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RESEARCH

# Obtention of green-synthesized silver nanoparticles and evaluation of its antimicrobial and antifungal activity against skin pathogenic microorganisms

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**ABSTRACT**

Bacterial resistance to antibiotics is a major public health problem. In this setting, silver nanoparticles (AgNPs), metal oxides and nanocarbons could be alternatives. Several issues arise when nanoparticles are obtained by chemical synthesis, including the use of toxic and hazardous chemicals. Otherwise, metallic nanoparticles are attractive in biomedical research. Hence, in this work, AgNPs were synthesized by the green route using olive and eucalyptus leaf extracts by an environmental-friendly technique. Both plants are locally obtainable, abundant, economical and eco-friendly. The synthesized AgNPs were characterized by ultraviolet-visible (UV-vis) spectrometry, scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) techniques. Their antibacterial and antifungal potential were tested by the well diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Klebsiella pneumoniae* and *Candida albicans*, and an o/w cream was formulated to be used as an alternative therapeutic approach against skin infections. AgNPs were validated by the UV-vis spectra obtained. FTIR spectrum assumed the polyphenols and proteins act as stabilizing and reducing agents in the synthesis of AgNPs. AgNPs were shown to have spherical morphologies by SEM analysis. The biosynthesized AgNPs showed their potential to inhibit growth of yeast, Gram-positive and -negative bacteria. The employed green synthesis route supported the obtention of biosynthetic AgNPs. Compared to metallic silver, AgNPs bear improved antibacterial and antifungal effect. The cream formulated shown properties consistent with future pharmaceutical applications. This combination of cutting-edge nanotechnology with traditional medicine provides a great opportunity to develop new antimicrobial agents.

*Keywords:* nanoparticles, AgNPs, cream formulation, plant extracts, Green-synthesized silver

**RESUMEN**

**Obención de nanopartículas de plata mediante síntesis verde y evaluación de sus actividades antifúngica y antibacteriana contra microorganismos patógenos de la piel.** La resistencia bacteriana a antibióticos es un problema de salud pública muy significativo. Por ello, las nanopartículas metálicas, entre ellas las de plata (AgNPs), los óxidos metálicos y los nanocarbonos, han emergido como alternativas. No obstante, para su síntesis se emplea químicos tóxicos y riesgosos. Por lo tanto, en este trabajo se sintetizó AgNPs mediante el método de síntesis verde con una técnica ecoamigable, a partir de extractos de hojas de olivo y eucalipto. Ambas plantas son de alta disponibilidad local, económicamente asequibles y ambientalmente seguras. Las AgNPs fueron caracterizadas mediante las técnicas de espectrometría ultravioleta visible (UV-vis), microscopía electrónica de barrido (SEM), difracción de rayos X (XRD) y expectroscopía infrarroja de la transformada de Fourier (FTIR). El potencial antibacteriano y antifúngico se evaluó mediante el método de difusión en pocillo contra *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Klebsiella pneumoniae* y *Candida albicans*. También se formuló una crema aceite en agua para el uso terapéutico contra infecciones de la piel. El método de síntesis empleado permitió obtener nanopartículas, luego validadas validadas por el espectro de UV-vis. El análisis FTIR indicó que los polifenoles y las proteínas actúan como agentes estabilizantes y reductores durante la síntesis. El análisis SEM mostró AgNPs de morfología esférica. Se demostró que las partículas mostraron potencial para inhibir el crecimiento de levaduras y bacterias Gram positivas y negativas, con mayor efecto que la plata metálica. La crema formulada permite desarrollos famacéuticos futuros. Esta combinación de la nanotecnología más actual con la medicina tradicional brinda una oportunidad para desarrollar nuevos agentes antimicrobianos.

*Palabras clave:* nanopartículas, AgNPs, formulación en crema, extractos de plantas, síntesis verde de plata

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## Introduction

The pharmaceutical industry has become increasingly interested in nanotechnological advances [1]. As a physical barrier and the site of the primary immune response, the skin and its underlying tissues are frequently the first line of protection against infections[2]. Bacteria, fungi, viruses, and parasites are the principle cause of skin infections [3]. Meanwhile, silver is one of the oldest metals with documented use

to treat skin contaminations[4]. More recently, a wide range of applications have been demonstrated for silver nanoparticles (AgNPs), most of them derived from their very small shape and size control, as a crucial factors to enhance its antimicrobial, physical, and chemical properties [5].

AgNPs are a special form of metallic silver, having less than 100-nm size, providing them a high surface area to volume ratio [6]. They can be synthesized by several physical, chemical and biological methods, the first two types been hazardous for the environment, because of using high level of heat, and the release of toxic by-products during the synthesis process, in addition to be expensive in comparison with biological methods [7]. Therefore, there is a growing need to develop environmentally friendly processes for nanoparticle synthesis devoid of toxic chemicals [8].

One such alternative to obtain AgNPs is green synthesis utilizing plant phytochemicals, which comprises three stages. First, $Ag^+$ oxidation occurs, in which Ag atoms are formed by reducing agents (plant extracts), and AgNPs are formed by nucleating Ag atoms. Second, the AgNPs expand to larger ones, along with the surface decrease of any Ag+ ion, giving rise to the creation of Ag atoms. Third, AgNPs are electrostatically stabilized, to monitor its scale. While the adsorption on the surface of the formed AgNPs of excessive, negatively charged reducing agent's ions occurs [9]. In this process, plant extracts' metabolites (i.e., phenols, alkaloids, proteins, and carbohydrates) mediate the synthesis and stabilize the green-synthesized metallic nanoparticles nanoparticles [10]. Among all synthesis methods, the biological process (also called green synthesis) is the simplest, cheapest and ecofriendliest, since it uses biological materials for synthesizing nanoparticles, such as microorganisms and plant-based tools [11, 12].

