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Biosynthesis of silver nanoparticles from phytochemicals and study of their physical and biological properties

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Abstract

Objective

This study investigates the physical and biological properties of silver Biosynthesized nanoparticles from phytochemicals. Phytochemicals in olive leaves serve as natural reducing agents and stabilizers in the green synthesis of AgNPs, which represents a green and ecofriendly approach compared to the conventional methods.

Materials and methods

Silver nanoparticles (AgNPs) were analyzed using a variety of analytical techniques. UV-Vis spectroscopy was performed in the 200-800 nm wavelength range to detect the characteristic plasmon resonance of the silver nanoparticles. Field-emission scanning electron microscopy (FE-SEM) was also used to determine the particle size and shape. X-ray diffraction (XRD) was employed for studying the structural and crystalline nature and obtaining information about their

phase composition and crystallinity. To ascertain the proper synthesis of AgNPs, Fourier-transform infrared (FTIR) spectroscopy was also performed within the spectral range of 4000-600 cm^{-1} to identify functional groups that belong to the nanoparticles and analyze their structural characteristics. 250 mL of distilled water was used to dissolve 6.25 g of nutrient agar to prepare the culture medium. To ensure complete dissolution, the solution was continuously stirred on a heating plate at a controlled temperature using a magnetic stirrer. The medium was then autoclaved for 15 minutes at 121 °C. After sterilization, the molten agar was cooled to a temperature between 45 and 50 °C before being aseptically transferred to sterile petri dishes. The media was then allowed to fully solidify by leaving the plates undisturbed at room temperature. Subsequently, 0.1 g of plant extract and 0.002 g of nanoparticles were dissolved twice in 1 mL of ethanol and 0.5 mL of distilled water, respectively.

Results

The results show that silver nanoparticles manufactured using olive leaves have great potential as natural antibacterial agents in pharmaceutical applications. This study demonstrated the novelty of the effectiveness of silver nanoparticles against the studied bacteria, as it was almost twice as effective as the plant extract. This contributes to other medical fields that are very important for the development of medicine at present.

Conclusions

The Nano properties demonstrated by various methods of examination lead to the possibility of obtaining silver nanoparticles with high properties and very small diameters from olive leaves that can be used in other medical fields that are very important for the development of medicine at the present time.

Keywords: AgNPs, antibacterial activity, FE-SEM, FTIR, phytochemicals

Paper Type: Research Paper.

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Introduction

Phytochemicals are low-molecular-weight (LMW) secondary plant metabolites that occur naturally; they are biologically active molecules and extremely significant in the normal cellular metabolic process and the encouragement of health as well as prevention of disease (Karunakaran et al., 2024 & Rodríguez-Negrete et al., 2024). Phytochemicals play a critical role to combat microorganisms and even outperform synthetic drugs at times. Most natural ingredients possess pronounced antimicrobial properties, including antibacterial properties, making them essential for both preventative and therapeutic use against infectious agents. (Badrzadeh et al., 2014 & Khameneh et al., 2021). Phytochemicals are characterized by their ability to target multiple pathways in microbes, making it more difficult for microorganisms to develop resistance compared to other antibiotics that target limited pathways. This multi-target activity renders them more effective and decreases the likelihood of new drug-resistant pathogens Bansal & Priyadarsini, 2021). Antibiotic treatment is seriously affected by the increasing number of diseases that resist a broad spectrum of drugs. Therefore, measures need to be taken in order to explore other sources of antimicrobial agents, such as plant-derived nanomaterial's (Zanjage & Khan, 2021; Karimi et al., 2023 & Sadou et al., 2024). Nanotechnology is the synthesis of materials at the nanoscale, usually between 1 and 100 nanometers, to create materials with properties far more advanced than those from which they were made. (Khan et al., 2022 & Asadi et al., 2023). This technology can have applications in medicine, agriculture, and the environment with solutions including drug delivery. The goal of nanotechnology is the development and application of methods for the fabrication of nano systems with the ability to interact at the molecular level with high specificity to produce maximum therapeutic effects with minimum side effects (Rehman et al., 2023). It is a critical element of environmental restorative technologies and waste bioactive product recyclers into more valuable and sustainable (Alqarni et al., 2022; Alotaibi et al., 2024; Al-Tarjuman et al., 2025). Nanoparticles such as gold (AuNPs), silver (AgNPs), iron (FeNPs), and palladium (PdNPs) have been promising to use in plant science (Mosqueda-Frómeta et al., 2025). These nanoparticles have the ability to enhance nutrient delivery and augment antimicrobial activity to microorganisms (Khan et al., 2022 & Hajishoreh et al., 2024). Silver nanoparticles (AgNPs) are one of the best superior and most essential nanomaterials with many compulsive nanomaterials that have multifunctional bio applications in a broad range (Hashmi et al., 2024). Phytochemicals are very important in nanoparticle synthesis using plants since extracts from the plants allow particle formation and stabilization in a green way (Sahu, 2023). The eternal olive tree (*Olea europaea*) is extremely valuable because it is one of the several medicinal plants that have been used for hundreds of years and are endowed with phenols possessing desirable bioactive properties (Kakakhel et al., 2021). Producing

