Journal of Applied and Natural Science
18(1), 16 - 28 (2026)
ISSN : 0974-9411 (Print), 2231-5209 (Online)
journals.ansfoundation.org

Research Article

Antibacterial activity of extracts and silver nanoparticles from *Vitex Agnus castus* against bacteria isolated from Hospital wastewater

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Article Info
https://doi.org/10.31018/jans.v18i1.7024
Received: July 24, 2025
Revised: January 09, 2026
Accepted: January 15, 2026

How to Cite

Jadoua, M. A. *et al.* (2026). Antibacterial activity of extracts and silver nanoparticles from *Vitex Agnus castus* against bacteria isolated from Hospital wastewater. *Journal of Applied and Natural Science*, 18(1), 16 - 28. https://doi.org/10.31018/jans.v18i1.7024

Abstract

The plants extract and silver nanoparticles are gaining popularity and are being used in medical technologies due to their antibacterial activity. This study aimed to evaluate the potential antimicrobial properties of an aqueous leaves extract of *Vitex agnus -castus* and biosynthesized Ag NP particles. The extract was made using three distinct methods, and silver nanoparticles were formed by adding 0.2 grams of silver nitrate to the extract. UV-Vis Spectroscopy, scanning electron microscopy (SEM), Energy-Dispersive X-ray spectroscopy (XRD), Fourier transform infrared (FTIR) spectroscopy, and Gas chromatography-mass spectrometry (GC-MS) were used to determine the characteristics of the silver nanoparticles. The results indicated that the Soxhlet extraction method produced the highest percentage of silver nanoparticles, measuring 56.13%. The antibacterial activity of both the extracts and the silver nanoparticles was tested against Gram-positive and Gram-negative bacteria isolated from hospital wastewater at three different concentrations: 25, 35, and 50 mg/mL. The extracts exhibited the strongest inhibition of 25 mm against *Enterobacter cloacae* strain NCTC 9394 (FP929040) when using an aqueous Soxhlet extract at a concentration of 35 mg/mL. Conversely, the lowest inhibition recorded was 11 mm against *Pseudomonas aeruginosa* strain WPB098 (CP031876) with an acetone Soxhlet extract at a concentration of 50 mg/mL. In addition, at a concentration of 35 mg/mL, the cold soak extract did not affect *Citrobacter sedlakii* strain NWPK (MW720666). The antibacterial activity of biosynthesized silver nanoparticles showed inhibition against *Pseudomonas aeruginosa* strain WPB098 (CP031876) reached 25 mm from the aqueous Soxhlet extract at a concentration of 50 mg/mL was combined with biosynthesized silver nanoparticles from the hot soaking extract at 50°C at a concentration of 50 mg/ml. Meanwhile, the lowest inhibitory against *Escherichia coli* strain S51 (CP015995) was 15 mm, which was achieved by using biosynthesized silver nanoparticles from aqueous Soxhlet extract at a concentration of 35 mg/mL. Biosynthesized silver nanoparticles from *Vitex agnus-castus* exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria, as promising antibacterial agents.

Keywords: Antibacterial, Hospital wastewater, Pathogenic bacteria, Silver nanoparticles, *Vitex Agnus- castus* L.

INTRODUCTION

The purified water is considered the most crucial natural resource for human survival (Tang *et al.,* 2022). The purity of water is critical to the health of all living things on Earth. The world's population continues to grow, as do industry, urbanization, and chemically enhanced agriculture, which damages water supplies (Mohammad *et al*., 2021; Rathi *et al.,* 2021). Microbial pollution of urban water is a global concern for public health safety, especially in developing countries. Microorganisms such as bacteria and viruses are the predominant pathogens in drinking water. The main sources of pathogens in water are livestock and wildlife feces, sewage, industrial wastewater, and pharmaceuticals. Poor management and ignorance contribute to waterborne diseases in humans by consumption of contaminated drinking water (Rani *et al.,* 2024; Al-Khafaji *et al.,* 2025). Several pathogenic bacteria in wastewater can cause diarrhoea, cholera, gastrointestinal disorders, and limit nutrient absorption, resulting in malnutrition (Zhan *et al.,* 2024). Traditional water treatment methods, such as charcoal filtration, sand filtration, and sedimentation, have limited promise for widespread use. As a result, the use of low-cost alternative materials with desirable qualities, such as photocatalytic activity, absorption capacity, quick adsorption kinetics, and separation and regeneration, has been highlighted. Recently, nanoparticles have been used to remove pollutants (Ajith *et al.,* 2021).

