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Biosynthesis of Silver Nanoparticles and Their Antibacterial Activity Against Urinary Tract Infection Bacteria

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Abstract

The rising use of antibiotics, particularly in developing nations, has made bacteria more skilled at creating resistance mechanisms, which has led to high infection and mortality rates. The World Health Organization states that one of the biggest risks to human health is antibiotic resistance, which ranks third in terms of causes of mortality after cardiovascular disease. The investigation and application of plant chemicals as natural antibiotics have continued in recent decades. Green synthesis of silver nanoparticles was carried out using volatile oils extracted from Citrus aurantium peels as a reducing agent. The particles were characterized by UV-Vis spectroscopy, FTIR, AFM, and colorimetric analysis. Results revealed that the size of the synthesized silver nanoparticles was 74.8 nm. particles irregular shapes and exhibited antimicrobial activity against antibiotic-resistant pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterococcus faecalis,* causing urinary tract infections (UTIs). The agar well diffusion method evaluated the effect of the synthesized silver nanoparticles at three concentrations of 25%, 50%, and 75%, respectively, against pathogenic bacteria. The results showed significant variations between the concentrations and bacteria. The 75% concentration showed the highest effect on all bacteria compared to other concentrations, and *S. aureus* was the most sensitive, with an inhibition zone of 15.583 mm, while *E. faecalis* was less affected, with an inhibition zone of 10.167 mm, and there was no significant difference with *E. coli*, with an inhibition diameter of 10.583 mm. The results obtained indicated that the manufactured silver nanoparticles will pave the way for successful and environmentally friendly treatment methods against pathogens that cause urinary tract infections. In addition, industrial waste can be used to make important treatments.

Keywords: Nanoparticles, Terpene, UTIs.

التخليق الحيوي لجسيمات النانو الفضية ونشاطها المضاد للبكتيريا ضد بكتيريا التهابات المسالك البولية

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الخلاصة

أدى الاستخدام المتزايد للمضادات الحيوية، وخاصة في الدول النامية، إلى جعل البكتيريا أكثر مهارة في خلق آليات المقاومة، مما أدى إلى ارتفاع معدلات العدوى والوفيات. تنص منظمة الصحة العالمية على أن أحد أكبر المخاطر التي تهدد صحة الإنسان هو مقاومة المضادات الحيوية، والتي تحتل المرتبة الثالثة من

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حيث أسباب الوفيات بعد أمراض القلب والأوعية الدموية. استمر التحقيق في المواد الكيميائية النباتية وتطبيقها كمضادات حيوية طبيعية في العقود الأخيرة. تم إجراء التخليق الأخضر لجسيمات النانو الفضية باستخدام الزيوت المتطايرة المستخرجة من قشور الحمضيات كعامل اختزال. تم تحديد خصائص الجسيمات من خلال التحليل الطيفي للأشعة فوق البنفسجية والمرئية، وFTIR، وAFM والتحليل اللوني. كشفت النتائج أن حجم الجسيمات النانوية الفضية المصنعة كان 74.8 نانومتر. الجسيمات ذات أشكال غير منتظمة وأظهرت نشاطًا مضادًا للميكروبات ضد مسببات الأمراض المقاومة للمضادات الحيوية مثل *Escherichia coli* و *Pseudomonas aeruginosa* و *Staphylococcus. aureus* و *Enterococcus faecalis* التي تسبب عدوى المسالك البولية (UTI). تم تقييم تأثير الجسيمات النانوية الفضية المصنعة بثلاثة تركيزات 25% و50% و75% على التوالي ضد البكتيريا المسببة للأمراض باستخدام طريقة انتشار الآجار. وأظهرت النتائج وجود فروق معنوية بين التراكيز والبكتيريا. حيث أظهر تركيز 75% أعلى تأثير على جميع البكتيريا مقارنة بالتركيزات الأخرى، وكانت المكورات العنقودية الذهبية الأكثر حساسية، حيث بلغ قطر تثبيطها 15.583 ملم، في حين كانت المكورات المعوية البرازية أقل تأثراً، حيث بلغ قطر تثبيطها 10.167 ملم، ولم يكن هناك فرق معنوي مع الإشريكية القولونية، حيث بلغ قطر تثبيطها 10.583 ملم. وأشارت النتائج المتحصل عليها إلى أن الجسيمات النانوية الفضية المصنعة سوف تمهد الطريق لطرق علاج ناجحة وصديقة للبيئة ضد مسببات الأمراض التي تسبب التهابات المسالك البولية. بالإضافة إلى ذلك، يمكن استخدام النفايات الصناعية في صنع علاجات مهمة..

Introduction

Among the most dangerous types of infections that threaten humanity as a result of bacterial resistance to antibiotics are urinary tract infections [1]. It affects people of all ages, and both males and females [2]. The development of this resistance in bacteria and other pathogens, such as *Candida albicans* is a result of the widespread and indiscriminate use of antibiotics [3]. Therefore, this study aimed to isolate resistant bacteria from patients with urinary tract infections and test alternative environmentally friendly treatments such as green synthesis of silver nanoparticles to overcome the antibiotic resistance problem [4]. Nanoparticles are particles with dimensions ranging from 1 to 100 nanometers and have unique chemical, physical, and biological properties that make them useful in many applications [5]. These particles are characterized by a large surface area-to-volume ratio, which makes them capable of interacting with biological systems and important in various medical and therapeutic applications, as they can be used to deliver drugs and target tissues and cells with high-efficiency nanoparticles have also become a crucial tool in the field of drug delivery, providing high efficacy in treatment, which has prompted recent research to focus on exploiting their unique properties to deliver drugs to specific sites in the body of the living organism [6]. The successful development of nanotechnology in the past few years has also provided new ideas and potential methods for diagnosing and treating many different diseases [7]. Nanotechnology researchers have expressed great interest in environmentally acceptable alternative techniques for manufacturing metal nanoparticles using natural plants. Green synthesis of metal nanoparticles aims to use non-toxic phytochemicals of plant components, such as terpenes, phenolics, and alkaloids, as a source of stabilizing and reducing agents [8]. These bioactive compounds present in plant parts can actually accelerate the conversion of metal ions into active nanoparticles in a standard and bio-friendly pathway [9]. Silver nanoparticles are known for their bioactivity against antibiotic-resistant bacteria. Due to its many different properties, it has found wide applications in the medical, biological, and environmental fields [10]. Therefore, this study aimed to isolate resistant bacteria from patients with urinary tract infections and test alternative environmentally friendly treatments, such as green synthesis of silver nanoparticles, because they are highly effective and have low toxicity to overcome the antibiotic resistance problem.