nanoparticles from plant products is a method for producing molecules with enhanced biological activity and is considered environmentally friendly, as it reduces health and environmental risks. (Khan et al., 2022). Our study demonstrated the antimicrobial potential of nanomaterial's synthesized from olive tree leaves. By using silver nanoparticles derived from olive leaves (as green nanoparticles), we aim to determine whether this nano formulation can enhance antimicrobial effects. Employing nanotechnology to enhance the stability and efficacy of bioactive compounds is an important and novel approach. Given the known antimicrobial properties of both olive extracts and silver nanoparticles, this approach may lead to effective solutions in combating bacteria.

Materials and methods

Preparing the leaves powder: The leaves were thoroughly washed with tap water to remove dust, and then rinsed with distilled water to remove any remaining impurities. Afterward, the samples were dried using sterile paper towels to ensure the removal of surface moisture. Once fully dried, the samples were prepared for subsequent analysis. In the drying phase, the collected samples were placed into paper bags perforated with multiple holes to allow proper air circulation. The bags were then transferred to an oven and incubated at a temperature range of 38°C to 40°C for one week, until the olive leaves were completely dried. After they were crushed using a grinder to make them into a powder. Then, the powder was made finer using a mortar and pestle to prepare it for the extraction step. **Biosynthesis of *Olea europaea* leaf extract:** In the extract preparation step, 60 grams of the powdered olive leaves were weighed using a sensitive balance. The powder was then soaked in 600 mL of ethanol and stored in a refrigerator for 24 hours to allow the solution of active compounds. After soaking, the mixture was first filtered using gauze and then filtered again using filter paper to remove any remaining solid particles. The filtered solution was then placed in a rotary evaporator, to remove the ethanol, leaving behind the concentrated plant extract in powdered form. About 2 g of plant extract powder was dissolved in 200mL of distilled water which heated at 70 °C for 25 minutes with continuous straining. After that, we used Whatman filter paper and centrifuge with 6000 rpm for 10 minutes, to remove any insoluble materials, then must be prepared (AgNO₃) solution use 0.086 g of (silver nitrate) dissolved in 500mL of distilled water (Rozhin et al., 2021). Now have two solutions mixing slowly 130mL of plant extract solution to 325mL of AgNO₃ solution under constant stirring. Keep the reaction at 75 °C for 20-60 minutes and adjust the pH to 8-10 by using NaOH, observe a color change from (yellow to dark brown), that indicates nanoparticle formation (Karahana et al., 2023). After that we used UV-Vis Spectroscopy that Peak around 400-450 nm confirms AgNPs formation, and again

Centrifuged at 8000-10,000 rpm for 10 min to collect nanoparticles at the end dry at 40-60°C in the oven, after drying stored until we used (Al-Nuaimy et al., 2025).

Characterization of Silver NPs (Ag NPs): Silver nanoparticles (AgNPs) were analyzed using a variety of analytical techniques. UV-Vis spectroscopy was performed in the 200-800 nm wavelength range to detect the characteristic plasmon resonance of the silver nanoparticles. Field-emission scanning electron microscopy (FE-SEM) was also used to determine the particle size and shape. X-ray diffraction (XRD) was employed for studying the structural and crystalline nature and obtaining information about their phase composition and crystallinity. To ascertain the proper synthesis of AgNPs, Fourier-transform infrared (FTIR) spectroscopy was also performed within the spectral range of 4000-600 cm^{-1} to identify functional groups that belong to the nanoparticles and analyze their structural characteristics.

Antibacterial sensitivity test: 250 mL of distilled water was used to dissolve 6.25 g of nutrient agar to prepare the culture medium. To ensure complete dissolution, the solution was continuously stirred on a heating plate at a controlled temperature using a magnetic stirrer. The medium was then autoclaved for 15 minutes at 121 °C. After sterilization, the molten agar was cooled to a temperature between 45 and 50 °C before being aseptically transferred to sterile petri dishes. The media was then allowed to fully solidify by leaving the plates undisturbed at room temperature. Subsequently, 0.1 g of plant extract and 0.002 g of nanoparticles were dissolved twice in 1 mL of ethanol and 0.5 mL of distilled water, respectively. Two concentrations were prepared for each solution: 100% and 50% (diluted with the same solvent). Small circular discs were made from filter paper and soaked in the prepared solutions. The discs were then placed onto the surface of nutrient agar plates that had been inoculated with *Staphylococcus aureus* and *Escherichia coli*. This process was done separately for both concentrations to test the antibacterial effect of each solution. Petri dishes were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured in millimeters (Abdellatif et al., 2022).

