GREEN CHEMISTRY: USING THE AQUEOUS EXTRACT OF THE LEAVES OF A MEXICAN OAK (*QUERCUS RUGOSA*; FAGACEAE) FOR SYNTHESIS OF SILVER NANOPARTICLES

Oropeza D.R., Reyes Ramírez L.A., García M.C., Morales G.C., Cervantes Tavera A.M., Hernández Santiago A.A., Arzola Flores J.A.

Meritorious Autonomous University of Puebla

av. San Claudio, S/N Col. San Manuel, Puebla, Pue. C.P. 71590, Mexico; e-mail: ximikad09@mail.ru

Received: 10.07.2019

Abstract. Currently, the silver nanoparticles (AgNPs) are highly recurrent in medicine due to its magnetic and optical properties, becoming important antimicrobial, antiviral and anticarcinogenic agents; nevertheless, the synthesis of said nanoparticles is based on chemical methods, applying reducing and stabilizing agents that are highly polluting to environment and harmful to health. However, plant extracts can be used as a "green" method alternative to chemical conventional synthesis. In the present work, the aqueous extract of the leaves of an Oak native to Mexico (*Quercus rugosa;* Fagaceae) was used as a reducing and stabilizing agent in AgNPs synthesis using silver nitrate as a precursor. Different tests were performed by varying the volume of the aqueous extract and the concentration of silver nitrate. Through UV-vis spectroscopy, the presence of biomolecules, such as polyphenols and flavonoids, was identified. The AgNPs were characterized by the identification of their plasmonic response.

Key words*: mexican oak; quercus rugosa; fagaceae; silver nanoparticles.*

INTRODUCTION

Nanoparticles of noble metals, mainly gold (AuNPs) and silver (AgNPs) exhibit interesting optical properties that can be utilized on medical applications in radiotherapy systems and thermal ablation; and given its nature of biocompatible materials, do not pose significant risks to human health and the environment [1]. AgNPs application in clinical treatments involves no side effects to health, since they have reported remains of silver in despicable concentrations in different organs and systems of rats [2]; nevertheless, the synthesis of said nanoparticles is based on the implementation of reducing agents and chemical stabilizes which may lead to the emergence of some toxic chemical species absorbed and consequently, generate counterproductive effects in medical applications [1]; inducing side effects on health and negatively impacting the environment. However, synthesis AgNPs through microorganisms or plant extracts could mitigate the effects of these problems, making the nanoparticles potentially more biocompatible and respectable with the environment [1, 3, 4].

It has been shown that the secondary metabolites of plants, such as flavonoids, polyphenols and terpenes, that could be closely related to the immune system of plants by having bactericidal and bacteriostatic properties [5-9], act as reducing and stabilizing agents in the synthesis of AgNPs, being a powerful "green" resource alternative to the conventional chemical synthesis of metallic nanoparticles [10].

In recent years, concern for the environment and human health has increased considerably, so in many investigations the extracts have been applied mainly leaves of different plants in the biosynthesis of metal nanoparticles for medical applications, particularly for their antimicrobial qualities: Shankar and collaborators in 2004 used the leaves of the neem of India in the synthesis of gold and silver nanoparticles [1], as well as Shakeel and collaborators in 2016 [11]. Mostafa and collaborators in 2014 used olive leaves in the synthesis of AgNPs to test their antibacterial effect; while Logeswari and collaborators in 2015 applied in the biosynthesis of AgNPs, the extracts of the leaves of different plants of medical-food interest (purple basil, eggplant, asiatic centella and orange tree). Bagherzade and collaborators in 2017 used the extract of saffron in their "green synthesis" experiments of AgNPs [12]. Researches on nanoparticle biosynthesis focuses principally on commercial plant species and in Mexico, these studies are precarious despite the great diversity of plants we possess; having a broad field of research poorly exploited in our native species with potential applications in bio and nanotechnology. The white oak (*Quercus rugosa*: Fagaceae Née, 1801) is a tree species endemic to Mexico that dwell in wooded regions with temperate climates [13], this tree possess bactericidal properties and its leaves have been used in traditional Mexican medicine to fight infectious diseases and wounds [14]. For all the above, the objective of the present study was to use the aqueous extract of white oak leaves as a reducing and stabilizing agent for the biosynthesis of AgNPs from silver nitrate as precursor.

