



## SYNTHESIS AND SIZE ESTIMATION OF SILVER NANOPARTICLES, BY REDUCTION WITH AQUEOUS EXTRACTS OF CALYCES LEAVES AND SEEDS OF *HIBISCUS SABDARIFFA* LINN: PROMOTION OF GREEN SYNTHESIS

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Short Report

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**Palabras clave:** *Nanopartículas, Reducción, Hibiscus sabdariffa, Síntesis Verde, Polifenoles.*

### ABSTRACT

The synthesis of silver nanoparticles (AgNPs) has had a positive impact on biomedical sciences, thanks to its diverse biological potentialities, among which its antimicrobial activity and in vitro interaction with various chemotherapeutic drugs stand out. The study aimed at the synthesis of AgNPs, using aqueous extracts of calyces, leaves and seeds of an organic cultivation of *Hibiscus sabdariffa* and a solution of silver nitrate ( $\text{AgNO}_3$ ). The concentration of total phenolic compounds and flavonoids was determined for each extract, by the methods of Folin-Ciocalteu and Marinova respectively, this with the purpose of guaranteeing the quality and content of compounds with reducing capacity. AgNPs synthesis conditions were optimized, referring to  $\text{AgNO}_3$  volume and concentration, extract volume (calyces, leaves and seeds), pH, heating time and temperature, using the statistical program StatGraphics. The analyzes yielded a concentration of  $17.42 \pm 0.12$  mg GAE / g (calyces),  $9.03 \pm 0.91$  mg GAE / g (leaves) and  $11.32 \pm 0.36$  mg GAE / g (seeds). The maximum absorption peaks were obtained at 424 nm for all the three extracts, with absorbances of  $1.5240 \pm 0.32 = 30-32$  nm (AgNPs-calyces),  $0.5674 \pm 0.24 = 12-14$  nm (AgNPs-leaves) and  $0.764 \pm 0.18 = 18-20$  nm (AgNPs-seeds). The findings of the study are related to the variation of phenolic compounds in the different parts of *H. sabdariffa*, which conditions the synthesis performance of AgNPs. The present study represents a valuable contribution in the "Green Synthesis", representing the first study in Venezuela in which AgNPs are synthesized from *H. sabdariffa*.



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## RESUMEN

La síntesis de nanopartículas de plata (AgNPs) ha tenido un impacto positivo en las ciencias biomédicas, gracias a sus diversas potencialidades biológicas, entre las que destacan su actividad antimicrobiana e interacción in vitro con diversas drogas quimioterapéuticas. El estudio tuvo como objetivo la síntesis de AgNPs, empleando extractos acuosos de cálices, hojas y semillas de un cultivo orgánico de *Hibiscus sabdariffa* y una solución de nitrato de plata ( $\text{AgNO}_3$ ). A cada extracto se le determinó la concentración de compuestos fenólicos totales y flavonoides, por los métodos de Folin-Ciocalteu y Marinova respectivamente, esto con el propósito de garantizar la calidad y contenido de compuestos con capacidad reductora. Se optimizaron las condiciones de síntesis de AgNPs, referente a volumen y concentración de  $\text{AgNO}_3$ , volumen de extracto (cálices, hojas y semillas), pH, tiempo y temperatura de calentamiento, empleando el programa estadístico StatGraphics. Los análisis arrojaron una concentración de  $17.42 \pm 0.12$  mg GAE/g (cálices),  $9.03 \pm 0.91$  mg GAE/g (hojas) y  $11.32 \pm 0.36$  mg GAE/g (semillas). Los picos máximos de absorción se obtuvieron a 424 nm para todos los tres extractos, con absorbancias de  $1.5240 \pm 0.32 = 30-32$  nm (AgNPs- cálices),  $0.5674 \pm 0.24 = 12-14$  nm (AgNPs-hojas) y  $0.7641 \pm 0.18 = 18-20$  nm (AgNPs-semillas). Los hallazgos del estudio están relacionados con la variación de compuestos fenólicos en las diferentes partes de *H. sabdariffa*, lo que condiciona el rendimiento de síntesis de AgNPs. El presente estudio representa un valioso aporte en la "Síntesis Verde", representando el primer estudio en Venezuela en la que se sintetizan AgNPs a partir de *H. sabdariffa*.