Results and discussions

UV-visible spectral analysis: The UV-Vis absorption spectrum of the liquid extract, recorded over a wavelength range of 200 to 800 nm, shows the presence of phytochemicals through distinctive absorption properties. As shown in Figure 1, the multiple absorption peaks are due to the concentration of the pure extract. Additionally, the absence of a distinct surface plasmon resonance (SPR) peak in the 400-450 nm region suggests that silver nanoparticles were not produced, as seen in Figure 2. However, the peak near 400 nm confirms the formation of silver nanoparticles. These results were consistent with the past findings of Anju et al. (2021) who

also reported that a peak was also observed in the UV-VIS absorption spectra around 300-400 nm.

Fourier-transform infrared (FTIR): As seen in Figures 3 and 4, the FT-IR spectra of the leaf extract and the artificial AgNPs displayed several prominent IR bands, at 3348, 2117, 1635, and 667 cm^{-1} , the strongest bands were visible. The N-H stretching vibration of primary amines is represented by the band at 3348 cm^{-1} . The C-N stretching of R-N=C=S is represented by the milled band at 2117 cm^{-1} . The broad band seen at 667 cm^{-1} is caused by the bending vibrations of N-H groups in proteins, while the sharp absorbance band at 1635 cm^{-1} may be attributed to N-H stretching. Evidently, these FTIR absorbance bands are related to the plant bio-active compounds of protein, glucomannan, starch, saponin, colchicine, flavonoids, polysaccharides, phenyl propanoid and glycerides (Zhang et al., 2014).

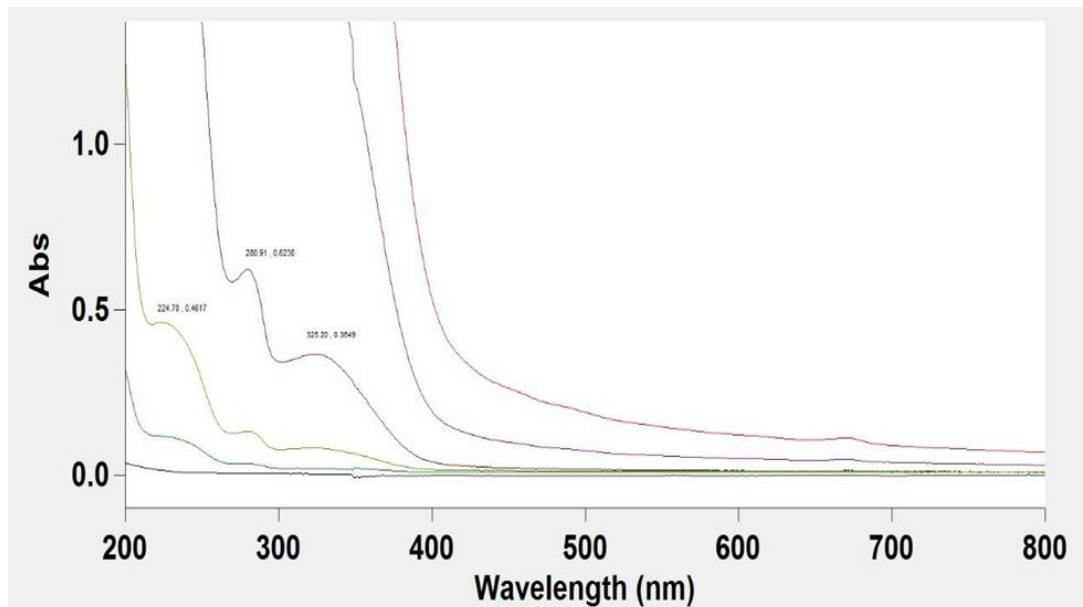


Figure 1. UV-Vis spectra of liquid extract recorded over a wavelength range of 200 to 800 nm