METHODOLOGY

1. Aqueous extract preparation. The dry leaves of white oak were collected of a mature specimen in the state of Puebla, Mexico. The leaves were ground to a fine powder and 2 grams of the vegetable powder were weighed and mixed with 10 mL of deionized water at 80-95 °C in a test tube and vortex was immediately applied for 5 minutes; subsequently it was centrifuged at 2500 rpm at 37 °C for 10 minutes. Again, 15 mL of deionized water at 80-95 °C was added to the tube with the mixture and vortexed for 5 minutes. Finally, it was centrifuged at 2500 rpm at 37°C for 15 min. The supernatant liquid was separated of the mixture to be applied immediately in the biosynthesis of the AgNPs.

2. Aqueous extract characterization. The characterization of the aqueous extract for the determination of the biocompounds with reducing and stabilizing effect was carried out by UV-vis spectroscopy [15].

3. Silver nanoparticles biosynthesis.

3.1 Tests of variation of the aqueous extract concentration. We based on the chemical synthesis method of Creighton [16], solutions of 20 mL of 0.3 M of $AgNO_3$ concentration were prepared to mix them with different volume of plant extract (1, 2, 3, 4 and 5ml) and were shacked at room temperature for 10 min. The formation of silver nanoparticles was determined by the change in the coloration of the solutions and the UV-vis spectrum. The solutions were monitored for 5 days to study their stability.

3.2 Tests of variation of silver nitrate molarity. In this case, the molarity was modified and solutions of 20 mL of 1, 2, 3, 4 and 5 M concentration of $AgNO_3$ were prepared to mix each with 3 ml of the aqueous extract of the white oak. The mixtures were shacked at room temperature for 10 min. The formation of AgNPs was determined by the change in coloration of the solutions and the UV-vis spectrum. The solutions were monitored for 5 days.

4. AgNPs characterization. The characterization of the nanoparticles obtained was carried out by UV-vis spectroscopy [21] for the identification of surface plasmon resonance reported from 400 to 470 nm [17, 18] for 5 days

RESULTS AND DISCUSSION

1. Determination of the vegetal biocompounds in the aqueous extract with reducing and stabilizing activity. To be able to use the aqueous extract of Q. rugosa, it was necessary to determine the presence mainly phenolic compounds, biocomponents with reducing and stabilizing effect in the biosynthesis of AgNPs. Aleixandre-Tudo and Du Toit in their research on the release of phenolic compounds from the solid parts of the grape berries during the wine making process published [19] report the absorbance peaks for different phenolic compounds (malvidin-3-glucoside, malvidin-3-pcoumarylglucoside, catechin, gallic acid, caftaric acid, coutaric acid, rutin, quercetin) that absorb in the range from 200 to 400 nm approximately (except for malvidin-3-glucoside and malvidin-3-pcoumarylglucoside, which range up to 600 nm), with slight variations depending on the identity of the phenolic compound. In this context, the resulting spectrogram for the aqueous extract white oak presents an absorbance peak in approximately 310 nm (Fig. 1), which suggests that some phenolic compounds that possess the fruits of the grape, are also present in the leaves of the tree used in this study. However, it is necessary to carry out the characterization by FT-NIR to confirm the presence of said bio components.