Regarding the antimicrobial activity of silver and its compounds, they are considered to be extremely toxic to major pathogenic microorganisms such as bacteria, fungi and viruses. Although the bactericidal and fungicidal silver mechanism of action is not completely understood, it has been suggested to inhibit cell transduction and induce cell lysis as well [13]. Thus, its proven antibacterial activities have attracted considerable attention, because they may provide a solution to the antibiotic resistance issue [14]. Commonly, silver nanoparticles have shown sufficient preservation efficacy against mixed bacteria and fungi, and did not penetrate normal human skin [15]. It is probable that 0.2 to 2 % of AgNPs will penetrate the skin (0.002-0.02 ppm). AgNPs did not demonstrate any toxicity at these stages. Furthermore, intact or partially damaged skin contacting nanoparticles (20 to 200 nm) do not penetrate the skin barrier and penetrate the lower strata, making them healthy as cosmeceuticals. The deeper layer of the stratum corneum could be penetrated by nanoparticles with a diameter less than 10 nm, while NPs greater than 40 nm wide could only penetrate 5-8 μm [16].

Furthermore, the plants used are important sources of phenolic compounds [17], which could aid on the intended therapeutic application of the AgNPs produced. For instance, the major active components in olive leafs comprise oleuropein and its derivatives, such as hydroxytyrosol and tyrosol, as well as cafeic acid, p-coumaric acid, vanillic acid, vanillin, luteolin, diosmetin, rutin, luteolin-7-glucoside, apigenin-7-glucoside, and diosmetin-7-glucoside [18]. On the other hand, eucalyptus leaves contain a wide array of active components, being rich in polyphenols, including flavonoids and tannic acids, organic acids and volatile oils, of potential applications [19].

Therefore, this work was aimed to produce AgNPs by the green synthesis technology from olive and eucalyptus leaf extracts, to characterize them by using nanotechnology tecniques, and to assess its antimicrobial activity against pathogenic yeast and bacteria. The results obtained supported the formulation of an antimicrobial cream which was rheologically characterized, of potential application.

## Materials and methods

### Plant material

Eucalyptus and olive leaves were collected from north Algeria in Ain lahjr at Bouira, Algeria, in the period of May-June, 2019. The Specimen voucher was deposited in the National Herbarium of the Research laboratory of Arid Zones LRZA Herbarium for authentication N°10-2019 Boui;95 MB/ol/LRZA/USTHB for olive and N°18-2019 Boui; MB/eu/LRZA/USTHB for Eucalyptus voucher. The plants extract solutions were prepared using 10.0 g of leaves that had been rinsed with deionized water, dried and reduced into powder. The powders were boiled in 100 mL of deionized water for 1 h, cooled at room temperature, and then filtered and stored in at 4 °C.

### Preparation of the aqueous extract

Comminuted plant material (50 g) was boiled in 1 L of sterile deionized water for 5 min and allowed to stand for 1 h at room temperature. It was then subsequently filtered through Whatman filter paper No. 1 and Millipore filter (0.22 μm). The obtained aqueous extract was frozen at –70 °C, lyophilized and stored at 4 °C in the dark under sterile conditions until use.

### Phytochemical study

The phytochemical components of olive and eucalyptus leaf extracts were investigated. Herbal extracts were screened to determining their active components for therapeutic purposes [20]. The presence of alkaloids, flavonoids, glycosides, steroids, saponins, tannin, coumarines and terpenoids were determined following the conventional approach [21].

### Green synthesis of silver nanoparticles

Silver nanoparticles were synthesized by processing starting materials silver nitrate ($AgNO_3$; Sigma-Aldrich). A solution of $AgNO_3$ (20 mM) was prepared, by dissolving 0.34 g of $AgNO_3$ in 100 mL of deionized water. Increasing volumes each plant leaf extracts (1, 2, 3, 4 and 5 mL) were chosen for eucalyptus extracts (referred as Euc.NP1, Euc.NP2, Euc. NP3, Euc.NP4 and Euc.NP5, respectively) and olive extracts (Ol.NP1, Ol.NP2, Ol.NP3, Ol.NP4 and Ol.NP5), and added to 5 mL of $AgNO_3$ solution for the synthesis of AgNPs. Final volume was was adjusted to 50 mL with deionized water and stirred for

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2 min. The reduction process of $Ag^+$ to $Ag^0$ nanoparticles was followed by color change of the solution from yellow to brownish-yellow to deep brown (Figure 1).

## Characterization of silver nanoparticles

The synthesized AgNPs were characterized through the following techniques using the UV-PROBE vers.2.34 (Shimadzu) spectrophotometer. The UV-vis spectra were documented at room temperature and used to measure absorbance and record optical density at 300-700 nm wavelengths. Fourier transform infrared spectroscopy (FTIR) with a resolution of 0.2 nm at 40-4000 $cm^{-1}$ (Nicolet 670, Madison, WI) was used to identify the possible biomolecules responsible for the reduction of silver ions and capping of the synthesized AgNPs at room temperature. A small amount of silver nanoparticles (0.01 g) dried at 60 °C for 4 h was combined with KBr to form a circular disk acceptable for FTIR measurements of capped silver nanoparticles. The morphological characteristics of the silver nanoparticles were identified by examining a freeze-dried sample of AgNPs under a Zeiss EVO-18 scanning electron microscope at 20 kV.