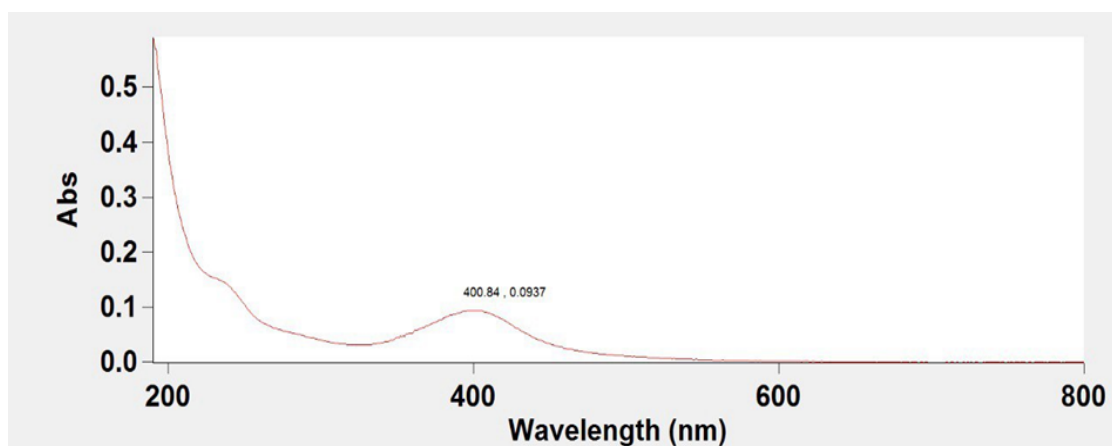


Figure 2. UV-Vis spectra of AgNPs , the peak near 400 nm confirms the formation of silver nanoparticles

Notably, in the synthesized Ag NPs spectrum, a new band was detected at 563 cm^{-1} , which corresponds to the formation metal (Ag) peak and N-H groups in proteins. This finding suggested a relative interaction between Ag NPs and the protein. However, in contrast to the spectrum of leaf extract, the spectrum of synthesized Ag NPs demonstrated a low transmittance rate, which was further supported by the formation of Ag NPs. This decrease in transmittance rate was related to a color change of synthesized Ag NPs solution (Al-Ansari et al., 2019). FTIR spectroscopy may be used to probe the chemical composition of the surface of nanoparticles. Vidhu et al., 2011 showed that (FTIR) technique was used to study the chemical composition of the surface of silver nanoparticles (AgNPs) prepared using aqueous seed extract of (*Macrotyloma uniflorum*) plant. Kong & Yu, 2007 found a strong and dense continuity of oxygen and carbon (C=O) in -COOH groups of the reducing agent at 1721 cm^{-1} . The C=O groups can act as capping bonds for nanoparticles, and (FTIR) spectra can confirm the presence of various C=O groups on the nanoparticle surface, preventing their aggregation and helping to stabilize them in the aqueous phase. Amine groups can bind proteins to silver nanoparticles, and they can also be anchored to the surface by associated proteins (Balaji et al., 2009 and Pasieczna-Patkowska et al., 2025).

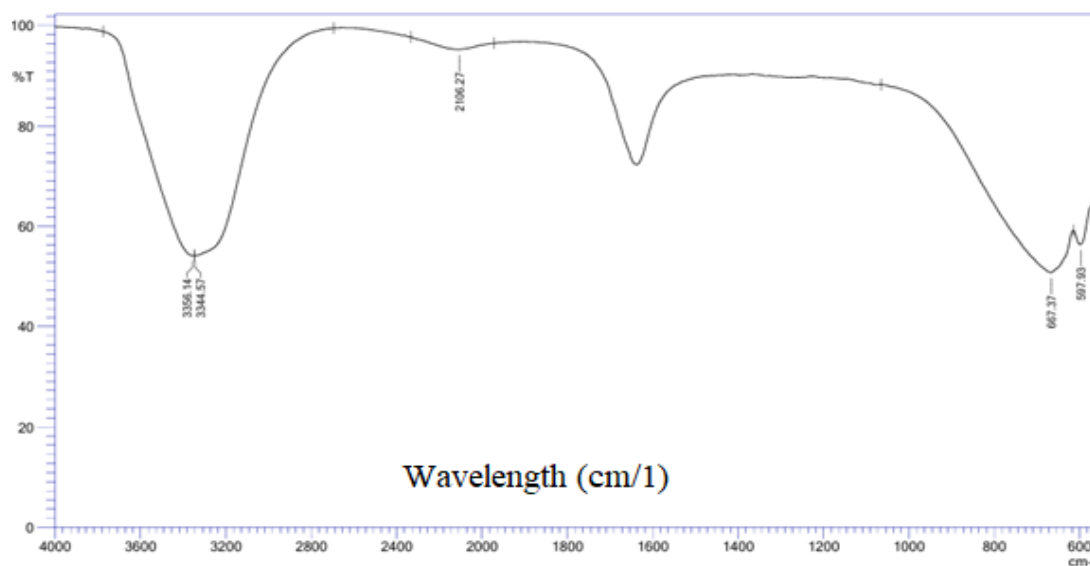


Figure 3. FTIR spectra of AgNPs, displayed several prominent IR bands, at 3348, 2117, 1635, and 667 cm^{-1} , the strongest bands were visible. The N-H stretching vibration of primary amines is represented by the band at 3348 cm^{-1}

