirocatecólicos)
Reducing Compounds. Fehling's Test	Positive
Flavonoids. Anthocyanidin Test	Positive
Saponins. Foam Test	Negative
Alkaloids. Dragendorff's Test	++ , +++
Essential Oils Test	Positive

Sources: Authors

3. Resultados

The initial weight of the Marcela leaves was 922 grams and they were dried in the oven at a temperature of 38º-40º for 3 days, being checked at 4-hour intervals to determine the mass variation.

Percentage of moisture removed

$$\%H = \frac{m_h - m_s}{m_h} * 100 = \quad (1)$$

$$\%H = \frac{m_h - m_s}{m_h} * 100 = \quad (2)$$

Where:
m_h: wet mass of Marcela leaves
m_s: dry mass of Marcela leaves

The dried leaves were subjected to grinding using a ball mill with the help of 50 ceramic balls of the same diameter for 20 minutes. Due to the velvety or woolly material of the leaves, they were further subjected to mechanical grinding using a conventional mill.

3.1. DPPH. Antioxidants

The DPPH sample was prepared to identify the inhibition due to the antioxidant content in the extract and to make an estimate, using methanol as a blank.

To obtain these results, the samples were prepared in triplicate with each volume of extract, resulting in favorable outcomes regarding the antioxidant content in the plant.

Tabla 4.
Percentage of inhibition of ethanolic extract from A. satureioides leaves.

50 microlitros	25 microlitros	15 microlitros
84.86%	74.43%	62.45%

Sources: Authors

3.2. Characterization of Quercetin by HPLC

The HPLC analysis was conducted in the Analytical Chemistry laboratories of the Faculty of Chemistry and Exact Sciences at the Universidad Técnica Particular de Loja (UTPL).

For this analysis, 1 microliter of a 20% ethanolic extract of Marcela leaf powder was used to determine quercetin as the primary reducing agent and the flavonoids present in the leaves of this plant.

The quantitative result of its composition was 49.7±5 mg/g of quercetin, and the highest saturation point occurs at a retention time of 0.4 minutes. [17].

D. RESULTADOS DE ANÁLISIS DE MUESTRAS EN LABORATORIO			
MUESTRA 1			
Numero de Componente	Tiempo de Retención	Composición %	Componente
1	0,4 min	0.497±0.05	QUERCETINA

Fig. 7. Report on the HPLC Analysis of Quercetin Present in Marcela Leaf Powder
Sources: [17]

3.3. Characterization of Nanoparticles by UV-VIS Spectrum

For this procedure, the Genesys 10-UV spectrophotometer was used with AgNPs samples. Ethanol was used as the blank.

Table 5.
UV-Vis Spectroscopy Analysis

Days	λ	Absorbance		Concentration (ppm)
	295	0.476	0.475	226.7
0	330	2.19	2.18	250.6
	325	2.346	2.35	286.5
	365	2.956	2.95	0.989
6	375	2.92	2.874	0.963
	345	2.939	2.94	0.985
	315	2.989	2.99	0.999
9	370	2.990	2.934	0.995
	375	2.995	2.972	1.009
	355	2.994	2.953	0.998
12	365	2.938	2.931	0.995
	395	2.951	2.949	0.998

Sources: Authors

To obtain the result, samples were prepared with three different concentrations of AgNO3 (0.05-0.1-0.15 M), with 0.1M AgNO3 proving to be the most stable, resulting in a concentration of 0.99 ppm in the sample.

The data obtained indicate that the wavelengths reached with higher concentrations were 365 and 375 nm, averaging 370 nm, which, according to the table, indicates that the nanoparticles obtained have a diameter of 10-15 nm.

3.4. Characterization of the Shape and Size of Silver Nanoparticles

The analysis was conducted at the Scanning Electron Microscopy Laboratory of the National Institute of Public Health Research (INSPI) using the JEOL Scanning Electron Microscope (JSM 5310). The samples were gold-coated in the JEOL Metallizer (FJFC-1200) with an exposure time of 30 minutes, revealing spherical shapes, with sizes ranging between 10-15 nm, focusing on images that show the least aggregation [18]

The following illustrations show the characterization of the silver nanoparticles:

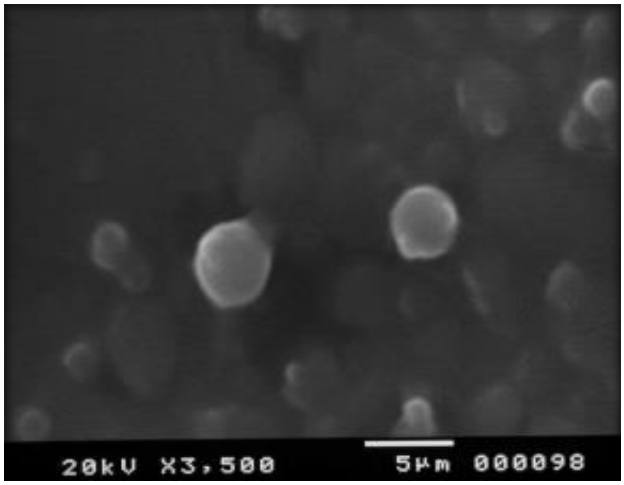

Fig. 8. Characterization of Silver Nanoparticles 1
Sources: Authors

1.5cm= 1.5e+7nm =
NPsAg =11.07nm (3)

1.5cm= 1.5e+7nm =
NPsAg =11.07nm (4)

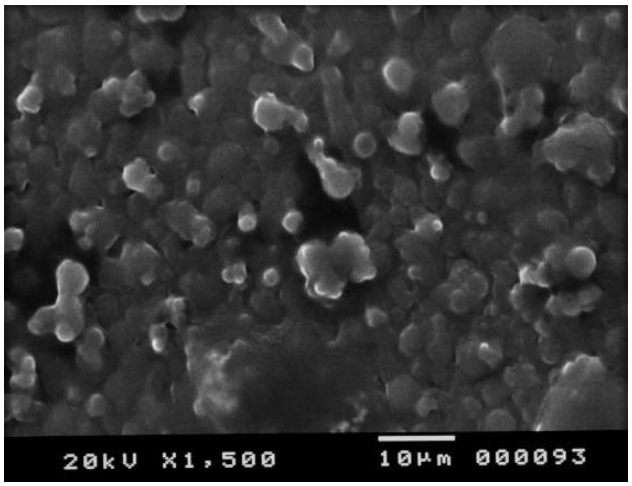

Fig. 9. Characterization of Silver Nanoparticles 2
Sources: Authors

2cm=2e+7nm=
NPsAg =12.44nm (5)

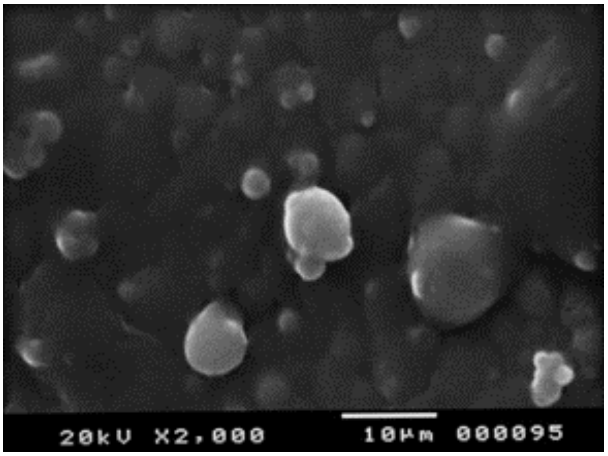

Fig. 10. Characterization of Silver Nanoparticles 3
Sources: Authors

2.5cm=2.5e+7nm=
NPsAg=13.79nm (6)

3.5. Bacterial inhibition

Tabla 6.
Inhibition Halo Values of AgNPs

Substances	E. coli (100µl) Values in cm		S. aureus (100µl) Values in cm	
AgNO3 1nM	1.055	1.10	1.02	1.025
Extract	1.15	1.20	1.48	1.44
NPsAg day 0	3.00	3.098	3.12	2.05
NPsAg day 6	2.58	2.78	2.18	2.02

Sources: Authors

The results from Table 6 confirm the microbial activity of AgNO3 and the Marcela leaf powder extract; however, the inhibition halo decreases over time, as can be observed from day 0 to day 6, but the activity is maintained.

4. Conclusions

The results obtained from the selection of *A. satureioides* include an initial sample of 968 grams of *A. satureioides* leaves, which resulted in a final sample of 692 grams after the drying process. The phytochemical analysis revealed the presence of several organic components: reducing compounds, flavonoid compounds, phenolic compounds, especially pyrocatechol tannins, catechins, alkaloids, and essential oils.

The characterization of AgNPs showed a concentration of 0.99 ppm in the sample with a particle diameter of 10-14 nm, predominantly in its most traditional form (spherical), with a favorable concentration of 0.1mM AgNO3. The concentration of quercetin present in the *A. satureioides* extract, determined by HPLC analysis, was 49.7±5 mg/g. The inhibition percentage in the *A.satureioides* leaf extract was determined using DPPH, yielding data of 84.46%, confirming the attribution of properties with anti-inflammatory, antibacterial, antioxidant, and antitumoral activity.

