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In vitro antimicrobial activity evaluation of AgNPs and lemon peel extracts

Evaluación de la actividad antimicrobiana *in vitro* de AgNPs y extractos de cáscara de limón

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Abstract

Quality assurance in fresh food, as well as biosafety for human consumption has led us to develop technological alternatives that guarantee food safety in the face of the SARS-CoV-2 pandemic. An alternative is nanomaterials, which have multiple properties or mechanisms of action, such as antimicrobials, antifungals and / or antivirals, making them an alternative as a biocidal agent of great interest in the current pandemic that humanity is experiencing. nowadays. In this work, a preliminary study is presented on the in vitro antimicrobial action mechanism of aqueous extracts of lemon peel mixed with a colloidal solution of silver nanoparticles (AgNPs) biosynthesized from plant extracts, and with a range of diameters between 20 and 50 nm. The microbiological tests in Escherichia coli (ATCC 25922) demonstrated a growth inhibitory effect for all treatments with AgNPs, while for the extracts a reduced inhibitory effect was observed in disk diffusion and null for the test in nutritive broth. As a result of preliminary studies in lemons, we conclude that the use of nanotechnology in colloidal solution for the agricultural area promotes food safety and its application can be extended to other agricultural products susceptible to contamination.

Agro-nanotechnology, Silver nanoparticles, Lemon, Antimicrobial activity, Food security

Resumen

El aseguramiento de la calidad en alimentos frescos, así como la bioseguridad para el consumo humano nos ha llevado a desarrollar alternativas tecnológicas que garanticen la seguridad alimentaria frente a la pandemia de SARS-CoV-2. Una alternativa son los nanomateriales, los cuales poseen múltiples propiedades o mecanismos de acción, tales como antimicrobianos, antifúngicos y/o antivirales, lo que los convierte en una alternativa como agente biocida de gran interés en la actual pandemia que vive la humanidad. en la actualidad. En este trabajo se presenta un estudio preliminar sobre el mecanismo de acción antimicrobiana in vitro de extractos acuosos de cáscara de limón mezclados con una solución coloidal de nanopartículas de plata (AgNPs) biosintetizadas a partir de extractos vegetales, y con un rango de diámetros entre 20 y 50 nm. Las pruebas microbiológicas en Escherichia coli (ATCC 25922) demostraron un efecto inhibidor del crecimiento para todos los tratamientos con AgNPs, mientras que para los extractos se observó un efecto inhibidor reducido en difusión en disco y nulo para la prueba en caldo nutritivo. Como resultado de los estudios preliminares en limones, concluimos que el uso de nanotecnología en solución coloidal para el área agrícola promueve la seguridad alimentaria y su aplicación puede extenderse a otros productos agrícolas susceptibles de contaminación.

Agro-nanotecnología, Nanopartículas de plata, Limón, Actividad antimicrobiana, Seguridad alimentaria

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Introduction

The Food and Agriculture Organization of the United Nations (FAO) has indicated that the global situation regarding the SARS-CoV-2 pandemic can cause a contraction in the supply and demand of agricultural products, even have an interruption in the chain of production, distribution and marketing in the food sector (FAO and ECLAC, 2020). This challenging public health situation has modified our environments and eating habits, including changes in the way we acquire food, its preparation and consumption. In this sense, due to confinement, food and nutrition could be adversely affected by having a decrease in family income due to economic depression and by a potential lack of availability and difficulty of access to healthy and safe food, especially in groups most vulnerable (Rodríguez, 2020).

However, for the vast majority of the population, quarantine translates into food insecurity in terms of a decrease in the amount of food available, of low nutritional quality or of poor cleaning, quite the opposite of what has been recommended regarding measures to avoid serious complications due to SARS-CoV-2 (Rodríguez, 2020; Devereux et al., 2020).

The agricultural and agri-food sector is one of the fastest growing fields in scientific research and its multiple applications aimed at innovating and generating technology that produces the quantity and quality of food sufficient to feed a rapidly growing world population (Lira, 2018). Lemon is a citrus fruit of the Citrus genus belonging to the Rutaceae family that is also rich in valuable nutritional components such as flavonoids and vitamin C; it also has antioxidant properties, which is why it is capable of preventing the negative activity of free radicals. As a disadvantage, it has the problem of accelerated deterioration. Its shelf life under environmental conditions is 10 to 15 days (Dolores, 2012). Mexico is a net exporter of lemon; in the last 5 years its imports have been almost nil and it participates with up to 34% of world production. On the other hand, it was reported in 2018 that the main lemon producing states were Veracruz, Oaxaca and Jalisco and that together they contributed 95% of the national volume in Mexico (SADER, 2019).