Field emission scanning electron microscopy (FE-SEM): Particle diameters were measured to know the particle size. Geethalakshmi et al., 2012, were found 15-75 nm spherical silver nanoparticles are evidenced in the field emission scanning electron micrographs obtained from

Olea europaea leaves extract and Deshpande et al., 2011, also found 90% silver nanoparticles derived from *Olea europaea* extract range from 12 - 50 nm in FESEM analysis. In our present work we are reporting FESEM images of functionalized silver nanoparticles synthesized from *Olea europaea* leaves are seen with core shell morphology of size 10 - 50 nm and it can be noted that there is minor variation in the particle size, two molecules, (13.8 and 20.6) nm, were identified as in Figure 5. The variation in particle size could be because the nanoparticles are being synthesized at different times. Higher resolution image taken at 200 nm shows a group of particles dispersed in an organic moiety to create a stable suspension. The particles are observed to be polydisperse in nature and are roughly spherical in shape (Ibrahem, 2025).

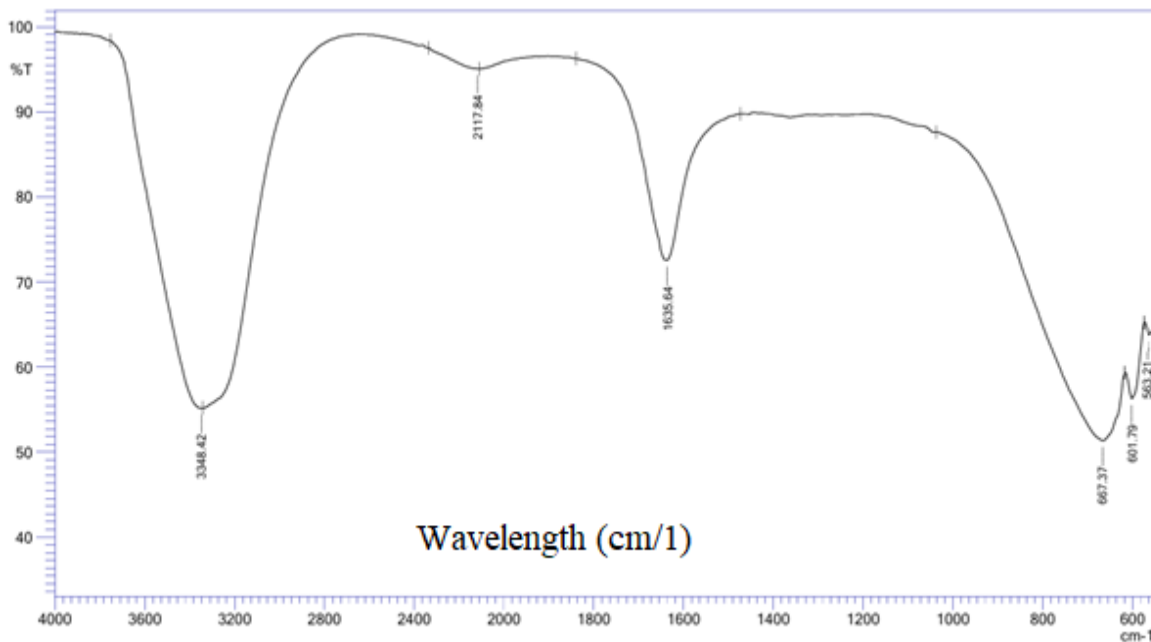


Figure 4. FTIR spectra of liquid extract, displayed several prominent IR bands, at 3348, 2117, 1635, and 667 cm⁻¹, the strongest bands were visible. The N-H stretching vibration of primary amines is represented by the band at 3348 cm⁻¹

X-ray diffraction (XRD): The principle of XRD for materials is based on Bragg's law, and it is expressed by

$$n\lambda = 2d_{(hkl)} \sin \theta$$

where λ is the wavelength of the X-ray, θ is the scattering angle, n is an integer representing the order of the diffraction peak, d is the interplane distance of the lattices, and (hkl) are Miller indices (Abbasi et al., 2016).

The X-ray diffraction (XRD) patterns of dried silver nanoparticles made with room-temperature olive leaf extract are displayed in Figure 6. X-ray diffraction patterns of the silver extract showed that the silver nanoparticles have a face-centered cubic (fcc) structure (Shameli et

al., 2010). Furthermore, the X-ray diffraction peaks at 2θ of (38.17, 44.31, 64.44, and 77.34) ° can be attributed to crystalline planes 111, 200, 220, 311, and 222, respectively.