## INTRODUCTION

Silver nanoparticles (AgNPs) have been one of the most attractive nanomaterials in biomedicine due to their unique physicochemical properties [1]. Other biological activities of AgNPs have been also explored, including promoting bone healing and wound repair, enhancing the immunogenicity of vaccines, and anti-diabetic effects [2- 4]. Globally, research focus on the synthesis of AgNPs with controlled size and shape, and a variety of specific synthetic methods have been developed, including, chemical, physical and biological methods [5,6] (Figure 1).

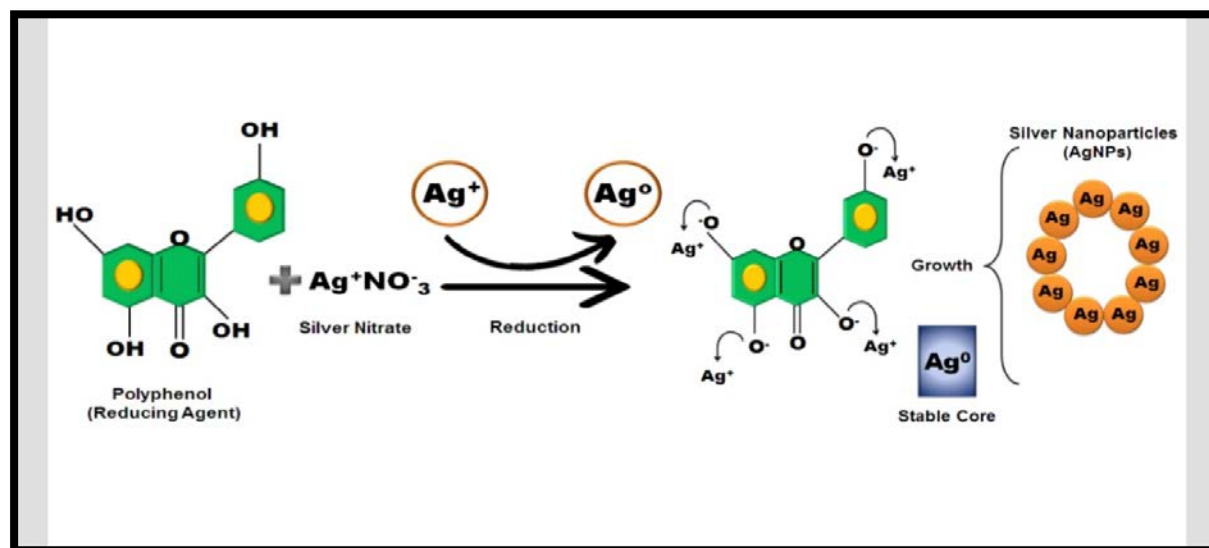


Figure 1. Silver Nanoparticle System Mechanism. Source: Own elaboration

The synthesis of AgNPs due to biological processes aims to change the reagents used in chemical synthesis with another type of substances that can perform the same role. The "green synthesis" of silver nanoparticles, in which plant extracts are used, has gained great interest in recent years. This type of synthesis is efficient both in terms of reaction time, as well as stability of nanoparticles that exclude toxic chemical agents [7]. In this sense *Hibiscus sabdariffa*, an easy-access and high-content plant in phenolic compounds, is an alternative as a bilingual material for



AgNPs synthesis [8,9]. Considering then the importance of AgNPs and their impact on biomedical sciences, the study aimed to synthesize and estimate the size of AgNPs, using aqueous extracts of calyces, leaves and seeds of *H. sabdariffa* obtained from an organic culture, thus promoting the "Green Synthesis".

## EXPERIMENTAL

### *Origin of plant material (PM)*

The calyces, leaves and seeds were obtained from an organic culture. The harvest was carried out on the researcher's own lands located in the Coropo sector, Aragua state, Venezuela (October 2019-March 2020).