Referencias

[1] K. K. Mahendra Rai, NANOTECHNOLOGY IN DIAGNOSIS, TREATMENT AND PROPHYLAXIS OF INFECTIOUS DISEASES, Chennai, India: Academic Press, 2015.

[2] L. Cardeño Calle y M. E. Londoño, «Síntesis verde de nanoparticulas de plata mediante el uso de ajo (Allium sativum),» *Revista Soluciones de Postgrado EIA*, vol. 6, nº 12, pp. 129-140, 2014.

[3] F. Rivera Megret, D. Tejera, J. A. Abin Carriquiri, G. P. dos Santos, M. Martínez Busi y F. Dajas Méndez, «Achyrocline satureioides (Lam.) DC. (marcela) reduces brain damage in permanent focal ischemia in rats,» *Revista Cubana de Plantas Medicinales,* vol. 18, nº 3, pp. 445-460, 2013.

[4] M. C. Sabini, L. N. Cariddi, F. M. Escobar, F. M. L. Comini, D. Iglesias, M. Larrauri, S. Núñez Montoya, J. Sereno, M. S. Contigiani, J. J. Cantero y L. I. Sabini, «Potent inhibition of Western Equine Encephalitis virus by a fraction rich in flavonoids and phenolic acids obtained from Achyrocline satureioides,» *Revista Brasileira de Farmacognosia,* vol. 26, nº 5, pp. 571-578, 2016.

[5] S. Oldenburg, «"Silver Nanoparticles:Properties and Applications",» *Carboh Resea,* p. 4, 2008.

[6] N. V. Ayala Nuñez, «Nanopartículas de plata como microbicidas: actividad y mecanismo de acción contra la infección por el virus de inmunodeficiencia humana (VIH) y diferentes bacterias resistentes a antibióticos.,» UANL, Mexico, 2010.

[7] Gabriela Margarita Miño Castro, «Investigación fitoquímica e identificación de principios activos en seis especies del género Baccharis,",» 2007.

[8] Alberto Corzo Lucioni, «Síntesis de nanopartículas de oro obtenidas por reducción de H[AuCl4],» *Revista de la Sociedad Química del Perú,* vol. 78, nº 2, pp. 79-90, 2012.

[9] M. A. Salguero Salas, «Síntesis y caracterización de nanopartículas de plata usando como reductores extractos de menta (Origanum vulgare) y cilantro (Coriandrum sativum), y como funcionalizante el látex de sangre de drago (Croton lechleri),» Pontificia Universidad Católica del Ecuador, Quito, 2016.

[10] W. A. Robledo Prada, «FENÓMENOS TAUTOMÉRICOS EN SISTEMAS HETEROCÍCLICOS AROMÁTICOS.,» Colombia, 2015.

[11] María Concepción Fraile Romero, «Estudio de las interacciones entre nanoparticulas de metales nobles y ADN,» Sevilla, 2016.

[12] Rodolfo Zanella, «Metodologías para la síntesis de nanopartículas:,» Mexico, 2012.

[13] D. A. Cruz Perdomo y M. C. Rodríguez, «Nanopartículas metálicas y plasmones de superficie: Una relación profunda.,» *Avances en Ciencias e Ingeniería,* vol. 3, nº 2, pp. 67-78, 2012.

[14] K. A. Castro Batioja, «Elaboración de nanopartículas de plata vía síntesis y compuestos orgánicos de púnica granatum y catálisis bacteriana de escherichia coli, staphylococcus aureus y aspergillus niger.,» Guayaquil, 2018.

[15] D. Retta, E. Dellacassa, J. Villamil, S. A. Suárez y A. L. Bandoni, «Marcela, a promising medicinal and aromatic plant from Latin America: A review,» *Industrial Crops and Products,* vol. 38, pp. 27-38, 2012.

[16] E. Susanti, Ciptati, R. Ratnawati, Aulanni'am y A. Rudijanto, «Qualitative analysis of catechins from green tea GMB-4 clone using HPLC and LC-MS/MS,» *Asian Pacific Journal of Tropical Biomedicine,* vol. 5, nº 12, pp. 1046-1050, 2015.

[17] J. A. Muñoz Muñoz, J. E. Morgan Machado y M. Trujillo González, «Validación de una metodología por HPLC para cuantificar quercetina total en extractos de Calendula officinalis,» *Revista Cubana de Farmacia,* vol. 49, nº 1, pp. 91-102, 2015.

[18] INSPI, «Determinación de tamaño y forma mediante Microscopia Electronica de Barrido,» INSPI, Guayaquil, 2019.