A peak was also observed at 2θ equal 22 suggesting that the crystallization of bio-organic phase occurs on the surface of the silver nanoparticles (Sathishkumar et al., 2009). Therefore, it is evident from the XRD pattern that the AgNPs made with olive leaf broth were primarily crystalline.

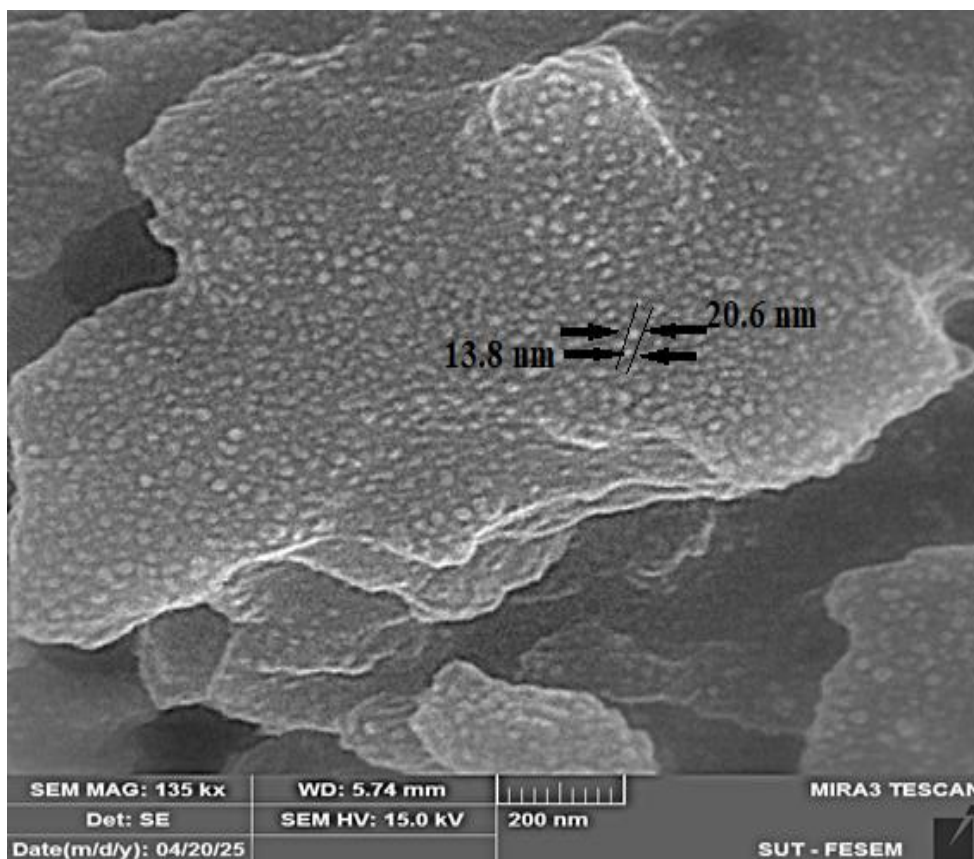


Figure 5. FESEM image of silver nanoparticles (AgNPs) synthesized by *Olea europaea* leaves with 0.01 M of silver nitration

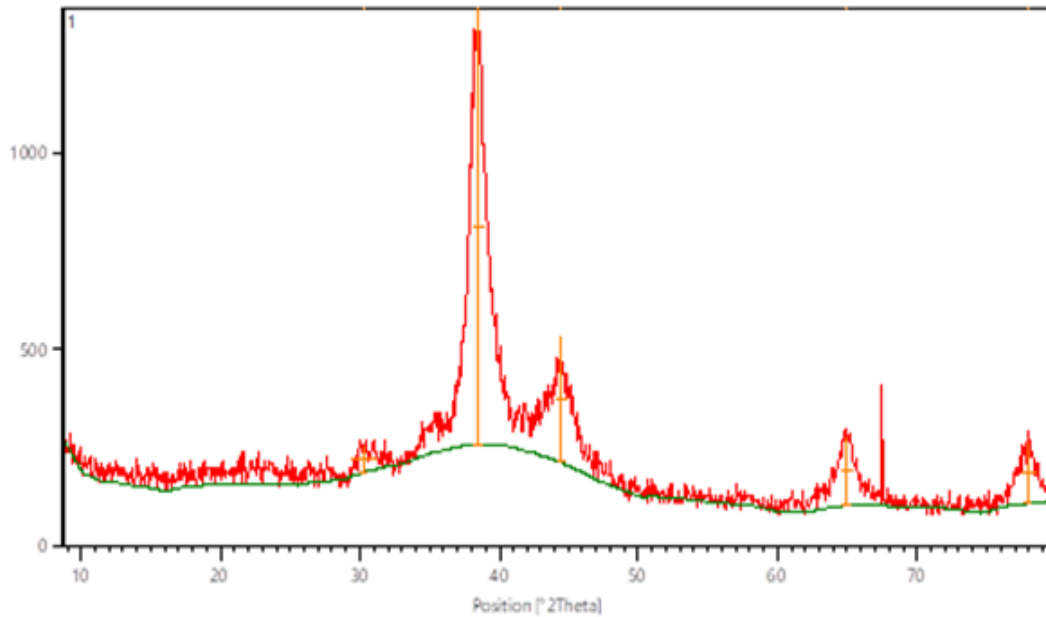


Figure 6. X-ray diffraction pattern of Ag nanoparticles prepared with ethanol olive leaf extract