80% of world lemon consumption is represented by Mexico, the European Union and the United States, where Mexico exported 733 thousand tons of lemon to the latter in 2018 (SADER, 2019). This implies challenges to ensure the safety of food that reaches other nations and that is marketed within the country that demand high quality standards (Shahidi, 2020; Hobbs, 2020).

Nanotechnology has emerged as a technological advance that can transform the agri-food sector by offering various applications during this new normal, as there is a wide range of opportunities providing tools for food preservation and reducing health risks (Lira, 2018, Hochella et al., 2008). Studies have been reported on the antimicrobial, antifungal, antiviral effect of metallic nanoparticles (Copper, Zinc, Silver) of even different morphology (Galdiero et al., 2014).

In this work, the antimicrobial effect of silver nanoparticles (AgNPs) synthesized by a green way (Martínez Espinosa et al., 2020) and in conjunction with aqueous extracts of 3 varieties of lemon grown in Mexico was evaluated: *Citrus aurantifolia*, *Citrus latifolia* and *Citrus lemon* in disk diffusion method and in broth dilution dilution on the way to exploring new antimicrobial agents to promote food safety of fresh produce.

Materials, methods and procedures

Biological samples used

Escherichia coli (ATCC 25922) was used as a control strain to be evaluated in the microbiological tests. For the plant extracts, three varieties of lemon were used (Figure 1): Mexican, green or bitter lemon (*Citrus aurantifolia*), Persian or seedless lemon (*Citrus latifolia*) and Eureka, yellow or Italian lemon (*Citrus lemon*). All the lemon samples were acquired in commercial establishments in the city of León, Guanajuato.

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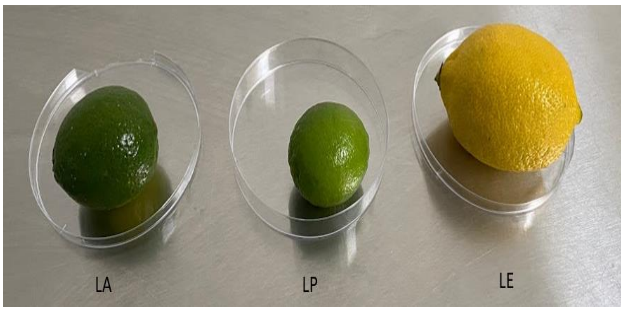

Figure 1 Variety of lemons used in each trial for the present study. LA: Bitter lemon (*Citrus aurantifolia*), LP: Persian lemon (*Citrus latifolia*) and LE: Eureka lemon (*Citrus lemon*) *Source: Own Elaboration*

Synthesis and characterization of AgNPs

AgNPs were synthesized based on a green synthesis protocol reported by Martínez et al. (Martinez Espinosa et al. 2020). The synthesis was carried out using a Geranium plant extract from an AgNO3 solution as a precursor solution. The colloidal nanoparticle solution was characterized by UV-Vis spectrophotometry (DU 730 Beckman Coulter spectrophotometer), by high resolution transmission electron microscopy (HRTEM-JEOL JEM 2100) equipped with a LaB6 source operated from 80 to 200 kV. As well as the EPA 6020A analytical method for Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Lemon extracts preparation

The lemons were disinfected with ethanol at 70 ° and 2.26 g of their peel were macerated and 10 mL of sterile distilled water were added, and then they were left to rest for 5 min. The supernatant was recovered and reserved for antimicrobial testing. In addition, a 140 mg / L AgNPs solution was prepared with distilled water as diluent for antimicrobial evaluation.

Microbiological disk diffusion assay

The EUCAST (2014) protocol was followed with modifications, for antimicrobial susceptibility studies. 90 mm petri dishes were inoculated with nutrient broth with agar-agar, by means of the streak technique with a sterile swab from an axenic culture of *Escherichia coli* (ATCC 25922) previously incubated with one week of growth. 3 filter paper sensidisks with 6 mm diameter were used, 50 μL of lemon peel extract were added to each one. In addition, amoxicillin 1 g/L was used as a positive inhibition control.