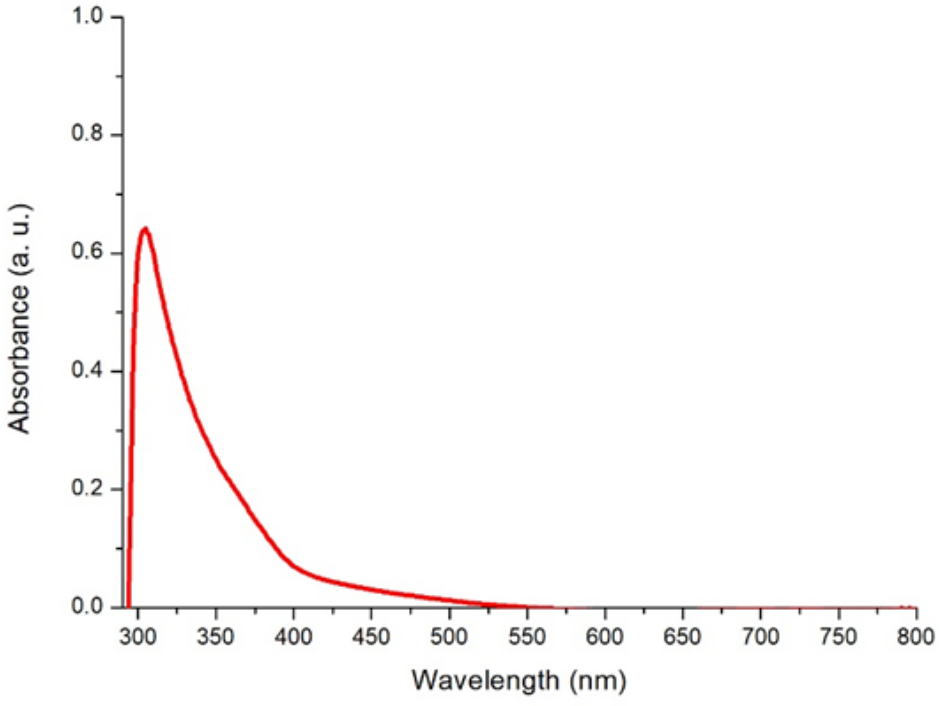

Figure 1. UV-vis spectrogram of the aqueous extract of *Q. rugosa*. A peak of absorbance is observed in approximately 310 nm, at which point they absorb some phenolic compounds, plant biocomponents that have reducing and stabilizing properties in the biosynthesis of AgNPs

2. Determination of surface plasmon resonance of AgNPs.

2.1. Tests of variation of the aqueous extract concentration. There are multiple investigations on the synthesis of AgNPs in which plant extracts are applied as reducing and stabilizing agents; however, the way in which the variation in the concentration of those extracts influences, as well as the concentration of the precursor in the growth of the AgNPs over time, is poorly studied and probably also depends on the plant species used. When the concentration of the precursor was set to 3 M, favorable results were observed in growth and surface plasmon morphology from the third day on all concentrations applied, especially in volume greater than 2mL of the extract (Fig. 2), but when the concentrations of the extract are little, in this case 1 and 2 mL, the growth stops in the fourth day, as the availability of the bio compounds with the reducing and stabilizing effect is probably finished (Fig. 2 A and B) At higher volume of the aqueous extract (3, 4 and 5 mL), the definition of the surface plasmons resonance improves over the time and greater growth is observed (Fig. 2 C, D and E).