Materials and Methods

Isolation and identification of bacteria

Urine samples were collected from patients with suspected urinary tract infections at Imam Ali General Hospital in Sadr City/Baghdad during the period from January to July 2014. The total number of samples was 370 samples. They were collected in sterile, tightly sealed containers with a capacity of 100 ml and cultured directly to MacConkey agar, blood agar, and mannitol salt agar, which are selective and differential media for isolating, purifying, and identifying bacterial species and determining the ability of each isolate to ferment lactose and mannitol. Then, they were re-cultured on a blood agar medium, and the isolates were examined in terms of shape, color, pigments, and hemolytic activity. All plates were incubated at 37 °C for 24 hours. A pure colony was transferred to a nutrient agar medium for preservation, and ViteK tests were performed to confirm the identity of the isolates [11].

Collection of Plant Material

Fruits of *C. aurantium* were collected from the fields of Baghdad, then cleaned well with tap water and left to dry. The plant was identified in the herbarium of the Biology Department in the College of Science at the University of Baghdad, where the peels are removed and dried in an electric oven at a temperature of 40ºC, then ground using an electric mill and stored in the form of powder in a tightly sealed glass container until use [12].

Extraction of essential oils

One hundred grams of *C. aurantium* peel powder was soaked in 2-liter bottles with 1500 ml of water and hydro-distilled for 8 hours in a Clevenger apparatus. The yield of essential oils was measured and collected in a sterile bottle until use [13].

Compositional analysis of essential oils using GC-MS

The active compounds of the essential oil extracted from the peels were tested at the Samarra University, College of Applied Sciences, Central Laboratory, using a GC-MAS device at a temperature of 60°C [14].

Biosynthesis of silver nanoparticles

First, silver nitrate (1 x 10-3) molarity, $AgNO_3$ stock solution was prepared in sterile deionized. The green synthesis of silver nanoparticles (AgNPs) was carried out by adding 1 ml of essential oil to 1 ml of silver nitrate solution with continuous stirring and leaving it in the light for 7 days until the color changed to brown, indicating nanoparticle formation [15].

Characterization of silver nanoparticles

Absorption spectra of the formed AgNPs were measured by a UV-Vis spectrophotometer (Shimadzu, model UV-1800 spectrophotometer) with distilled water as a blank sample. After diluting a small portion (0.5 ml) of the sample with 5 ml of deionized water, the absorbance of the solution was measured. FTIR examination was carried out in the Central Chemistry Laboratory, University of Baghdad, College of Science, using a device (Shimadzu UV-1700, Japan). Spectroscopy (FTIR) is responsible for the detection of the functional groups contributing to the reduction and stabilization of AgNPs, where a drop of oil was placed on a cell of potassium bromide salt and placed inside the device, and the data was read. Particle size, shape, and surface morphology of silver nanoparticles were analyzed using atomic force microscopy (AFM) [16].

Evaluation of the effectiveness of silver nanoparticles against bacteria
Well Diffusion Method

The antibacterial activity of the synthesized AgNPs was tested using the agar well diffusion method against *P. aeruginosa*, *E. coli, E. faecalis*, and *S. aureus*. Pure bacteria were cultured on Mueller Hinton agar using sterile cotton swabs. Then, 5 mm diameter wells were made on Mueller Hinton agar using a cork borrer. Each well was filled with a specific concentration of AgNPs per well (25%, 50%, and 75%), and after 24 h of incubation, the inhibition zone diameter was measured. Three replicates were performed for each experiment [17].

Results and discussion

Yield of essential oil

The yield of essential oil after condensation and collection in a Clevenger tube was 2 ml for dry weight and 1 ml for fresh weight of peels. The results of this study were similar to the report presented Dao *et al.,* [18], in which they used three sizes of original raw materials, cut and ground fibers. The percentage of essential oils ranged between 1.6%, 1.8%, and 2.4%, respectively. The researcher explained that the difference in essential oil yield is due to the nature of the fruits, extraction process, and processing conditions. He pointed out that fine grinding of the raw materials facilitates their extraction during the distillation process, which increases the efficiency of the extraction process.

Identification of active compounds in the essential oil of C. aurantium peels

The chemical components of bitter orange peel oil and their percentages based on GC-MS are shown in Table 1. The results revealed that D-limonene accounted for 86.65% of the total volatile components, which is the largest percentage among all 23 components.

Table 1: Major compounds in *C. aurantium* peels essential oil analyzed by gas chromatography-mass spectrometry.