### *Sample preparation for extraction*

For extracts 2,5 grams of plant material were weighed. This was poured into a 400 mL Beaker, to which 200 mL of distilled water previously heated to the boiling point was added. The sample was slightly stirred for 4 min and filtered using Whatman No. 4 paper [10].

### *Determination of total phenolics*

For the determination of total phenolics, 50  $\mu$ L were mixed with 250  $\mu$ L of the Folin-Ciocalteu 1 N reagent (Analytical grade, Merck). It was left to stand for 8 minutes and then 750  $\mu$ L of 20%  $\text{Na}_2\text{CO}_3$  and 950  $\mu$ L of distilled water were added. Was incubated for 30 min at room temperature and the absorbance was read on a Genesis 20 UV/VIS spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA). A calibration curve for Gallic Acid (Sigma-Aldrich, Germany) was prepared with concentrations of 50, 100, 200, 300, 400, 500 and 1000 ppm. The results were expressed in mg of Gallic Acid Equivalents (GAE) /g of PM [11].



Figure 2. Leaves and calyces.

### *Determination of flavonoids*

A volume of 100  $\mu$ L of sample was mixed with 30  $\mu$ L of 5% w/v  $\text{NaNO}_2$ , 30  $\mu$ L of 10% w/v  $\text{AlCl}_3$ , 200  $\mu$ L of 1 M NaOH and adjusted with distilled water to a final volume of 1 mL. The reading was performed at 510 nm in a Genesis 20 UV / VIS spectrophotometer and was compared with a standard curve with standard (+)-catechin (Sigma Aldrich, USA). The results were expressed in mg of Catechin Equivalents (CE) / g of PM [12].

### *Synthesis of AgNPs-UV-VIS spectrophotometry*

Nanoparticle formation was analyzed by UV-VIS Spectrophotometry (Thermo Scientific, Waltham, Massachusetts, USA). A spectral scan of each synthesis was carried out, obtained with the extracts of the various parts of the plant, in a range of wavelengths from 420 to 430 nm, estimating the size of the nanoparticles from (2 to 40 nm). For the analysis of the nanoparticles, 2 ml of each synthesis were taken and diluted with distilled water to a final volume of



4 mL. 1 mL of the previous dilution was placed in a cell with 1 cm of optical path and the spectral scan was carried out [13,14].

### Optimisation of the synthesis of silver nanoparticles and Statistical analysis

The parameters to be optimised were: concentration of silver nitrate, volume of extract, pH, heating time and temperature. The StatGraphics programme was used with a screening design class. All determinations (phenolic compounds, flavonoids) and the spectral sweep corresponding to each synthesis were performed in quintuplicate and values were expressed as mean  $\pm$  standard deviation. Statistical differences were determined by analysis of variance (ANOVA) using Statistic 9.0 for Windows.

## RESULTS AND DISCUSSION

### Total phenolics and flavonoids of the extracts

Prior to the synthesis and estimation of the size of the AgNPs, the concentration of total phenolics was determined in quintuplicate, obtaining a concentration of  $17.42 \pm 0.12$  mg GAE / g PM (calyces),  $9.03 \pm 0.91$  mg GAE/g PM (leaves) and  $11.32 \pm 0.36$  mg GAE/g PM (seeds), observing a statistical difference ( $p=0.035$ ). For flavonoids it was of  $12.17 \pm 0.22$  mg CE/g PM (calyces),  $6.45 \pm 0.67$  mg CE/g PM (leaves) and  $9.32 \pm 0.28$  mg CE/g PM (seeds) ( $p=0.045$ ). The difference observed is similar to that reported in several studies carried out with *Hibiscus sabdariffa*, which indicate that the concentration of phenolic compounds and flavonoids it varies in the different parts of the plant, also conditioned by various factors (plant genetics, climate, soil conditions, cultivation) [15-17].

### Optimal conditions for the synthesis of AgNPs

As indicated above, the material used corresponds to an organic culture of *H. sabdariffa*. In this sense, when using different parts of the plant, the appropriate conditions for the synthesis of nanoparticles were optimized [18-20] (Table 1).