This process was repeated for the three lemon varieties, as well as for the 140 mg / L AgNPs solution. Finally, the inhibition halo was recorded after 24 h of incubation at 37 ° C.

Antimicrobial test in nutritive broth

For the evaluation of the antimicrobial activity, tubes were prepared with nutrient broth, the substance to be evaluated (lemon peel extract and / or AgNPs solution at 140 mg / L, as the case may be) and *Escherichia coli* inoculum (ATCC 25922). The microorganism was previously prepared following the standard with modifications from EUCAST (2014). The 20 ml Falcon tubes were incubated at 37 ° C at 10 RPM in a horizontal position for 24 h. 100 μL of each tube were inoculated into 90 mm petri dishes with agar-agar and nutrient broth by means of the plate extension technique, after 24 h of incubation. This test was repeated for each variety of lemon extract and the antibiotic amoxicillin at 1 g / L was used as a positive inhibition control and sterile distilled water was used as a negative control.

Results

Synthesis and characterization of AgNPs

The colloidal solution of nanomaterials obtained by the aforementioned green synthesis protocol was characterized in order to ensure the range of diameters reported, this due to being a biological methodology some properties may change. The UV-vis spectrophotometry analysis showed the typical absorption peak at 435 nm (Figure 2A), while the HR-TEM technique showed some nanoparticles with a compact, contracted hexagonal structure with a particle diameter distribution between 20-50 nm. (Figure 2B-D). While the colloidal silver concentration was 1,474.6 mg / L according to the ICP-MS analytical method (Martínez-Espinosa et al., 2020).

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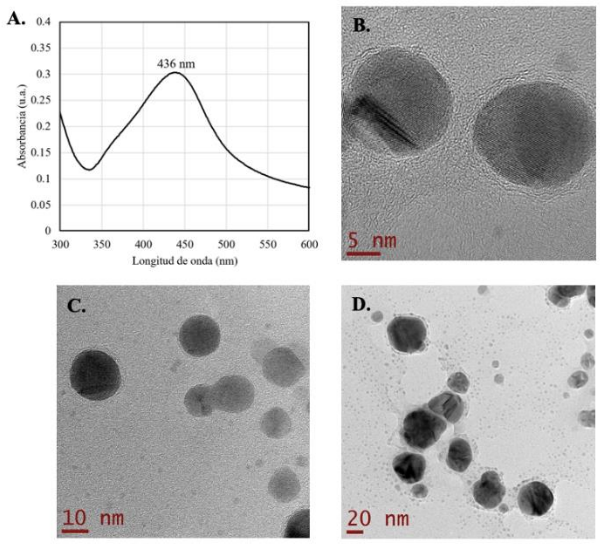

Figure 2 UV/Vis and HR-TEM absorption spectra of silver nanoparticles synthesized by reducing $AgNO_3$ by a green methodology. A) UV-Vis spectrum of the AgNPs solution. B, C and D) Micrographs of AgNPs obtained by green synthesis registered by the high resolution microscope JEOL-JEM-2100
Source: Own Elaboration

Microbiological test by disk diffusion

For the in vitro antimicrobial activity test where filter paper disks impregnated with the substance to be evaluated (lemon peel extract or AgNPs solution) shown in Figure 3 were used, the presence of inhibition halos of 1 mm in diameter for treatment with the AgNPs solution. On the other hand, with the Persian lemon extract (EP) an inhibition halo with a diameter of 0.5 mm was obtained. For the treatments with the extracts of lemon peel eureka and bitter (EE and EA) they did not show antimicrobial activity for any of their respective tests. In parallel, the microbiological test for the control sample with amoxicillin treatment 1 g / L presented an inhibition halo of 42 mm.