No	Compound name	Ret time	Area%	formula
1	(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-en	3.567	1.73	C10H16
2	beta.-Phellandrene	4.054	0.49	C10H16
3	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-meth	4.126	0.31	C10H16
4	beta.-Myrcene	4.292	6.46	C10H16
5	D-Limonene	5.106	86.65	C10H16
6	beta.-Ocimene	5.214	0.70	C10H16
7	alpha.-Methyl-.alpha.-[4-methyl-3-penteny	5.542	0.40	C10H18O2
8	alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl	5.784	0.18	C10H18O
9	1,6-Octadien-3-ol, 3,7-dimethyl-	6.000	0.99	C10H18O
10	Neryl nitrile	6.396	0.06	C10H15N
11	endo-Borneol	7.184	0.06	C10H18O
12	L-.alpha.-Terpineol	7.638	0.40	C10H18O
13	Decanal	7.894	0.17	C10H20O
14	Acetic acid, octyl ester	8.081	0.07	C10H20O2
15	D-Carvone	8.459	0.11	C10H14O
16	Geraniol	8.861	0.09	C10H18O
17	Linalyl acetate	8.990	0.40	C12H20O2
18	Geranyl acetate	11.323	0.20	C12H20O2
19	Caryophyllene	12.358	0.27	C15H24
20	2-Dodecenal	12.868	0.06	C12H22O
21	.beta.-copaene	13.435	0.13	C15H24
22	Nerolidyl acetate	14.763	0.05	C17H28O2
23	Cycloheptane, 4-methylene-1-methyl-2-(2-m	16.313	0.04	C17H28O2

Peak area showed the relative amount of each component, as shown in Figure 1, and the highest percentage was D-limonene, which accounted for 86.65% of the total components, while β-myrcene ranked second with 6.46% and then (1R)-2,6,6-Trimethylbicyclo [3.1.1] hept-2-en with 1.73%.

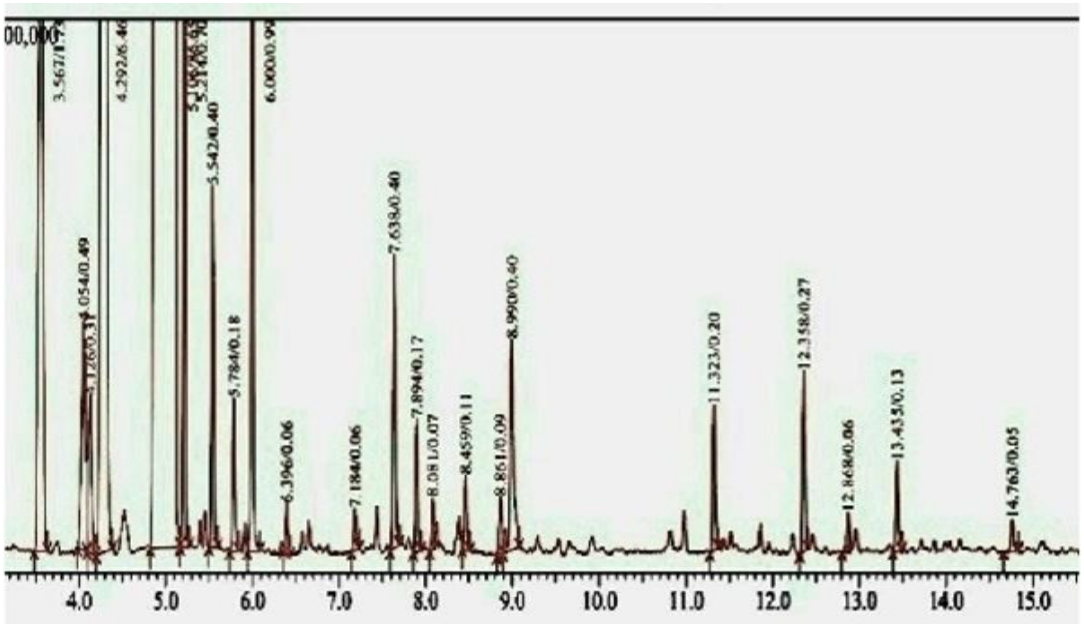

Figure 1: Typical GCMS chromatogram of *C. aurantium* essential oil.

The results of the present study are consistent with those of Maksoud *et al.,* [19], who indicated that oxidized monoterpenes and monoterpene hydrocarbons were the major chemical groups of bitter orange peel oils. They indicated that limonene was the major component of bitter orange peel oils, where they found limonene (94.67%) and myrcene (3%). Hung *et al.,* also indicated that the chemical composition of essential oils is affected by a variety of factors, including genetic differences, culture environment, and extraction techniques [20]. Although the current results are consistent with those of many previous studies, the variation may be due to a variety of factors, including the species analyzed, location, season, environmental factors such as soil type and climate, genetic traits, processing and extraction methods, and the part of the plant used to extract the oil [21].

Biosynthesis of silver nanoparticles

Color change from transparent or light yellow to dark brown upon mixing terpene oil and silver nitrate, as shown in Figure 2, which is an initial indicator of the formation of silver nanoparticles.

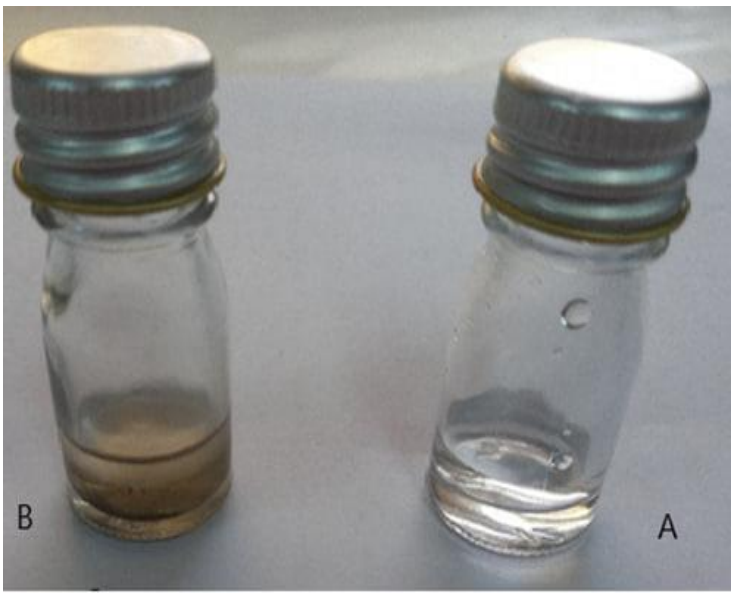

Figure 2: Synthesis of AgNPs according to color change. A: Color of the reaction mixture before the reaction. B: Color of the reaction mixture after the reaction.