Antimicrobial activity of olive extract (*Olea europaea*) and synthetic silver nanoparticles: Two strains of bacteria; *Staphylococcus aureus* (Gram-positive) and *Escherichia coli* (Gram-negative) were used to test the antibacterial properties of olive extract and synthetic silver nanoparticles. The results showed a difference in the size of the inhibition zones depending on the treatment concentration and type of bacteria. Table 1 illustrates the case of *Staphylococcus aureus*, where a 50% concentration of *O. europaea* extract produced a larger inhibition zone (5 mm) compared to a 100% concentration which was (2 mm). This unexpected decrease in inhibition at higher concentration may suggest that certain compounds in the extract become less effective due to saturation, compound aggregation, or reduced diffusion in the agar medium. On the other hand, the application of AgNPs resulted in increased inhibition with higher concentration (1 mm) at 50% and 6 mm at 100%, indicating that the antibacterial effect of AgNPs against *S. aureus* improves with increased concentration. For *Escherichia coli*, both *O. europaea* extract and AgNPs showed increased inhibition with higher concentrations. The extract produced inhibition zones of (2 mm) at 50% and (6 mm) at 100%, while AgNPs produced (4 mm) and (11 mm) inhibition zones at 50% and 100%, respectively. These results demonstrate that both agents are more effective at higher concentrations in inhibiting *E. coli* growth, with AgNPs showing a notably stronger effect. The silver nanoparticles can damage the membrane of cell and can attack the respiratory chain through direct contact (Franci et al., 2015; Sheng and Liu, 2017; Swolana and Wojtyczka, 2022; Omar, 2024). Compared to silver ions, silver nanoparticles (AgNPs) possess significantly higher antibacterial properties. Although Gram-negative bacteria and drug-resistant strains are highly resistant, the antibacterial effect of colloidal nanosilver against these

bacteria has been confirmed (Ji et al., 2020). This broad spectrum of activity against morphologically and metabolically diverse microorganisms and potent antibacterial activity appear to be associated with a multidirectional range of antimicrobial activity (Franci et al., 2015). The bactericidal activity, which is detrimental to bacterial cell survival, results from the release of silver ions from nanoparticles (Leng et al., 2020).

Table 1. Inhibition zone of *O. europaea* extract and AgNPs Against *S. aureus* and *E. coli*.

Bacteria species	Treatment type	50 % Conc. (mm)	10 % Conc. (mm)
<i>Staphylococcus aureus</i>	<i>Olea europaea</i> extract	5 mm	2 mm
<i>Staphylococcus aureus</i>	Silver nanoparticles (AgNPs)	1 mm	6 mm
<i>Escherichia coli</i>	<i>Olea europaea</i> extract	2 mm	6 mm
<i>Escherichia coli</i>	Silver nanoparticles (AgNPs)	4 mm	11 mm

Conclusion: Relying on green sources to manufacture nanoparticles is not difficult if financial and incentive capabilities are available. This study demonstrated that nanoparticles manufactured from olive leaf sources are effective against pathogenic bacteria and have an inhibitory power that is twice that of the plant extract alone. The Nano properties demonstrated by various methods of examination lead to the possibility of obtaining silver nanoparticles with high properties and very small diameters from olive leaves that can be used in other medical fields that are very important for the development of medicine at the present time.

Importance of this study to the Sustainable Development Goals (SDGs): This study aligns with the United Nations Sustainable Development Goals by addressing key scientific challenges. Specifically, it contributes to achieving Sustainable Development Goal 3 (Good Health and Wellbeing).

Authors' Contributions

Yaseen Noori Mahmood ALShekhany: The conception and research design, methodology, research administration and supervision, the drafting of the paper; revising it critically for intellectual content. Dawod Noori M. Shekhani: Methodology, formal analysis and the final approval of the version to be published. Sundus Jassim Muhammad Aljory: Methodology, investigation and writing-original draft. Shalaw Kamal Salih: Methodology, analysis and interpretation of the data and field follow-up.

Data Availability Statement

The data and samples for this study are available at the Scientific and Health Research Center at Koya University and are available from the authors upon reasonable request.