A nanoparticle, or ultrafine particle, is a particle of matter having dimensions of one nanometer to one hundred nanometers (Altammar, 2023). Nanoparticles can be crystalline (single or polycrystalline solids) or amorphous. They can be either solitary or aggregated, and homogeneous or composed of several layers (Joudeh and Linke, 2022). The term "green synthesis" refers to ecologically benign techniques for manufacturing materials, particularly nanoparticles such as AgNPs, using biological systems such as plants, algae, fungi, and bacteria. Green synthesis seeks to eliminate waste, mitigate environmental impacts, and rely on sustainable, renewable resources. The core concepts of green synthesis include the utilization of renewable resources such as plant extracts and microorganisms (Fahim *et al.,* 2024). Plant extracts are frequently selected for their simplicity, convenience of use, scalability, chemical diversity, and cost-effectiveness. Furthermore, plant extracts can be derived from various sources, including stems, roots, fruits, seeds, leaves, flowers, agricultural waste, peels, and bark. These advantages make plant extract-based nanoparticle synthesis more appealing, particularly for large-scale manufacturing for fine nanoparticle applications in agriculture and biomedicine (Oselusi *et al*., 2025). The plant parts used in the process can be cleaned, cooked in distilled water, and then filtered. Following that, nanoparticles are produced by adding the appropriate extract solution to the desired nanoparticles, and the solution's colour change is usually associated with their presence (Mohammadidargah *et al.,* 2024).

Silver nanoparticles have become increasingly popular in the medical sciences due to advances in nanotechnology and their high antibacterial potential. Green synthesis methods for nanoparticles are preferred over physical and chemical methods due to their lower cost, reduced energy use, and greater time efficiency (Al-assdy *et al.,* 2025). They do not use toxic solvents or environmentally hazardous components and are regarded as environmentally and biologically friendly. Biologically produced silver nanoparticles exhibit excellent therapeutic properties, including antioxidant, anticancer, anti-inflammatory, antidiabetic, antiparasitic, and antibacterial effects (Ghani *et al.,* 2022). The present study aimed to evaluate the potential of an aqueous extract of *Vitex agnus-castus* (Chasteberry) leaves and to manufacture silver nanoparticles using the green synthesis method from $AgNO_3$, and to determine the antimicrobial properties of these compounds against bacteria isolated from local hospital wastewater.

MATERIALS AND METHODS

Preparation of plant extracts and nanoparticles

Collection of plant samples

Vitex agnus-castus plants were collected from the Qarmat_Ali is an Iraqi town located in Al-Hartha District, Basrah. (30.56953.9″N, 47.74971.2″E) during November and December 2024. The collected plant leaves were placed in plastic bags and transferred to the laboratory, where they were washed with distilled water to remove accumulated dust, then allowed to dry for a week. Following that, the plant was ground in a ceramic mortar and the powder was stored in plastic containers.

Extraction techniques

Several methods were used to prepare the aqueous extract of the selected plant, including:

Cold soaking: This method involves placing 20 grams of plant powder in a 500 ml conical flask with 150 ml of distilled water and allowing it to sit for 24 hours. Afterward, the mixture is filtered through filter paper, and 25 ml of the extract was reserved for further use, while the remaining solution was allowed to dry (Sankeshwari *et al.,* 2018).

Hot soaking: Twenty grams of powdered plant were placed in a 500 mL conical flask with 150 mL of distilled water. The mixture was heated on a magnetic stirrer plate at 50°C for two hours. Afterwards, filter the mixture through filter paper, reserving 25 mL for later use, and allow the remainder to dry (Logeswari *et al.,* 2015).

Extraction using the soxhlet apparatus: Aqueous, alcoholic, and acetone extracts were prepared by taking 20 grams of powder from each plant and placing it in a thimble. Water, alcohol, and acetone were used as solvents; 25 ml of each was collected for later use, while the remainder was dried in dishes, ground, and stored in plastic containers (Mokaizh *et al.,* 2024).