Table 1. Optimal conditions for the synthesis of AgNPs

Optimization	Calyces	Leaves	Seeds
*AgNO <sub>3</sub> Solution (mL)	2.7	3.5	2.8
Time (min)	4.3	5.5	4.7
Temperature (°C)	91	82	84
Volume Extract (mL)	4.7	6.8	8.0
+pH	7.3	6.6	7.1

\*Silver nitrate concentration: 1.2 mM (Calices), 0.98 mM (leaves), 1.25 mM (Seeds). +pH adjusted with sodium hydroxide.

UV-VIS spectroscopy is a very powerful tool to monitor the synthesis of the AgNPs as the metallic nanoparticles possess a property known as surface plasmon resonance which is primarily because of the oscillation of the free electrons present on the surface of the metallic nanoparticles when they are excited by any external energy source[1]. The three solutions yielded maximum absorption peaks at 424 nm, observing that the synthesis of AgNPs, using the calyces, originated the highest absorbance value (Figure 3). All this shows that the characteristic peak for the formation of silver nanoparticles is around 422-426 nm, where the size of silver nanoparticles is estimated to be between 2 and 40 nm. [18,19].

On the other hand, the morphology of the synthesized nanoparticles plays a fundamental role in its biological activity[21-24]. Although the morphology of the AgNPs was not characterized, what was found in the study represents an important contribution to the green synthesis, leaving pending further studies, not only the characterization of the AgNPs, but also their biological potential.

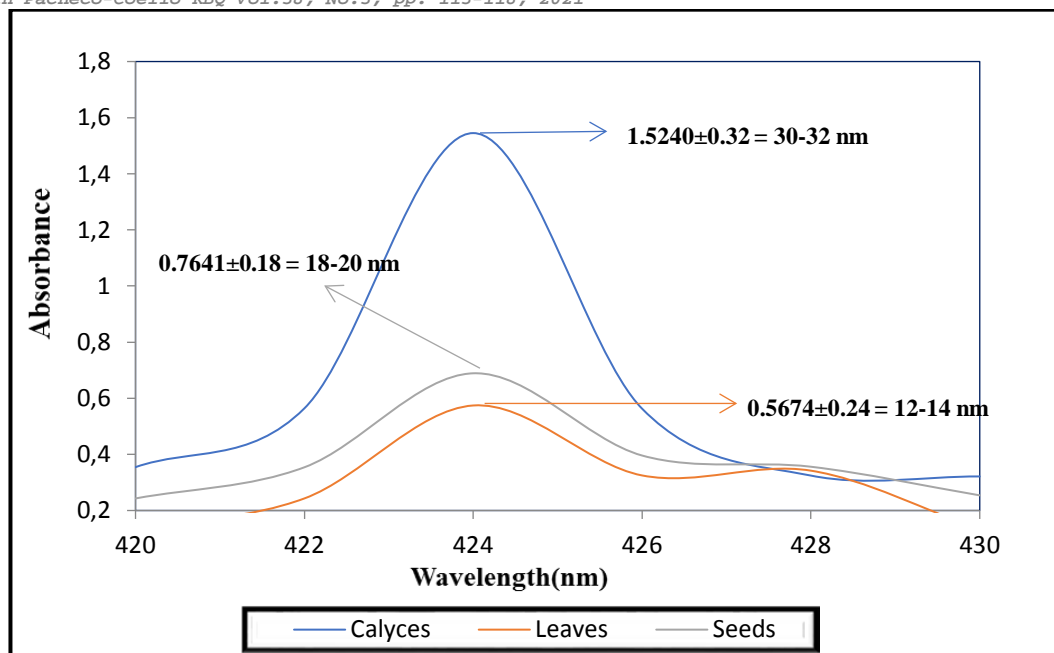


Figure 3. Absorption spectrum of AgNPs.

## CONCLUSION

In conclusion, it must be said that the various parts of *H. sabdariffa* constitute an excellent alternative for the synthesis of silver nanoparticles, allowing to appreciate that its performance is conditioned to the presence of compounds with reducing capacity. Finally, this study provides interesting data for those researchers whose synthesis of nanoparticles is based on the "Green Synthesis".

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