Furthermore, the shape, morphology and elemental mapping of the AgNPs was studied using a scanning emission electron microscopy (NOVA NanoSEM 450). For this purpose, the freeze-dried sample was sonicated for a sufficient time. The smear was made on a platinum grid and allowed to dry overnight under vacuum. The grid was then covered with a thin film of palladium and finally subjected to FESEM. The crystalline nature of AgNPs was confirmed by the XRD pattern obtained by using a Rigaku-MiniFlex 600 X-ray Diffractometer at 2θ range from 0 to 100°. The sample for the XRD measurement was prepared by casting the powder of silver nanoparticles on a glass slide and then air-drying it under ambient conditions. The pattern was recorded by CuKα radiation with λ of 1.5406 Å at a 40 kV voltage and 15 mA current with a scan rate of 10°/min.

## *In vitro* inhibitory activity

To detect the activity of AgNPs, standard well agar diffusion method was carried out against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Klebsiella pneumoniae* and *Candida albicans* obtained from the Pasteur Institute in Algiers and provided by the National Pharmaceutical Control Laboratory (LNCCP). The antibacterial activities of the AgNPs was verified by the agar well diffusion method. Using this method, a Petri dish containing Mueller-Hinton liquefied medium was inoculated with 1 µL of each bacterial suspension adjusted and diluted to $10^7$ c.f.u. (0.5 McFarland standard diluted to 10 %), and a $10^4$ c.f.u. final inoculum was applied per spot. Wells, 6 mm diameter, were made aseptically with corn borer after complete solidification of the liquefied inoculated medium. In each plate, silver solution, plant extracts and green synthesized AgNPs topical formulations were placed carefully. Plates were kept for pre-diffusion for 30 min at room temperature and then incubated at 37 °C for 24 h, and the inhibition zones measured. Each test was carried out twice. Gentamycin and ciprofloxacin were used as positive controls for bacteria.

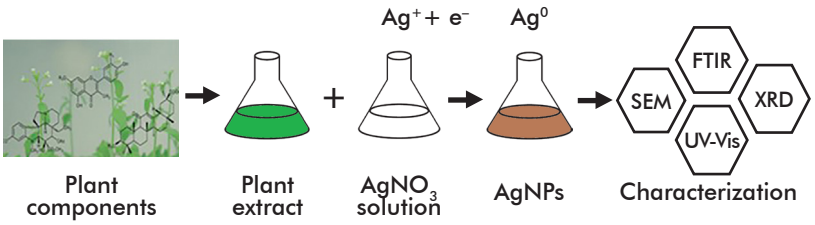


Figure 1. Diagram of the synthesis process and characterization of silver nanoparticles (AgNPs) by reduction of $Ag^+$ into $Ag^0$. AgNPs: Ag nanoparticles. SEM: scanning electron microscopy. FTIR: Fourier transform infrared spectroscopy. XRD: X ray diffraction. UV-vis: ultraviolet-visible spectrometry.

## Preparation of topical formulations

Statistical analyses were performed using the R system, version 3.1.0 (2014-04-10). Statistical differences were established for $p \leq 0.05$ for all comparisons. The variables used for statistical interpretation were: body weight, average consumption of drinking water and food, respectively, histopathological findings and morphometry immunotoxicological studies. In all cases, the measures of central tendency and dispersion (mean, standard deviation, maximum and minimum values) were estimated as descriptive properties Topical formulations were prepared with biosynthesized AgNPs containing more than 60 % water, 0.4 % xanthan and 4% glycerin [17]. Ingredients comprising oil phase ingredients, including vegetal oil and beeswax, were heated in a vessel at 72 °C. Similarly, all the aqueous phase ingredients (including AgNPs from eucalyptus or olive leaf extracts, glycerin and xanthan) were heated at 72 °C in a separate vessel (see composition). Under continuous stirring, the oil phase was added to the aqueous phase and the cream cooled to 35 °C, followed by adding additives. The cream was transferred to a clean jar, taped to evacuate air and labeled with the manufacturing date [17]. The cream composition was in percentage of sufficient quantity for (SQF): solution of nanoparticles (100 %), glycerin (3-6 %), xanthan (0.2-0.4 %), wax (5-7 %), vegetal oil (10-30 %), vitamin E (0.3 %), essential oil (4 %) and fragrance (1.5 %).

Subsequently, 5 ± 0.01 g of the cream was weighed accurately in a 100 mL beaker for pH measurement, and 45 mL of water added and the cream on it. The pH of the suspension was determined at 27 °C using a pH meter. Rheological measurements of the cream were performed using a rheometer (Physica MCR 301 rheometer) with cone and plate geometry. The rabbit irritation test was carried out using the established procedure [22]. The cream formulated (500 mg) was applied on the right and left hind limbs of the rabbit, the site of approximately 25 $mm^2$, and covered with a patch (semi-occlusive), using a non-occlusive bandage to wrap the test areas. After 24 h, the patch and test materials were removed and the sites were examined for skin irritation. The reactions were assessed using the scoring system and response categories according to OECD test guideline 404 [22].

## Statistical analysis

All experiments were carried out in triplicate. For the experiments on antimicrobial activity, Arithmetic

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mean values were considered for data analysis. For the comparison of the data obtained by silver nanoparticles analyzed by SEM, the unpaired "t" test was performed. All statistical analyses were performed using GraphPad InStat software [DATASET1.ISD].