Green synthesis of silver nanoparticles

The silver nanoparticles were made using a process (Ahmed *et al.,* 2016) that involved adding 25 ml of the chosen plant's non-dried extract to a 500 ml glass flask containing 150 ml of deionized distilled water. Then, 0.2 g of silver nitrate was added and heated to 50 degrees Celsius on a magnetic stirring plate while stirring. The mixture was then stored in an area for 2 days. It was subsequently placed in Petri dishes and allowed to dry at room temperature. Finally, the particles were scraped and put in plastic bins.

Characterization of plant extracts and silver nanoparticles AgNPs

A variety of techniques were used as mentioned following to determine and confirm the nano characteristics of plant extracts and AgNPs. The optical properties of AgNPs were investigated with a UV-vis spectrophotometer. Fourier transform infrared (FTIR) spectroscopy was used to analyze the dried material of plant extracts and AgNPs. X-ray diffraction (XRD) and scanning electron microscopy (SEM) were used to characterize the structural properties and particle size and shape of AgNPs. Gas chromatography-mass spectrometry (GC-MS) were used to determine the characteristics of the silver nanoparticles (Hamid *et al*., 2024).

Isolation and identification of bacterial species

Collection of wastewater samples

Wastewater samples were collected from Ports Teaching Hospital and Al-Fayhaa Hospital in Basrah Governorate between 1 November and 30 December, 2024. The samples were obtained at depths of 10-15 cm, with 0.5 L collected at several points using a sterile plastic container. They were placed in sterile laboratory plastic containers, transferred to the laboratory, and kept in the refrigerator at 4°C until the laboratory analyses were completed.

Isolation of bacteria

A serial dilution method was used to isolate bacteria from a wastewater sample. Initially, 1 ml of wastewater was added to 9 ml of sterile distilled water to obtain a 10^{-1} dilution. To obtain a 10^{-2} dilution, 1 ml was transferred from the first tube to a second tube containing 9 ml of sterile distilled water. This process was repeated to obtain dilutions up to 10^{-6}. Next, the nutrient agar plates were inoculated under sterile conditions with 0.1 ml of each sample, with two replicates per dilution. The samples were evenly spread across the plates using a glass spreader. The plates were incubated at 37°C for 24 hours, then examined after 24 hours.

Purification of bacterial isolates

The bacterial isolates were purified by transferring a portion of each colony to a new plate containing nutrient agar. The plates were then incubated at 37 °C for 24 hours. Their purity was confirmed by Gram staining the bacteria and examining them under a light microscope. The pure colonies were then grown on slant nutrient agar and kept in the refrigerator at 4°C (Mohammad and Alyousif, 2022).

Diagnosis of isolated bacteria

The pure bacterial isolates were activated on nutrient agar and transferred to Al-Fayhaa Hospital in a cooling container for identification using the VITEK 2 system. The VITEK 2 system was used according to the manufacturer's instructions, with ID Gram Positive and ID Gram Negative cards used for identification. The bacterial isolates including *Leuconostoc mesenteroides* strain F17, *Kocuria rosea* strain MG2, *Staphylococcus hominins* strain MK-1, *Escherichia coli* strain S51, *Enterobacter cloacae* strain NCTC 9394, *Pseudomonas aeruginosa* strain WPB098, *Citrobacter sedlakii* strain NWPK, *Morganella morganii* strain UM869, and *Serratia marcesceens* strain UMH5.

Antibacterial activity of plant extracts and silver nanoparticles

The agar well diffusion method was employed to assess the activity of plant extracts and silver nanoparticles, with various concentrations (25, 35, and 50 mg\ mL). These extracts were dissolved in 1 mL of DMSO and left to stand for at least 3 hours to fully dissolve. The bacterial species isolated from hospital wastewater isolates including *Leuconostoc mesenteroides* strain F17 (CP178852), *Kocuria rosea* strain MG2 (JX534199), *Staphylococcus hominins* strain MK-1 (JX961712.1), *Escherichia coli* strain S51 (CP015995), *Enterobacter cloacae* strain NCTC 9394 (FP929040), *Pseudomonas aeruginosa* strain WPB098 (CP031876), *Citrobacter sedlakii* strain NWPK (MW720666), *Morganella morganii* strain UM869 (ON533444.1), and *Serratia marcesceens* strain UMH5 (CP0189171) were activated individually on Mueller-Hinton agar. 0.1 mL of dissolved extract and silver nanoparticles was added to the wells of the cultured bacterial plates, which were then incubated at 37 °C for 24 hours. The inhibition zones of plant extracts and silver nanoparticles against bacterial colony growth were determined by measuring the diameter of each inhibition zone for each bacterial isolate (Alyousif *et al.,* 2023).