Characterization of AgNPs

Results in this study were similar to those of Agustina *et al.,* [22]. The wavelength range of the nanoparticles was the highest peak at 350-500 nm. The researcher indicated that the color change was the first evidence of the formation of AgNPs, which are characterized by their light brown color, and the higher the concentration of particles, the darker the color becomes. In addition, they are characterized by a phenomenon called surface plasmon resonance. This property appears on the surfaces of some metals and is a result of the collective movement of free electrons present in the nanoparticle when light falls on them. This means that each nano-sized metal absorbs electromagnetic radiation at a specific wavelength. The properties of this absorption vary according to the size, shape, and structure of the particles, which can be determined from the ultraviolet and visible spectra. For AgNPs, the optimal absorption peak is in the wavelength around 450 nm. Figure 3 shows the ultraviolet and visible absorption spectra of silver nanoparticles made from terpene extract as a reducing agent, with the highest peak at 396 nm.

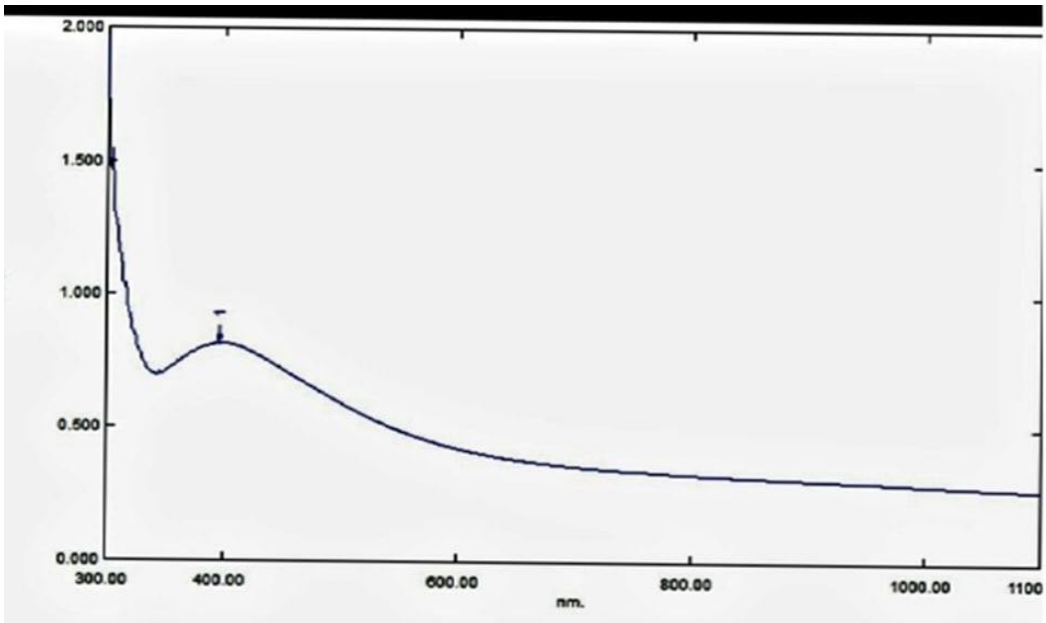

Figure 3: UV Vis absorbance of AgNPs

Results of FTIR

Results in Figure 4 showed the distribution of functional groups of the volatile oils extracted from bitter orange peels and the ranges in which each functional group falls before its participation in the green synthesis of silver nanoparticles, while Figure 5 shows a schematic diagram of the hypothetical process of AgNPs formation. It is known that plant organic components interact with metal salts ($AgNO_3$) via these functional groups, thus mediating the formation of AgNPs [23].

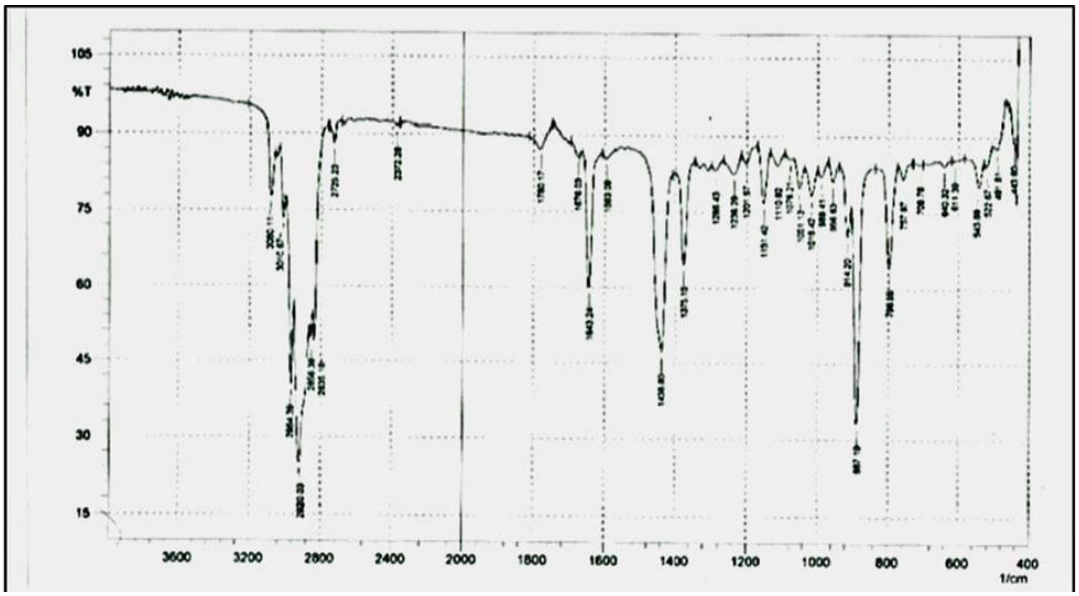

Figure 4: FTIR examination of *C. aurantium* essential oil compounds before silver nanoparticles formation