## Results and discussion

### Phytochemical screening

In recent years, the World Health Organization (WHO) has emphasized the importance of research and development of new antimicrobial drugs, as morbidity and healthcare costs have increased due to the rise of pathogenic resistant microbial strains. So, the selection of plant material for this study was based on the ancestral knowledge of indigenous people in northeastern Algeria about medicinal plants for the treatment of skin infections. Because of their various pharmacological effects on a range of illnesses, phytochemicals have received a lot of research to date [23]. Hence, the present step of research had been going on to find out the active ingredients present in olive and eucalyptus leaves. Phytochemical screening allowed us to highlight the presence of some secondary metabolites (Table 1) in the olive and eucalyptus leaves. These plants contain a wide range of secondary metabolites such as tannins, alkaloids, flavonoids, saponin, and terpenoids, all of which have therapeutic uses. The relative presence of secondary metabolites in olive and eucalyptus leaf extracts were similar, except for alkaloids, which were not detected in olive leaves, and steroids, absent in eucalyptus leaves. Based on the intensity of colors in the test, eucalyptus leaves had the highest amounts of flavonoids compared to olive leaves.

The abundance of these active biomolecules or secondary metabolites (flavonoids, alkaloids, terpenoids, and steroids) in olive and eucalyptus leaves, which act as reducing or capping agents for metal nanoparticles, makes feasible the use of these medicinal plants as a source for metal nanoparticle synthesis. It is considered that bio-molecules found in plant extracts can act as both reducing and stabilizing agents in the production of AgNPs. Plant-mediated synthesis has advantages over physical and chemical synthesis in that it is environmentally benign, affordable, and does not require high pressure, energy, temperature, or hazardous chemical agents [24].

### Characteristics of the synthesized AgNPs

#### *Color change*

In this work, the ionized chemical groups present in the aqueous extract allowed the rapid formation of AgNPs when plant extract was mixed with $AgNO_3$ solution. The color change indicated the presence of AgNPs in the solution. The aqueous extracts of olive and eucalyptus leaves was mixed with $AgNO_3$ solution and incubated in the dark. Following the addition of different concentrations of plants extracts to silver solution replicated thrice, the color of the solutions changed from pale yellow to yellowish brown to deep brown depending on the extract concentration indicating silver nanoparticle formation (Figure 2). The color change is due to excitation of surface plasmon vibration in the silver nanoparticles [25]. The intensity of the color increased during the period of incubation (Table 2).

Table 1. Phytochemical screening of secondary metabolites in olive and eucalyptus leaf extracts

| Phytochemicals | Test | Plant leaf extract | |
|---|---|---|---|
| | | Olive | Eucalyptus |
| Flavonoids | Shinoda test | Moderate | High |
| Saponins | Frothing test | Trace | Moderate |
| Steroids | Salkowski test | Trace | Absent |
| Alkaloids | Hager's test | Absent | Trace |
| Tannins | Braymer's test | Trace | Moderate |
| Phenols | $FeCl_3$ test | Trace | Moderate |
| Terpenoids | Killer-Killani's test | Trace | Trace |

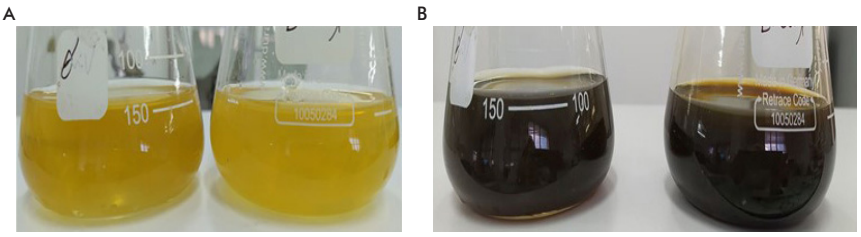


Figure 2. Color change of plant extract solution 24 h after adding it to a silver solution. A) Olive extract solution. B) Eucalyptus extract solution.

It was also observed that synthesis AgNPs from eucalyptus leaf extract lasted within 7-10 min, faster than AgNPs synthesized from olive leaf extract (20-30 min). This result redives from eucalyptus being rich in secondary metabolites such as alkaloids, flavonoids, saponins, steroids, tannins, and phenolic acids, more than olive leaves as mentioned above. Several studies have shown that many of these metabolites act as both reducing and stabilizing agents and inhibit the aggregation and agglomeration of the novel metallic NPs by nonhazardous means [1].

#### *UV-vis spectrum*

The synthesis of AgNPs was confirmed by the UV-vis spectrum recorded against water (Figure 3). Following the addition of plants extracts to silver solution, the color of the solutions changed from pale yellow to yellowish brown to deep brown, depending on the extract concentration, indicating silver nanoparticle formation [26]. The color change is due to excitation of surface Plasmon vibration in the silver nanoparticles. UV-vis spectrum of olive and eucalyptus extracts as well as Ag solution showed no peak of absorption (Figure 3A). It can be observed that AgNPs obtained from the eucalyptus leaves extract were 430-445 nm in mean diameter (Figure 3B), as

Table 2. Color change of Ag solutions during the synthesis of Ag nanoparticles from olive and eucalyptus leaves

| Time (h) | Plant leaf extract AgNPs | |
|---|---|---|
| | Olive | Eucalyptus |
| 0 | No change | No change |
| 0.05 | No change | No change |
| 0.10 | No change | Pale yellow |
| 0.15 | No change | Yellowish brown |
| 0.20 | Pale yellow | Brown |
| 0.30 | Yellowish brown | Reddish brown |
| 12 | Brown | Tinge brown |
| 24 | Tinge brown | Deep brown |