RESULTS AND DISCUSSION

Characterization of plant extracts

Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry revealed the existence of several aromatic and aliphatic chemicals. The components of the *V. agnus-castus* plant's Cold extract were characterized, revealing that there are compounds with high concentrations belonging to the organic categories of phenols, esters, carboxylic acids, and heterocyclic compounds, with concentration ratios ranging from 23.51% to 1.31% as indicated in Fig. 1. The results of the analysis of *V. agnus-castus* extracts using GC-MS were consistent with the study conducted by (Khames and Ahmed, 2025; Jasim *et al*., 2024), which evaluates the synergistic and antibacterial effects of *V. agnus-castus* leaf extract on multidrug-resistant *P. aeruginosa* isolates. The combining *Vitex*

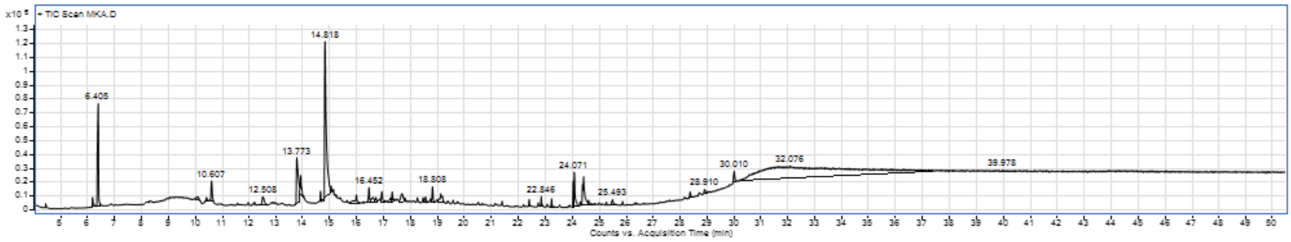

Fig. 1. Chromatogram of Cold-soaked extract of *Vitex agnus-castus* plant

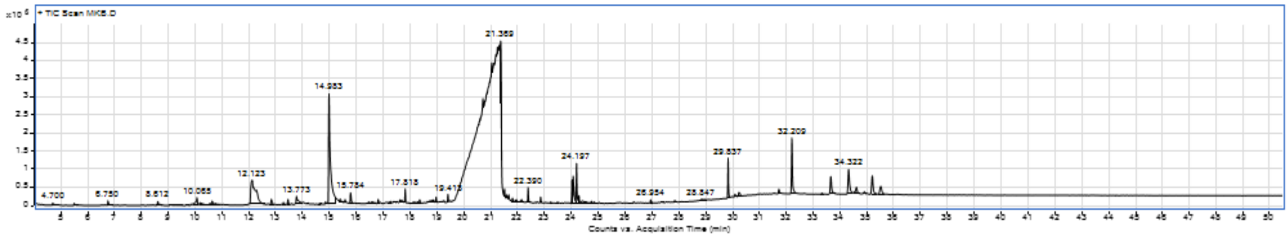

Fig. 2. Chromatogram of Hot-soaked extract at 50 C° of *Vitex agnus-castus* plant