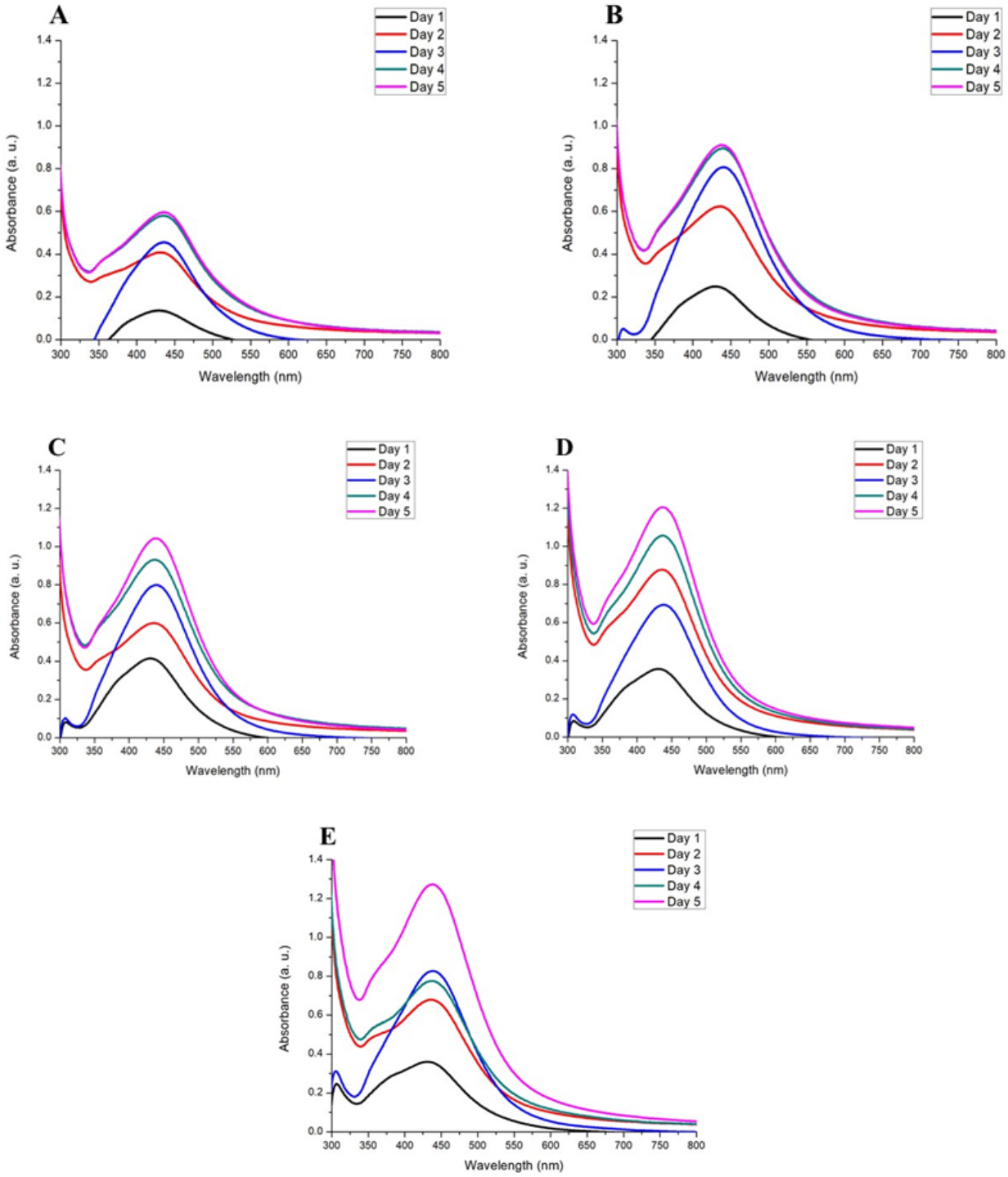

Figure 2. UV-vis spectrograms of 1, 2, 3, 4 and 5 mL of the aqueous extract of *Q. rugosa* with 3 M of $AgNO_3$ evaluated for 5 days (A, B, C, D and E respectively). The surface plasmon morphology is adjusted from the third day with marked in growth depending on the volume of the aqueous extract

2.2. Tests of variation of the silver nitrate molarity. When the concentration of the $AgNO_3$ is varied and the volume of the aqueous extract is set at 3 mL, a greater growth and better morphology of the surface plasmon resonance is observed on the third day, especially in concentrations greater than 1 M; however, the growth decreases in the fourth day and increases again to the fifth day. This phenomenon is probably due to the nanoparticles are broken and formed again due to their instability (Figure 3 A, B, D and E). In the case of the 3 M concentration of $AgNO_3$, the growth increases gradually without showing backs, which suggests that the nanoparticles are more stable and grew steadily during the 5 days; this indicates that 3 M is the ideal concentration of $AgNO_3$ for the adequate synthesis of nanoparticles (Fig. 3 C).