Figure 5 indicates that terpenoid extract has a dominant role in reducing silver. FTIR analysis was performed in diffuse reflectance mode operating at a resolution of 4 cm-1 in the 400 to 4000 cm-1 range to evaluate the functional groups that may be involved in forming the nanoparticles. This revealed different bands representing broad bands from 3300 to 3500 cm-1, indicating the interaction of the nanoparticles with the N-H group. An intermediate band at 2922 cm-1 also appears, indicating the stretching of C-H molecules in different ranges for many compounds. This indicates the interaction of AgNPs with the (N-H) group, which was confirmed Aref *et al.,* [24]. An intermediate band at 2922 cm-1 also appeared, indicating the stretching vibrations of the C-H group. This means that all the molecules of the terpene extract from bitter orange peels precisely participated in the reduction and fixation of silver nitrate, resulting in the crystallization of the phytochemical layer on its surface [25].

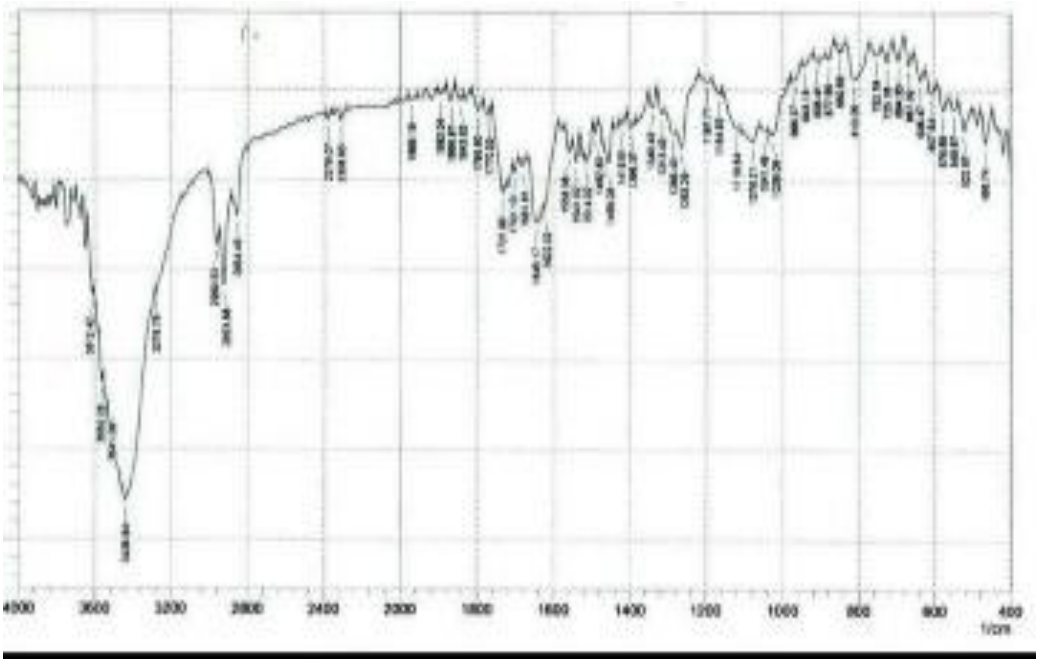

Figure 5: FTIR examination of *c. aurantium* essential oil compounds after formation of AgNPs.

Characterization of AgNPs using atomic force microscope (AFM)

Figure 6 shows the surface topography of the synthesized AgNPs, and Atomic Force Microscopy (AFM) analysis shows 2D and 3D images of AgNPs, spherical, single, or aggregated. AFM analysis also showed that the average size of the AgNPs was 74.8 9 nm (Figure 7). This result is consistent with Alaa *et al.,*[26], who showed that the biosynthesized silver nanoparticles were almost spherical, single (69 nm).