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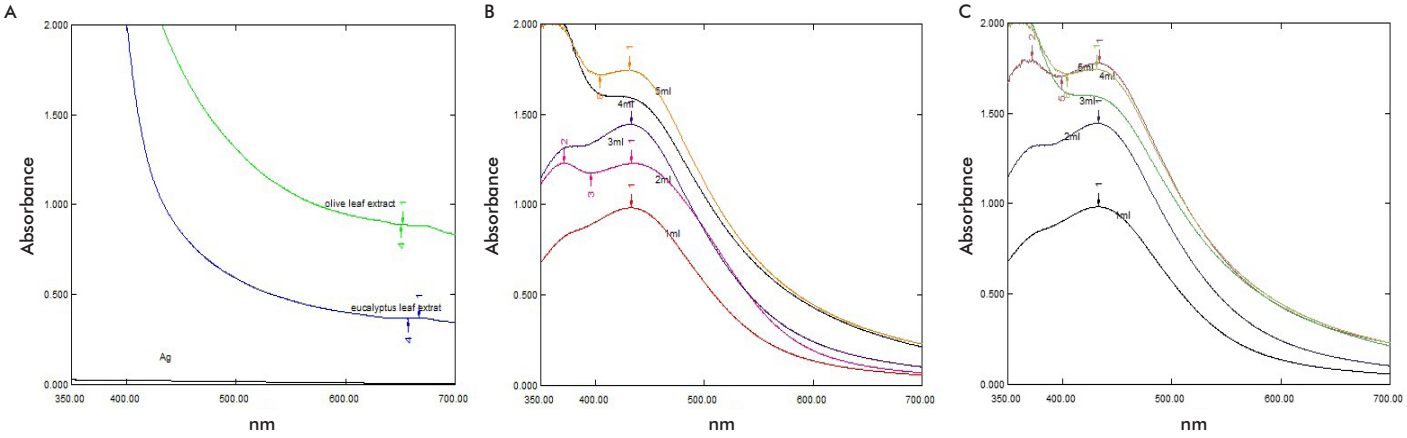


Figure 3. Ultraviolet visible spectroscopy spectrum of silver nanoparticles (AgNPs) biosynthesized by the green synthesis method from olive and eucalyptus leaf extracts. A) Eucalyptus and olive leaves extracts. B) AgNPs from olive leaf extracts. C) AgNPs from olive leaf extracts.

determined by surface plasmon resonance (SPR), and AgNPs obtained from olive leaves were 440-455 nm (Figure 3C).

As the leaf extract concentration increases, the peak of absorption becomes sharper and a blue shift, this last suggesting a decrease in the mean diameter of the silver nanoparticles. Furthermore, the blue-shifted and sharp narrow SPR band suggested the formation of AgNPs of spherical and homogeneous distribution [27]. These findings suggest that the concentration of leaf broth strongly affect the average particle size of the synthesized AgNPs. From this analysis, an absorbance peak was found at around 430-455 nm, which was specific for AgNPs. Based on the UV-vis spectra, the wave length of the AgNPs' resonance absorption peak is typically in the 400-500 nm range. The observation clearly shows the efficient AgNPs synthesis [28]. In agreement with previous reports, the increase in intensity may be attributed to the increasing number of nanoparticles created as a result of the reduction of silver ions in the aqueous solution [29].

### *FTIR spectroscopy*

FTIR is a method used to identify the functional groups present, based on the frequencies at which infra-red radiation is absorbed by the different bonds in the sample [30]. Therefore, FTIR measurements were performed to identify the biomolecules responsible for capping and stabilizing AgNPs. The FTIR spectrum (Figure 4A) for the eucalyptus extract showed a very strong peak at 3225 $cm^{-1}$, which was assigned as –OH stretching in alcohols and phenolic compounds [31]. The medium intense band 1625$cm^{-1}$ assigned to the stretching vibrations of C=C is characteristic of aromatic C=C bending shifted to a lower region after the formation of silver nanoparticles [11]. The vibrational bands corresponding to bonds such as –C-C and –C=O are derived from the compounds such as flavonoids and terpenoids in the eucalyptus extract. Therefore, it can be assumed that these biomolecules are responsible for capping and stabilization. The eucalyptus extract is rich in polyphenols such as tannic acid and flavonoid, and, therefore, it is reasonable to deduce that the polyphenols play complex roles in the reduction of Ag ions [32].

The peak of IR bands (Figure 4B) observed at 3429 $cm^{-1}$ and 1750 $cm^{-1}$ in olive leaf extract are characteristic of the O–H and C=O stretching modes for the OH and C=O groups, possibly due to oleuropein, apigenin-7-glucoside and/or luteolin-7-glucoside, which could be responsible for the formation of AgNPs. The medium band at 1624 $cm^{-1}$ corresponds to amide I arising from carbonyl stretch in proteins [33]. In the case of nanoparticles (Figure 4D), a large shift in the absorbance peak with decreased band intensity was observed from 3429 to 3351 $cm^{-1}$ and 1751 to 1755 $cm^{-1}$, implying the binding of silver ions with hydroxyl and carboxylate groups of the extract [34]. The spectra also illustrate a prominent shift in the wave numbers corresponding to amide I (1625 $cm^{-1}$) and amide II (1550 $cm^{-1}$) linkages. This validates that free amino (–NH2) or carboxylate ($–COO^{–}$) groups in compounds of the olive leaf extract have interacted with the surface of AgNPs, making AgNPs highly stable [35].

### *SEM*

Particle size analysis of the prepared silver nanoparticles using olive and eucalyptus leaf extracts are shown in Figure 5A and B, respectively, measured using a scanning electron microscope (SEM). The structure and shape of the green synthesized AgNPs using olive and eucalyptus extracts were further validated by FE-SEM (NOVA NanoSEM 450) micrographs. There were shown quasi-spherical nanoparticles of average size of 35 ± 2 nm from olive leaf extract (Figure 5A) and spherical nanoparticles of average diameter of 22 ± 1 nm from eucalyptus leaf extract, in good agreement with the average crystallite size estimated from XRD analysis. These results confirm that biomolecules act as reducing agents and cap the nanoparticles' surface, protecting them from aggregation. Similar results were previously reported with a significant extract ratio as required for the formation of symmetrical nanoparticles [36].