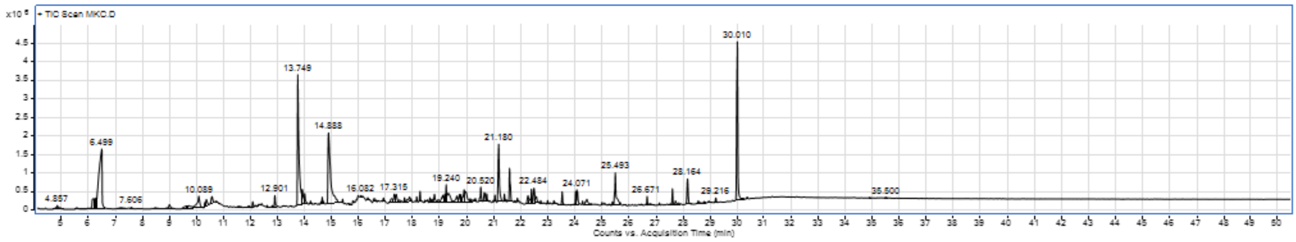

Fig. 3. Chromatogram of soxhlet extract of the *Vitex agnus-castus* plant

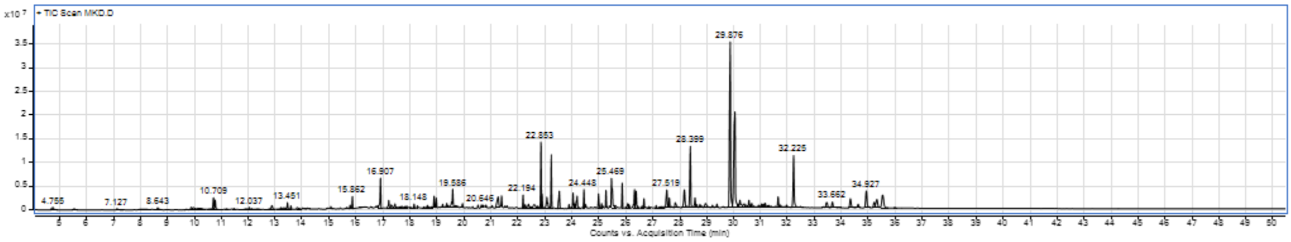

Fig. 4. Chromatogram of the alcoholic extract of the *Vitex agnus-castus* plant

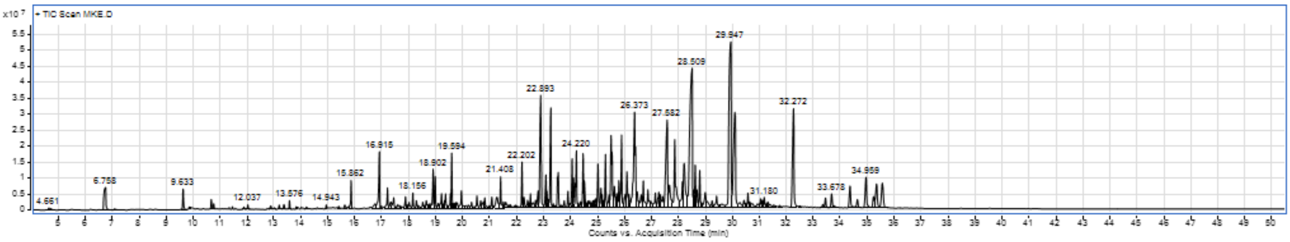

Fig. 5. Chromatogram of acetone extracts of the *Vitex agnus-castus* plant

extract with antibiotics has the potential to be beneficial as a therapeutic agent against drug-resistant *P. aeruginosa.*

The plant components of a 50 °C hot extract from *Vitex agnus-castus* contained active chemicals such as alcohols, phenols, non-homogeneous cyclic compounds, and amides, with concentrations ranging from 41.51% to 0.79%, as shown in Fig. 2.

The plant components of the aqueous extract of the *V. agnus-castus* plant using a Soxhlet apparatus. Active compounds such as alcohols, phenols, non-homogeneous cyclic compounds, and fluorine-substituted aromatic compounds are present, with concentration ratios ranging from 12.90% to 1.05% as in Fig. 3.

The plant components of the alcoholic extract of the *Vitex agnus-castus* plant were obtained using a Soxhlet apparatus. Active compounds such as alkenes, phenols, non-homogeneous cyclic compounds, fluorine-substituted aromatic compounds, and cholesterol derivatives are present, with concentration ratios ranging from 1.39% to 14.13% as indicated in Fig. 4.

The plant components of the acetone extract of the *V. agnus-castus* plant using the Soxhlet apparatus, revealing the presence of active compounds such as alkenes, phenols, non-homogeneous cyclic compounds, fluorine -substituted aromatic compounds, cholesterol derivatives, and lactone compounds with concentrations ranging from 1.70 to 8.02% as shown in Fig. 5.