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Ethical Considerations

Ethical approval was not required for this study as it focused exclusively on plant material. All experimental procedures were conducted in accordance with the biosafety and health regulations of the Scientific and Health Research Center at Koya University, and the experiments were carried out in approved containment facilities to ensure that the bacteria used in this study did not release into the environment.

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No funding was received for this study.

Conflict of Interest

The authors declare that they have no known conflicting financial interests or personal relationships that could affect the work described in this paper.

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زیست سنتز نانوذرات نقره از ترکیبات فیتوشیمیایی و بررسی ویژگی‌های فیزیکی و زیستی


آن‌ها

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چکیده

هدف: این مطالعه به بررسی ویژگی‌های فیزیکی و زیستی نانوذرات نقره زیست‌سنتز شده از ترکیبات فیتوشیمیایی می‌پردازد. ترکیبات فیتوشیمیایی موجود در برگ زیتون به‌عنوان عوامل کاهنده و پایدارکننده طبیعی در سنتز سبز نانوذرات نقره (AgNPs) عمل می‌کنند که در مقایسه با روش‌های متداول، رویکردی سبز و سازگار با محیط زیست محسوب می‌شود. **مواد و روش‌ها:** نانوذرات نقره (AgNPs) با استفاده از مجموعه‌ای از روش‌های تحلیلی بررسی شدند. طیف‌سنجی UV-Vis در بازه طول موج ۲۰۰ تا ۸۰۰ نانومتر برای شناسایی رزونانس پلاسمونی مشخصه نانوذرات نقره انجام شد. همچنین از میکروسکوپ الکترونی روبشی با گسیل میدانی (FE-SEM) برای تعیین اندازه و شکل ذرات استفاده گردید. پراش پرتو ایکس (XRD) به‌منظور

بررسی ساختار و ماهیت بلوری و به‌دست‌آوردن اطلاعاتی درباره فاز و میزان بلورینگی نانوذرات به کار رفت. برای اطمینان از سنتز صحیح AgNPs، طیف‌سنجی مادون قرمز تبدیل فوریه (FTIR) در محدوده طیفی ۴۰۰۰ تا ۶۰۰ در سانتی‌متر انجام شد تا گروه‌های عاملی مرتبط با نانوذرات شناسایی و ویژگی‌های ساختاری آن‌ها تحلیل شود. برای تهیه محیط کشت، ۶/۲۵ گرم نوترینت آگار در ۲۵۰ میلی‌لیتر آب مقطر حل شد. به‌منظور حل کامل، محلول با همزن مغناطیسی روی صفحه گرم‌کن در دمای کنترل‌شده به‌طور مداوم هم زده شد. سپس محیط کشت به مدت ۱۵ دقیقه در دمای ۱۲۱ درجه سانتی‌گراد اتوکلاو گردید. پس از استریلیزاسیون، آگار مذاب تا دمای ۴۵ تا ۵۰ درجه سانتی‌گراد سرد و سپس به‌صورت آسپتیک به پتری‌دیش‌های استریل منتقل شد. محیط کشت در دمای اتاق و بدون جابه‌جایی قرار داده شد تا کاملاً جامد گردد. در ادامه، ۰/۱ گرم عصاره گیاهی و ۰/۰۰۲ گرم نانوذره به‌ترتیب در ۱ میلی‌لیتر اتانول و ۰/۵ میلی‌لیتر آب مقطر، هر کدام دو بار حل شدند.

نتایج: نتایج نشان داد که نانوذرات نقره تولیدشده با استفاده از برگ زیتون، پتانسیل بالایی به‌عنوان عوامل ضدباکتری طبیعی در کاربردهای دارویی دارند. این مطالعه نوآوری اثربخشی نانوذرات نقره را در برابر باکتری‌های مورد بررسی نشان داد، به‌طوری‌که اثر ضدباکتریایی آن‌ها تقریباً دو برابر عصاره گیاهی بود. این یافته‌ها می‌تواند به سایر حوزه‌های پزشکی که در توسعه کنونی علم پزشکی اهمیت بالایی دارند، کمک کند.

نتیجه‌گیری: ویژگی‌های نانویی نشان‌داده‌شده توسط روش‌های مختلف بررسی، بیانگر امکان دستیابی به نانوذرات نقره با خواص مطلوب و قطر بسیار کوچک از برگ زیتون است؛ نانوذراتی که می‌توانند در سایر زمینه‌های پزشکی مهم برای پیشرفت علم پزشکی در زمان حاضر مورد استفاده قرار گیرند.

کلمات کلیدی: ترکیبات فیتوشیمیایی، فعالیت ضدباکتریایی، نانوذرات نقره (AgNPs)، FTIR، FE-SEM

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