### Antimicrobial assay

The efficacy of synthesized nanoparticles was tested against *E. coli*, *S. aureus*, *P. aeruginosa*, *Salmonella*, *K. pneumoniae* and *C. albicans* by the well diffusion

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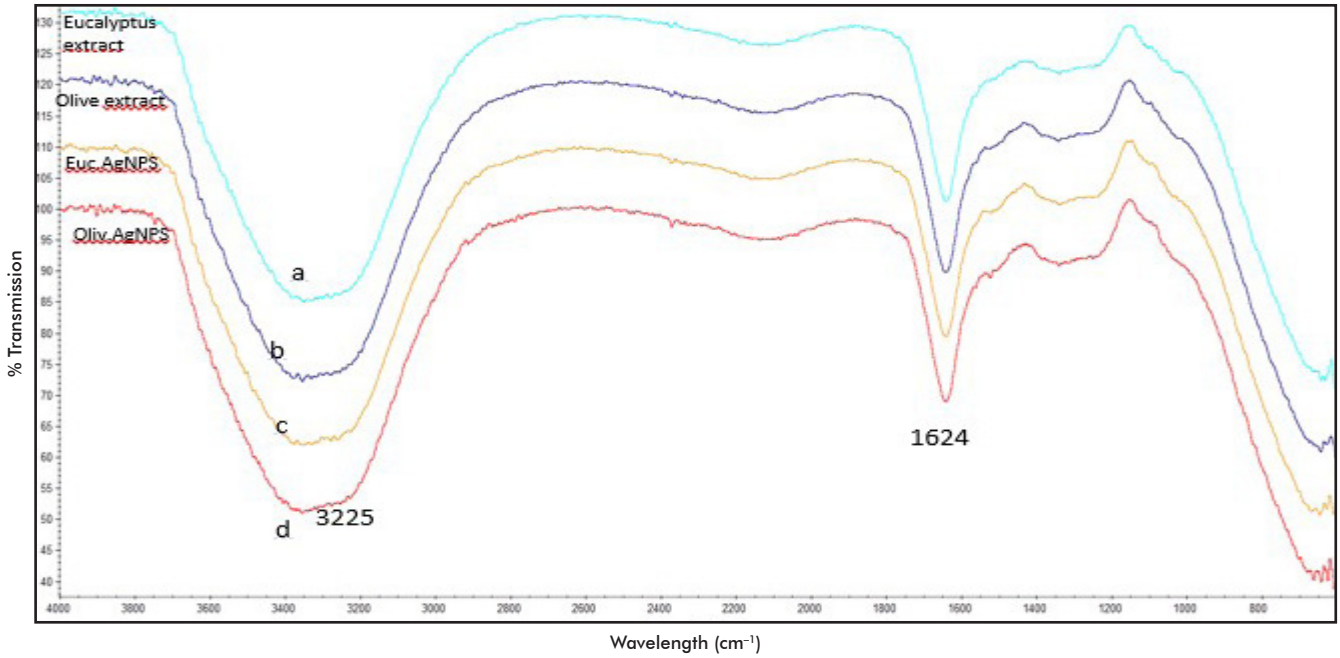


Figure 4. Fourier Transform Infrared spectroscopy (FTIR) analysis of olive and eucalyptus leaf extracts and derived biosynthesized silver nanoparticles (AgNPs).

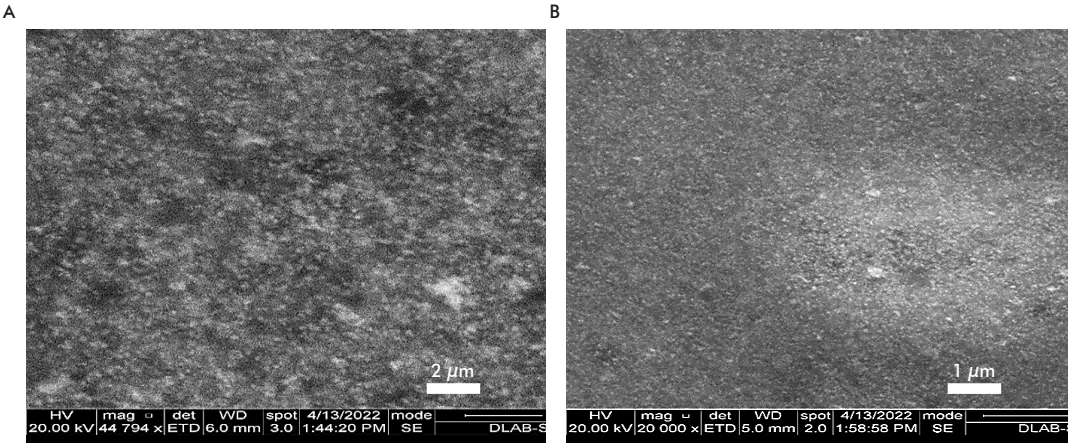


Figure 5. Scanning electron microscopy images of silver nanoparticles (AgNPs) prepared biosynthesized from plant leaf extracts. A) Olive AgNPs. B) Eucalyptus AgNPs.

method (Figures 6 and 7). Different concentrations (1, 2, 3, 4 and 5 mL of extract added per well) were tested to confirm the inhibition zone. The maximum antimicrobial activity was recorded, in decreasing order, against *Salmonella* with 26 mm (5 mL olive leaf extract), followed by *S. aureus* 25 mm (all olive leaf extracts' concentrations), *E. coli* 23 mm in 5 mL of eucalyptus extract and *C. albicans* 21 mm with 5 mL olive leaf extract. The mean zone of inhibition in the well diffusion methods were recorded (Figure 6). The efficacy of $AgNO_3$ solution tested against *E. coli*, *S. aureus*, *P. aeruginosa*, *Salmonella*, *K. pneumoniae* and *C. albicans* revealed the zone of inhibition of 12, 15, 0, 10, 13 and 13mm in diameter, respectively. Meanwhile, eucalyptus and olive leaf aqueous extracts had no antimicrobial activity. Antimicrobial activity of AgNPs against *E. coli*, *S. aureus* and *C. albicans*, tested by well diffusion method, revealed a 25-mm inhibition zone with 5 mL of olive AgNPs and 23-mm with eucalyptus AgNPs against *S. aureus*. Therefore, the antibacterial potential increases with the augmentation of the concentration. The 5-mL sample showed the highest UV absorbance, indicating creation of large amounts of AgNPs and demonstrated the highest activity against all bacteria [4].