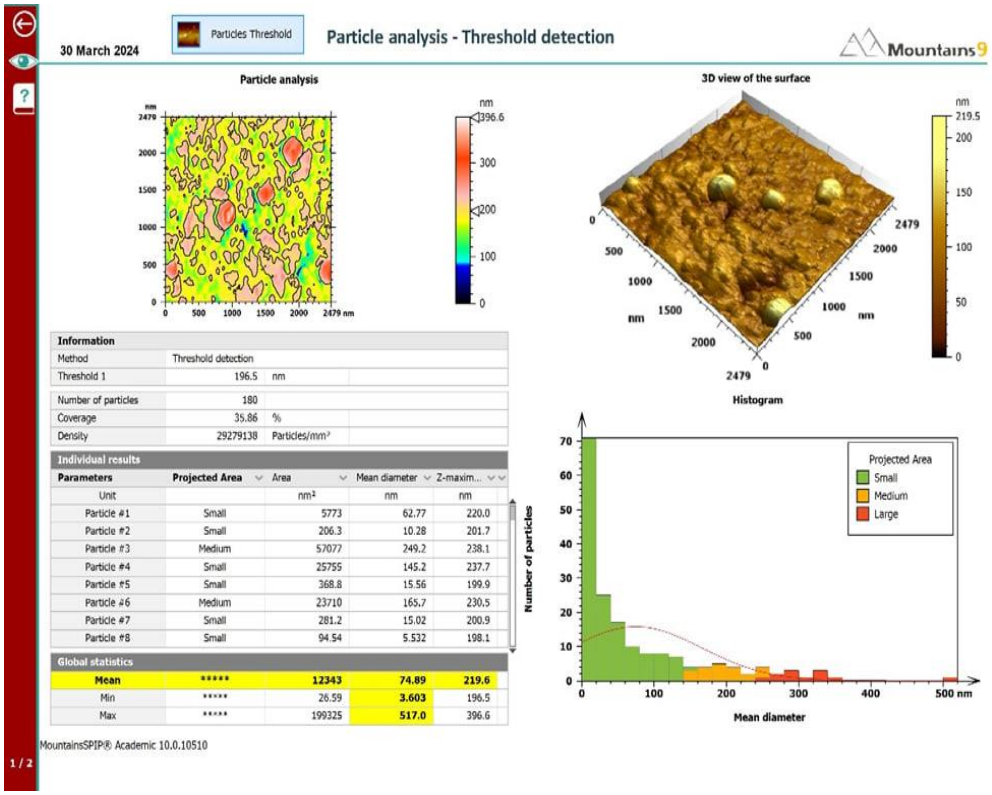

Figure 6: Average particle size of silver nanoparticles

Isolation of bacteria and sensitivity testing

Total number of samples, which amounted to 370 samples taken from patients with urinary tract infections, the number of isolates causing infection was 162, including 86 isolates of Gram-negative, *P. aeruginosa, E. coli*, and 63 isolates of Gram-positive bacteria, *E, faecalis*, and *S. aureus*, and the remaining 13 isolates included *C. albicans*. Mueller-Hinton agar was subjected to antimicrobial susceptibility testing by disk diffusion. This technique was used to evaluate the susceptibility of urinary tract infection agents according to the 2024 Clinical and Laboratory Standards Institute (CLSI) recommendations. All isolates were resistant to the antibiotics used in the study.

Table 2. Results of antibiotic susceptibility testing

Antibiotic	The symbol	Concentration in µg	*E. coli*	*P. aeruginosa*	S. aureus	*E. faecalis*
Ampicillin	AM	10	R	R	R	R
Augmentin	AUG	10	R	R	R	R
Cefotaxime	CAZ	30	R	R	R	R
Ceftriaxone	CTR	30	R	R	R	R
Ciprofloxacin	CIP	5	R	R	R	R
Nitrofurendtin	FT	30	R	R	R	R
Amikacin	AK	30	R	R	R	R
Ampicillin	AM	10	R	R	R	R

R: resistance

Evaluation of the inhibitory effect of silver nanoparticles against bacteria

Table 3 shows the effectiveness of nanoparticles against antibiotic-resistant bacteria taken from patients suffering from urinary tract infections. The study showed the presence of inhibition zones for AgNPs at all concentrations used against bacteria and that the inhibition zone increases with increasing concentration, meaning that the effectiveness of AgNPs against bacteria also increases.

Table 3: Antibacterial activity of silver nanoparticles (inhibition zone in mm)

Name of bacteria	Inhibition zone				Mean
	0.25Cons.	0.5Cons.	0.75Cons.	control	
E. coli	**11.667**	**14.333**	**16.333**	**0.000**	**10.583**
P. aeruginosa	**15.000**	**16.667**	**19.333**	**0.000**	**12.750**
S. aureus	**18.333**	**20.667**	**23.333**	**0.000**	**15.583**
E. faecalis	**12.000**	**13.333**	**15.333**	**0.000**	**10.167**
Lsd5%	**1.046****				**0.523****
Mean	**14.250**	**16.250**	**18.583**	**0.000**	
Lsd5%	**0.523****				

Cons: concentration

The present study showed that AgNPs were able to inhibit the growth of bacteria at all concentrations used. Results in Table 3 indicated that there were no significant differences between *E. coli* and *E. faecalis*. The least sensitive was if the inhibition diameter reached (10.583 mm) and (10.167 mm) respectively, at all concentrations, while the inhibition diameter for *P. aeruginosa* (15.000 mm) reached (16.667 mm) and (19.333 mm) at a concentration of 25%, 50%, and 75% respectively. This is consistent with a study by Rashad *et al*., [27] on the effect of AgNPs against *P. aeruginosa* if the inhibition zones ranged from

16.33 mm to 19.67 mm, while *S. aureus* was more sensitive compared to other types of bacteria, achieving the highest inhibition rate (23.333 mm) at a concentration of (75%). The nanoparticles synthesized from citrus fruits significantly affect the pathogens of UTI. Since AgNps have multiple mechanisms, they increase cell permeability, damage the cell wall and membrane of bacteria, penetrate cells, affect metabolic pathways, and damage DNA and protein [28]. The inhibition zone of the nano-extract against bacteria is clearly shown in Figure 7, which shows the sensitivity of bacteria to the extract concentrations.