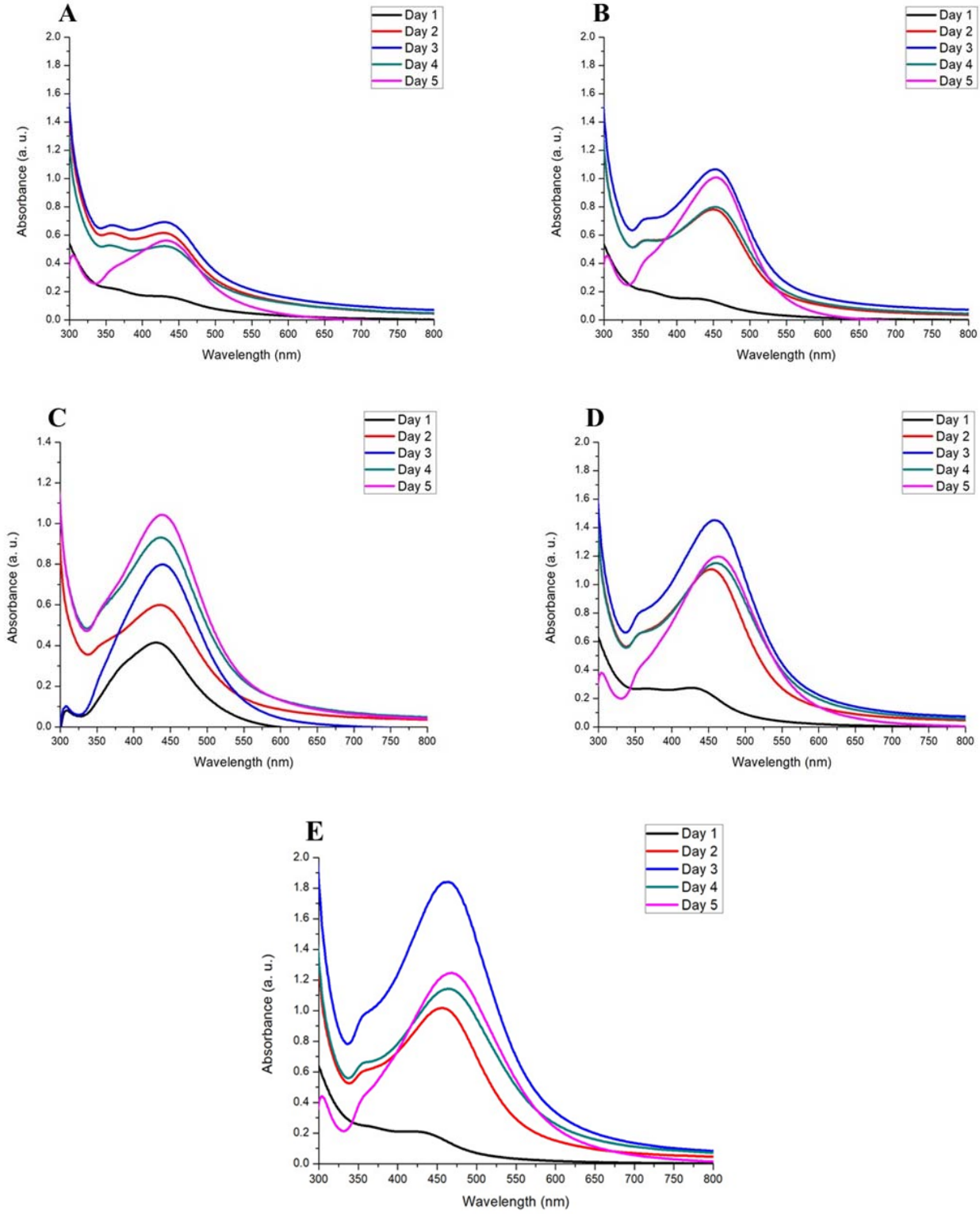

Figure 3. UV-vis spectrograms of 1, 2, 3, 4 and 5 M of the $AgNO_3$ with 3 mL of the white oak aqueous extract evaluated for 5 days (A, B, C, D and E respectively). The highest growth is observed on the third day, with a decrease in all concentrations in the fourth day (A, B, D and E) except for the 3 M concentration that presents a gradual growth (C)

CONCLUSIONS

Due to the aqueous extract of white oak leaves contains phenolic compounds, is an excellent agent with reducing and stabilizing effect, being a viable alternative friendly to the environment and human health that can be applied in the "green synthesis" of AgNPs.

The formation and particularly the growth of the nanoparticles over time is determined by the volume of the aqueous extract and the concentration of the precursor; being lower in low volume; in addition, the better defined surface plasmons resonance were obtained from the second day in high concentrations of both elements in the biosynthesis of AgNPs; however, the concentration of 3 M of the $AgNO_3$ is the most adequate, and the high concentrations of the aqueous extract can vary in the 3 M colloidal solution to obtain the successful biosynthesis of AgNPs. However, it is necessary to carry out the characterization by transmission electron microscopy to study the average size of the AgNPs.

It is highly recommendable to continue with researches on the potential applications of endemic plant species in biotechnology so that the manufacturing processes are more respectful with the environment and human health.

References:

1. Shankar S.S., Rai A., Ahmad A., Sastry M. *Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using Neem (Azadirachta indica) leaf broth*. India: Materials Chemistry Division, National Chemical Laboratory, 2004.

2. Kim Y.S., Kim J.S., Cho H.S., Rha D.S., Kim J.M., Park J.D., Choi B.S., Lim R., Chang H.K., Chung Y.H., Kwon I.H., Jeong J., Han B.S., Yu I.J. Twenty-Eight-Day Oral Toxicity, Genotoxicity, and Gender-Related Tissue Distribution of Silver Nanoparticles in Sprague-Dawley Rats. Incheon Institute, Korea. *Journal de Toxicología por Inhalación*, 2008, pp. 575-583.

3. Vilchis-Nestor A.R. et al. *Solventless synthesis and optical properties of Au and Ag nanoparticles using Camellia sinensis extract*. Faculty of Chemistry, Autonomous University of the State of Mexico, Toluca, Mexico, 2008.

4. Mittal A.K. et al. Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances Magazine*, 2013, pp. 346-356.

5. Mensah J.K., Okoli R.I., Ohaju-Obodo J.O., Eifediyi K. Phytochemical, nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *African Journal of Biotechnology,* 2008, vol. 7, no. 14, pp. 2304-2309

6. García A.Á., Pérez-Urria Carril E. Metabolismo secundario de plantas. *Reduca (Biología). Serie Fisiología Vegetal*., 2009, vol. 2, no. 3, pp. 119-145. [García A.Á., Pérez-Urria Carril E. Secondary plant metabolism. *Reduca (Biology). Plant Physiology Series*, 2009, vol. 2, no. 3, pp. 119-145. (In Span.)]

7. Pérez-Alonso N., Jiménez E. Producción de metabolitos secundarios de plantas mediante el cultivo *in vitro*. *Biotecnología Vegetal*, 2011, vol. 11, no. 4, pp. 195-211. [Pérez-Alonso N., Jiménez E. Production of secondary metabolites of plants through in vitro culture. *Plant Biotechnology*, 2011, vol. 11, no. 4, pp. 195-211. (In Span.)]