In the case of *P. aeruginosa*, $AgNO_3$ solution as well as AgNPs synthesized from olive leaf extract at different concentrations showed no activity against it. On the contrary, the AgNPs synthesized from eucalyptus extracts at different concentrations present a good antibacterial activity against *P. aeruginosa*

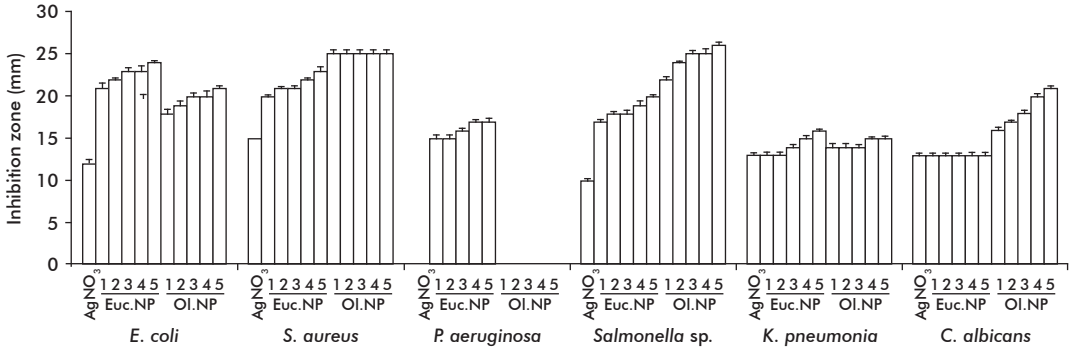


Figure 6. Antimicrobial activities of $AgNO_3$ solution and silver nanoparticles' solutions prepared with different volumes (1, 2, 3, 4 or 5 mL) of olive (Ol.NP) and eucalyptus (Euc.NP) leaf extracts, against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Klebsiella pneumonia* and *Candida albicans*. Error bars stand for standard deviation.