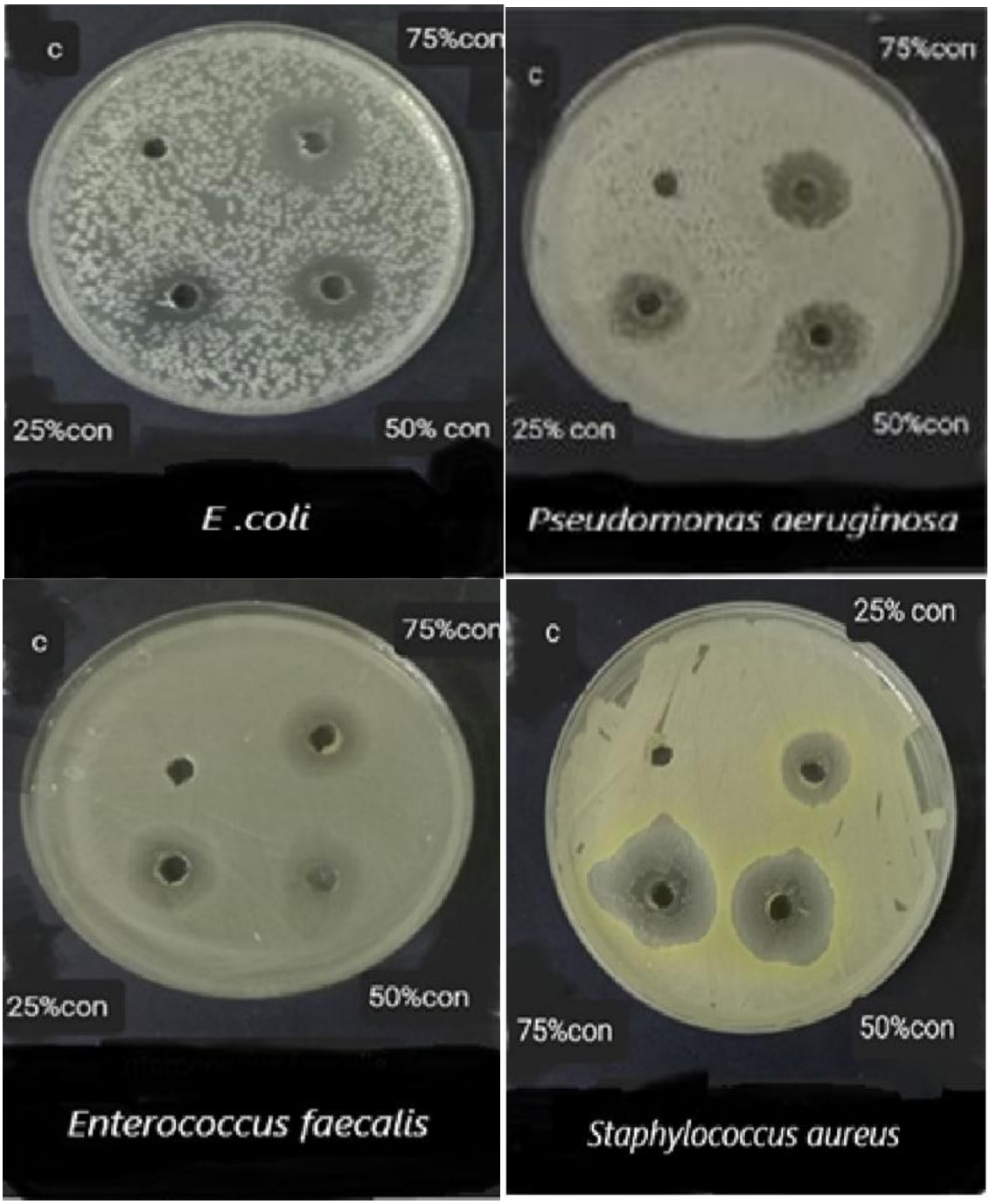

Figure 7: Effect of silver nanoparticles against bacteria isolated from patients with urinary tract infections.
Con: concentration, C: control

Conclusion

The study proved that *C. aurantium* peels contain compounds that effectively reduce nitrates and produce silver nanoparticles that are effective against pathogens that antibiotics may fail to eliminate. In other words, silver particles Biosynthesized from bitter orange peels can be used as an effective medicine against bacteria that cause urinary tract infections.

Ethical approval

Ethical approval numbered Ref.: CSEC/0225/0016 was obtained on February 4, 2025, by the Research Ethics Committee of the Faculty of Science.

Conflicts of interest

There is no conflict of interest.

References

[1] E. M. Mlugu, J. A. Mohamedi, R. Z. Sangeda, and K. D. Mwambete, "Prevalence of urinary tract infection and antimicrobial resistance patterns of uropathogens with biofilm forming capacity among outpatients in Morogoro, Tanzania: a cross-sectional study," *BMC Infectious Diseases*, vol. 23, no. 1, p. 660, 2023.

[2] M. Hadadi-Fishani, A. Khaledi, and Z. S. Fatemi-Nasab, "Correlation between biofilm formation and antibiotic resistance in *Pseudomonas aeruginosa*: a meta-analysis," *Le Infezioni in Medicina*, vol. 28, no. 1, pp. 47-54, 2020.

[3] Z. A. S. Ali and A. M. Y. Al-Araji, "Evaluation of copper nanoparticle synthesis by Fusarium oxysporum antibacterial activity against *Staphylococcus aureus*," in *IOP Conference Series: Earth and Environmental Science*, vol. 1325, no. 1, p. 012046, IOP Publishing, 2024.

[4] E. G. Sweedan and S. M. A. Majeed, "Effects of Silver Nanoparticles Synthesized from Phenolic Extract of *Agaricus bisporus* Against Pathogenic Bacteria and Yeasts," Nano Biomedicine & Engineering, vol. 15, no. 1, 2023.

[5] A. S. Jadou and A. B. Rusol, "Extraction of synephrine from waste peels of *Citrus sinensis* and green synthesis of gold nanoparticles from it against Dermatophytes," *Plant Science Today*, vol. 11, no. 2, 2024.

[6] K. A. Aadim and I. K. Abbas, "Synthesis and Investigation of the Structural Characteristics of Zinc Oxide Nanoparticles Produced by an Atmospheric Plasma Jet," *Iraqi Journal of Science*, Vol. 64, No. 4, pp. 1743-1752, 2023.

[7] Z. S. Qasim and K. Y. Abid, "The Antibacterial Activity of *Angelica glauca* in form of Silver Nanoparticles," *Iraqi Journal of Pharmaceutical Sciences*, vol. 32, no. 3, pp. 149–155, 2023.

[8] S. Thiyagarajan and S. Kanchana, "Green synthesis of silver nanoparticles using leaf extracts of Mentha arvensis Linn. and demonstration of their in vitro antibacterial activities," *Brazilian Journal of Pharmaceutical Sciences*, vol. 58, p. e19898, 2022.

[9] H. Munir, M. Bilal, S. I. Mulla, H. Abbas Khan, and H. M. Iqbal, "Plant-mediated green synthesis of nanoparticles," in *Advances in Green Synthesis: Avenues and Sustainability*, Cham: Springer International Publishing, 2021, pp. 75-89.