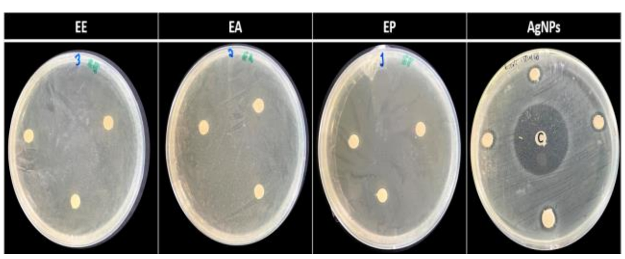

Figure 3 Antimicrobial activity test for lemon peel extracts and AgNPs solution on sensidisks. EE: Eureka Lemon Peel Extract, EA: Bitter Lemon Peel Extract, EP: Persian Lemon Peel Extract, AgNPs: 140 mg/L and C: Control with amoxicillin 1 g/L
Source: Own Elaboration

Antimicrobial test by dilution in nutrient broth

Regarding the antimicrobial susceptibility test in nutritive broth (Figure 4) for all treatments with the extracts of eureka, bitter and Persian lemon peel (EE, EA and EP) without the addition of AgNPs, they showed extended growth in plaque. The treatments that included the AgNPs solution (EE + AgNPs, EA + AgNPs, EP + AgNPs and AgNPs) did not show bacterial growth in any of the plates after 24 h of incubation in the 20 ml Falcon tubes. Regarding the control sample with antibiotic, it did not show bacterial growth, while the negative control plate showed extended bacterial growth.

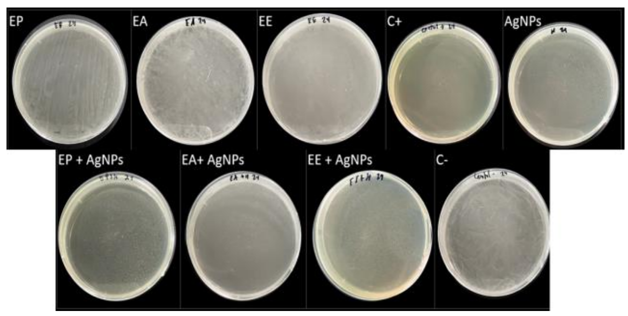

Figure 4 Antimicrobial sensitivity test for lemon peel extracts and AgNPs in broth. EE: Eureka Lemon Peel Extract, EA: Bitter Lemon Peel Extract, EP: Persian Lemon Peel Extract, EE + AgNPs: Eureka Lemon Peel Extract with AgNPs 140 mg/L, EA + AgNPs: Extract of Bitter Lemon Peel with AgNPs 140 mg/L, EP + AgNPs: Persian Lemon Peel Extract with with AgNPs 140 mg / L, AgNPs: AgNPs Solution 140 mg/L, C-: Control sterile distilled water, C +: Control amoxicillin 1 g/L
Source: Own Elaboration

Discussions and conclusions

More sustainable methods of nanomaterial production are preferred due to the great concern of global contamination, which makes employing a green method for the synthesis of AgNPs a promising alternative approach for different biomedical and biotechnological applications. This is due to the fact that it avoids the use of toxic reagents, which generate polluting by-products, dispensing with the comprehensive management of hazardous waste. As well as a more efficient energy consumption, compared with the traditional synthesis by chemical or physical means (Beyene et al., 2017; Singh et al., 2019).

The AgNPs used in the present study resulted in a diameter range between 20-50 nm, which showed antimicrobial activity in vitro against *Escherichia coli* at a concentration of 140 mg / L demonstrated by the disk diffusion test with the presence of inhibition halos (Figure 3), previous studies that have highlighted the powerful antimicrobial effect of AgNPs are corroborated (Wang et al., 2019, Duhan et al., 2019). While the aqueous extracts of lemon peel did not show antimicrobial effect against E. coli., With the exception of the Persian lemon extract (EP), with a lower inhibitory effect than that of AgNPs (Fig. 3), these results can be attributed to the low concentration of the biochemical components (flavonoids and organic acids) in the extracts obtained from the lemon peel (Londoño, 2006).

Although water, ethanol, and methanol are considered good extraction solvents for citrus, some authors Muhammed et al. (2020) recommend including a distillation to concentrate active compounds responsible for the antimicrobial effect of citrus peel. In the case of the antimicrobial activity test in nutritive broth, an inhibition effect of bacterial growth was not observed either (Figure 3), only the treatments with AgNPs and the control with antibiotics showed inhibition, which coincides with previous reports (Kalwar et al., 2018, Lira et al., 2018).

The antimicrobial effect on other citrus fruits and vegetables could be investigated because this study complements the research on the use of AgNPs in the area of food safety (Beyene et al., 2017, Simbine et al., 2019). Since it is possible that AgNPs at suitable doses are a viable solution to reduce the microbial load in different agricultural products to meet the high standards of food safety.

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Conflicts of interest

The authors declare that does not exist an interest conflict.

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