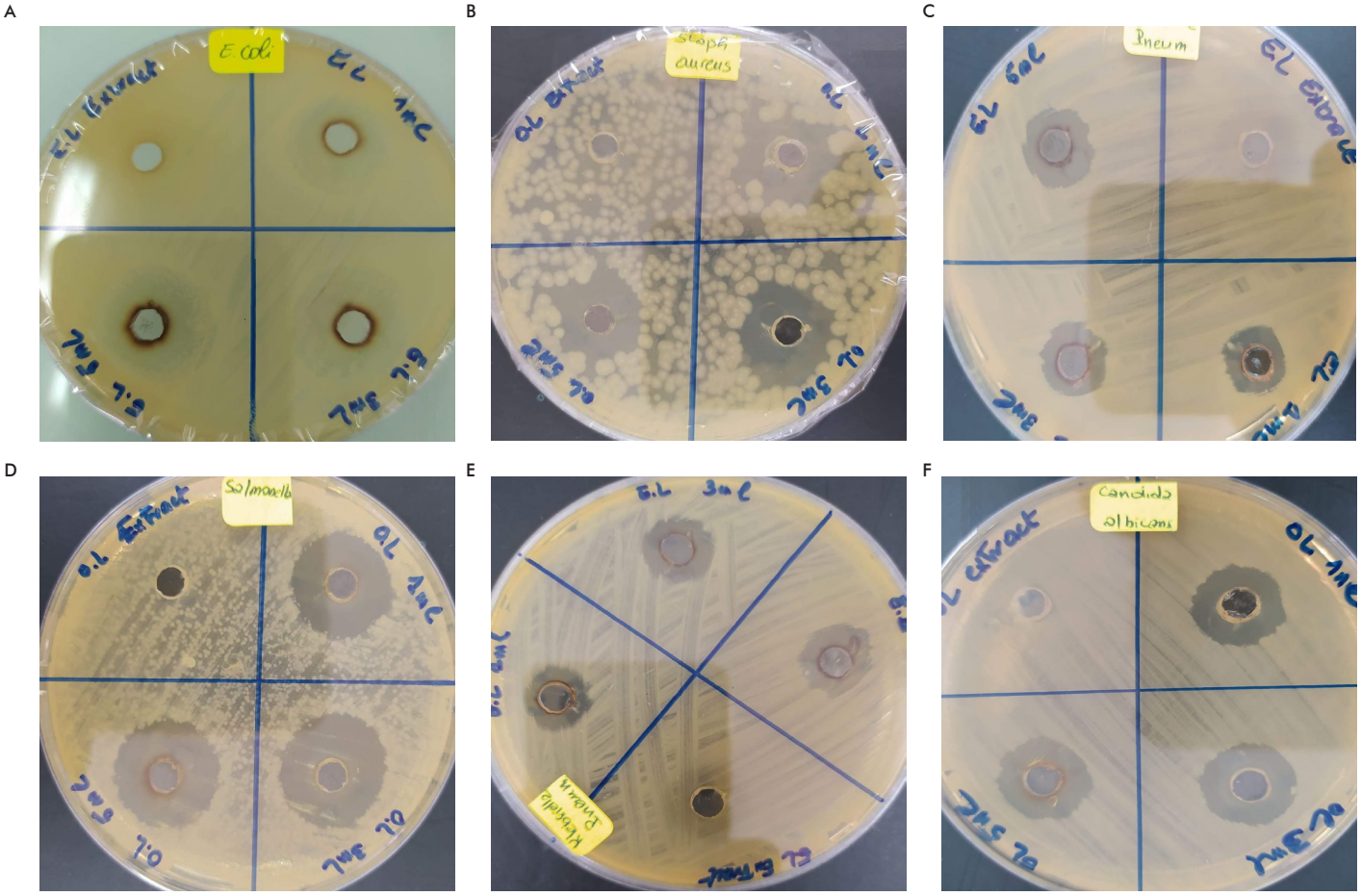


Figure 7. Antimicrobial activities of silver nanoparticles' solutions prepared with different volumes (1, 2, 3, 4 or 5 mL) of olive (Ol) and eucalyptus (Euc) leaf extracts against different microorganisms. A) *Escherichia coli*. B) *Staphylococcus aureus*. C) *Pseudomonas aeruginosa*. D) *Salmonella* sp. E) *Klebsiella pneumoniae*. F) *Candida albicans*.

with inhibition zone varying between 15 to 17 mm, and 13 to 16 mm against *K. pneumoniae* as shown in table 3. This could suggest that AgNPs react in synergy with eucalyptus extracts at different concentrations concerning its increases activity. In fact, the antibacterial activity of AgNPs is greatly influenced by a number of parameters, including the concentration as shown in this work. It was also found that the AgNPs synthesized by the green process display better antibacterial and antifungal activities as compared to metallic silver, making them a better option for its application in the field of nanomedicine. Noteworthy, *Salmonella* and *K. pneumoniae* were not evaluated for products indicated to skin diseases, but they could be

present on the skin and passed into the organism thereafter as opportunistic infections.

Silver ions and AgNPs have been shown to have strong antimicrobial activities [37]. The mechanism(s) of its bactericidal action could be due to permeation of such nanocomposite beyond the muscles' surfaces, most probably through the microbial membrane, causing their accumulation in cell membrane and nanoparticle internalization [38]. The AgNPs preferentially target the respiratory chain, finally leading to cell necrosis after cell division. In this sense, it has been shown that the release of silver ions into bacterial cells increase their bactericidal activity [16]. Coincidently, studies on staphylococci suggested that the primary mechanism of resistance is the expression of active efflux pumps, which are the first line of defense against antimicrobials [39]. Hence, AgNPs have an advantage because the development of microbial resistance against them is not probable, due to their mechanism of action. Such nanoparticles could be used to provide antimicrobial properties to materials for endodontics, allowing them to be used at different stages of treatment [40].

The data obtained by the chemical and biological approaches were statistically analyzed using the unpaired t test. A two-tailed p-value of 0.7865 was obtained. This suggested that the difference between the two standard errors was not considered significant.

### Cream formulation

Cream formulations of AgNPs synthesized from olive or eucalyptus leaf extracts could be useful in the treatment of skin infections. The AgNPs herbal cream (Figure 8) displayed acceptable physicochemical and safety profiles (pH 4.51-5.81), as well as significant in vitro antimicrobial activity (data not shown). Furthermore, the cream's antibacterial activity *in vitro* increased as the extract concentration content increased. The rabbit skin irritation test showed an index in the negligible category for primary irritation (0.1). The rheological investigation of the formulated cream (Figure 8) showed that due to shear-thinning, the curve illustrated is of a non-Newtonian fluid, resulting in a uniform application and good occlusion. Viscosity (1243.33 ± 33.52 cP) shows the adhesion ability of creams when applied to the skin and enables a longer administration of the product at the application site.. However, a lower viscosity is required when the topical cream is applied to the skin to promote penetration, hydration, and occlusion [36].

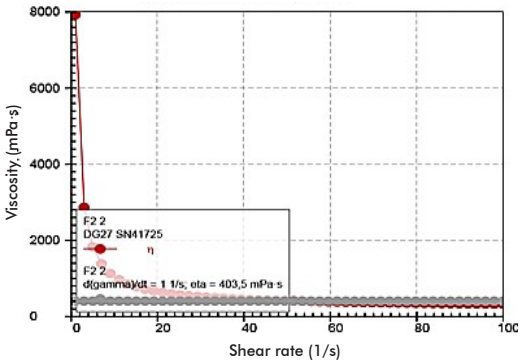


Figure 8. Silver nanoparticles cream and comparison of viscosity against the shear rate of the cream.

## Conclusions

Here we have shown that silver nanoparticules (AgNPs) can be synthetized successfully by using olive and eucalyptus leaf extracts. The AgNPs obtained were characterized by UV-vis spectrometry and FTIR, these last spectra showing that polyphenols and proteins act as reducing and stabilizing agents in AgNPs synthesis. The antibacterial potential of synthesized AgNPs was compared with that of aqueous extracts by well diffusion method, and AgNPs significantly inhibited bacterial growth against multidrug-resistant *S. aureus*, *P. aeruginosa*, *E. coli*, *Salmonella*, and *C. albicans*. This study revealed that the aqueous leaf extract has no effect at the concentrations used for preparation of the AgNPs nanoparticles. Thus AgNPs showed broad spectrum of antibacterial and antifungal activities at very low concentrations. Moreover, a cream formulation based on the synthesized AgNPs for skin infections was formulated as an alternative therapeutic approach, which reological properties must be improved for their successful therapeutic implementation.

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## Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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