[10] S. A. Farhan, "Silver Nanoparticles as a selective probe for Mercury Ions: A Review," *Iraqi Journal of Science*, *Science*, Vol. 63, No. 7, pp. 2774-2782, 2022.

[11] A. Said, M. S. El-Gamal, M. Abu-Elghait, and S. S. Salem, "Isolation, identification and antibiotic susceptibility pattern of urinary tract infection bacterial isolates," *Letters in Applied NanoBioScience*, vol. 10, pp. 2820-2830, 2021.

[12] T. Chatzimitakos, V. Athanasiadis, K. Kotsou, E. Bozinou, and S. I. Lalas, "Response surface optimization for the enhancement of the extraction of bioactive compounds from *Citrus limon* peel," *Antioxidants*, vol. 12, no. 8, p. 1605, 2023.

[13] E. A. Elhawary, N. Nilofar, G. Zengin, and O. A. Eldahshan, "Variation of the essential oil components of *Citrus aurantium* leaves upon using different distillation techniques and evaluation of their antioxidant, antidiabetic, and neuroprotective effect against Alzheimer's disease," *BMC Complementary Medicine and Therapies*, vol. 24, no. 1, p. 73, 2024.

[14] S. D. Shin, C. S. Kim, and J. H. Lee, "Compositional characteristics and antibacterial activity of essential oils in citrus hybrid peels," *Food Science and Technology*, vol. 42, p. e95921, 2021.

[15] A. M. Hasan, S. M. A. Majeed, and R. M. Al-Bahrani, "Investigating the inhibitory effect of silver nanoparticles against some species of *Candida* and pathogenic bacteria," *Cihan University-Erbil Scientific Journal*, vol. 4, no. 1, pp. 32-36, 2020.

[16] M. Sharifi-Rad, P. Pohl, and F. Epifano, "Phytofabrication of silver nanoparticles (AgNPs) with pharmaceutical capabilities using *Otostegia persica* (Burm.) Boiss. leaf extract," *Nanomaterials*, vol. 11, no. 4, p. 1045, 2021.

[17] H. Moussa, S. Hamid, A. Mameri, S. Lekmine, H. Tahraoui, M. Kebir, ... and A. Amrane, "From Green Chemistry to Healthy Environments: Silver Nanoparticles as a Dual Antioxidant and Antibacterial Agents for Advancing Biomedicine and Sustainable Wastewater Treatment," *Bioengineering*, vol. 11, no. 12, p. 1205, 2024.

[18] T. Dao, H. X. Phong, M. Cang, L. G. Bach, and N. V. Muoi, "Kinetic modeling of essential oil hydro-distillation from peels of Pomelo (*Citrus grandis* L.) fruit grown in Southern Vietnam," *Sains Malaysiana*, vol. 50, pp. 3251-3261, 2021.

[19] S. Maksoud, R. M. Abdel-Massih, H. N. Rajha, N. Louka, F. Chemat, F. J. Barba, and E. Debs, "Citrus aurantium L. active constituents, biological effects and extraction methods. An updated review," *Molecules*, vol. 26, no. 19, p. 5832, 2021.

[20] . H. R. Hung, H. J. Lin, E. C. Lee, W. J. Lu, Y. T. Lin, B. B. Huang, and H. T. V. Lin, "Effect of lemon essential oil on the microbial control, physicochemical properties, and aroma profiles of peeled shrimp," *LWT*, vol. 173, p. 114340, 2023.

[21] H. Boughendjioua, N. E. H. Mezedjeri, and I. Idjouadiene, "Chemical constituents of Algerian mandarin essential oil by GC-MS and FT-IR analysis," *Current Issues in Pharmacy and Medical Sciences*, vol. 33, no. 4, pp. 197-201, 2020.

[22] T. E. Agustina, W. Handayani, and C. Imawan, "The UV-VIS spectrum analysis from silver nanoparticles synthesized using *Diospyros maritima* Blume. leaves extract," *3rd KOBI Congress, International and National Conferences (KOBICINC 2020)*, 2021.

[23] A. Dhaka, S. C. Mali, S. Sharma, and R. Trivedi, "A review on biological synthesis of silver nanoparticles and their potential applications," *Results in Chemistry*, p. 101108, 2023.

[24] M. S. Aref and S. S. Salem, "Bio-callus synthesis of silver nanoparticles, characterization, and antibacterial activities via *Cinnamomum camphora* callus culture," *Biocatalysis and Agricultural Biotechnology*, vol. 27, 2020.

[25] A. M. H. Al-Rajhi, S. S. Salem, A. A. Alharbi, and T. M. Abdelghany, "Ecofriendly synthesis of silver nanoparticles using Kei-apple (*Dovyalis caffra*) fruit and their efficacy against cancer cells and clinical pathogenic microorganisms," *Arabian Journal of Chemistry*, vol. 15, no. 7, 2022.

[26] O. Alaa and N. Hassan, "Synthesis and characterization of silver nanoparticles using prodigiosin pigment and evaluation of their antibacterial and anti-inflammatory activities," *Iraqi Journal of Science*, Vol. 62, No. 4, pp. 1103-1120, 2021.

[27] F. F. Rashad, S. S. Obaid, and N. A. Al-kadhi, "Synergistic effect of synthesized silver nanoparticles combined with amoxicillin/clavulanic acid against *Pseudomonas aeruginosa* isolated from clinical samples," *Medical Journal of Babylon*, vol. 20, no. Supplement 1, pp. S173-S179, 2023.

[28] A. H. Hamad, M. A. Chawsheen, and A. A. Al-Naqshbandi, "Role of Laser Produced Silver Nanoparticles in Reversing Antibiotic Resistance in Some Multidrug-Resistant Pathogenic Bacteria," *ARO - The Scientific Journal of Koya University*, vol. 10, no. 1, pp. 104-110, 2022.