8. Moudi M. [et al.] Vinca alkaloids. *Int J Prev Med*, 2013, vol. 4, no. 11, pp. 1231-1235.

9. Cano T. Caracterización de una espirolactona sesquiterpénica α-metilénica obtenida de Ambrosia arborescens Milerl y evaluación de su actividad biológica en Tripanosoma cruzi. *Revista Soc-Quim Perú*, 2014, pp. 124-135. [Cano T. Characterization of an α-methylenic sesquiterpenic spirolactone obtained from Ambrosia arborescens Milerl and evaluation of its biological activity in Trypanosoma cruzi. *Soc-Quim Peru Magazine*, 2014, pp. 124-135. (In Span.)]

10. Neira I. *Green synthesis of nanoparticles to remove dyes in Aqueous Media*. University of Coruña Science Faculty. Department of Physical Chemistry and Chemical Engineering. Spain, 2015.

11. Ahmed S. [et al.] Green synthesis of silver nanoparticles using Azadirachta indica aqueous leaf extract. *Journal of Research in Radiation and Applied Sciences*, 2015, pp. 1-7.

12. Bagherzade G. [et al.] Green synthesis of silver nanoparticles using aqueous extract of saffron (Crocus sativus L.) wastages and its antibacterial activity against six bacteria. *Asian Pac. J. Trop. Biomed*., 2017, vol. 7, no. 3, pp. 227-233.

13. Batis A. [et al.] *Árboles y Arbustos Nativos Potencialmente Valiosas para la Restauración Ecológica y la Reforestación*. Instituto de Ecología, UNAM – Conabio, 1999. [Batis A. [et al.] *Potentially Valuable Native Trees and Shrubs for Ecological Restoration and Reforestation*. Institute of Ecology, UNAM - Conabio, 1999 (In Span.)]

14. Biblioteca de la Medicina Tradicional Mexicana. *Atlas de las Plantas de la Medicina Tradicional Mexicana.* Universidad Nacional Autónoma de México. 2009. Recuperado el 25 de marzo del 2019 de: http://www.unamenlinea.unam.mx/recurso/biblioteca-digital-de-la-medicina-tradicional-mexicana_80999. [Library of Traditional Mexican Medicine. *Atlas of the Plants of Traditional Mexican Medicine*. National Autonomous University of Mexico, 2009. Retrieved on March 25, 2019 from: http://www.unamenlinea.unam.mx/recurso/biblioteca-digital-de-la-medicina-tradicional-mexicana_80999 (In Span.)]

15. Olsen E.D. *Metodos ópticos de análisis*. Editorial Reverté, Barcelona, España, 1990, 681 p. [Olsen E.D. *Optical methods of analysis*. Editorial Reverté, Barcelona, Spain, 1990, 681 p. (In Span.)]

16. Mongue M. Nanopartículas de plata: metodos de síntesis en disolución y propiedades bactericidas. *An. Quim*., 2009, vol. 105, no. 1, pp. 33-41. [Mongue M. Silver nanoparticles: methods of synthesis in solution and bactericidal properties. *An. Quim*., 2009, vol. 105, no. 1, pp. 33-41. (In Span.)]

17. Edison T.J.I., Sethuraman M.G. Biogenic robust synthesis of silver nanoparticles using Punica granatum peel and its application as a green catalyst for the reduction of an anthropogenic pollutant 4-nitrophenol. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2013, vol. 104, pp. 262-264.

18. Thakur M., Pandey S., Mewada A., Shah R., Oza G., Sharon M. Understanding the stability of silver nanoparticles bio-fabricated using Acacia arabica (Babool gum) and its hostile effect on microorganisms. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2013, vol. 109, pp. 344-347.

19. Aleixandre-Tudo J.L., du Toit W. *The Role of UV-Visible Spectroscopy for Phenolic Compounds Quantification in Winemaking. Frontiers and New Trends in the Science of Fermented Food and Beverages*. IntechOpen, 2018.