Fourier transform infrared (FTIR) spectroscopy

The extracts of the cold and hot *V. agnus-castus*, as well as Soxhlet, exhibited distinct absorption bands that corresponded to different functional groups in the organic components adsorbed on the silver surface. All aqueous extracts revealed bands at 3200 cm^{-1}, which corresponded to the stretching vibration of the phenolic or alcoholic O-H bond. The bands at 2900-2800 cm^{-1} correspond to the stretching vibration of C-H bonds in aliphatic molecules. Furthermore, bands in the 1680-1690 cm^{-1} range correlate to the stretching vibration of carbonyl bonds in various ester and ketone compounds, as shown in Fig. 6.

The alcoholic and acetone extracts of the plant *V. agnus-castus* revealed distinct absorption bands that corresponded to different functional groups in chemical components. The extracts revealed bands between 3300 and 3200 cm^{-1}, which corresponded to the stretching vibration of the phenolic or alcoholic O-H bond. The bands at 2910-2920 cm^{-1} correspond to the stretching vibration of C-H bonds in aliphatic molecules. Additionally, bands between 1692 and 1730 cm^{-1} correspond to the stretching vibration of carbonyl bonds in several ester and ketone molecules. The bands in the 1000 cm^{-1} range correspond to the stretching vibration of the C-O and C-N bonds in heterocyclic aromatic organic compounds, as illustrated in Fig.7. The results of the analysis of *V. agnus-castus* extracts using FTIR were consistent with the study conducted by Abada *et al*. (2024) , Muhammad-Ali *et al.* (2025), who also showed that the active groups of *V. agnus-castus* extracts matched the current study.

Silver nanoparticles synthesis using plant extracts and their characterization

The present study revealed that adding an aqueous plant extract of *V. agnus-castus* to silver nitrate (0.2 grams) caused the mixture to change from green to brown, indicating excitation of silver nanoparticles' plasmonic resonance, as shown in Fig. 8.

UV-Vis Spectroscopy

UV-Vis spectroscopy was used to monitor the bioreduction of silver ions to silver nanoparticles. The absorption peaks of the silver nanoparticle combination demonstrated silver surface plasmon resonance at 410, 420, and 430 nm, as shown in Fig. 9, which showed the ultraviolet and visible light ranges, demonstrating how silver nanoparticles are made from the *V. agnus-castus* plant using cold soaking, hot soaking 50 C°, and Soxhlet extraction methods. The maximum absorption values were obtained at 410, 420, and 430 nanometers, which are within the diagnostic range for silver nanoparticles (400-450 nanometers). The results of the present work are consistent with those of Hashemi *et al*. (2021), as the UV-visible spectrum of biosynthesized silver nanoparticles from Alcea rosea showed the largest absorption peak at 425 nm.

Energy-dispersive X-ray (EDX) analysis

The main structures of AgNPs derived organically from the plant *V. agnus-castus* using three different extraction procedures (cold soaking, hot soaking at 50 °C, and Soxhlet extraction) were characterized using EDX. The results showed that AgNPs obtained using the Soxhlet extraction procedure yielded the highest proportion of silver nanoparticles (56.13), compared with the other two methods, indicating that this method was the preferred method for nanoparticle synthesis (Ghavam, 2023), as shown in Table 1 and Fig. 10.

Scanning electron microscope (SEM)

The silver nanoparticles biosynthesized from *V. agnus-castus* plant with distinct morphologies and surface sizes were characterized by scanning electron microscopy, which revealed that the silver nanoparticles had a smooth, spherical shape. The average size of the silver nanoparticles biosynthesized from the cold-soaked extract of the plant was 38.48 nanometers, whereas the average size of the silver nanoparticles biosynthesized from the hot-soaked extract at 50 C° was 51.30 nanometers. The silver nanoparticles biosynthesized from the Soxhlet extract measured 44.67 nanometers. The results of the current study agree with those of Ghabban *et al.* (2022), who reported that the silver nanoparticles produced by the prickly caltrop plant were spherical and ranged in size from 30 to 40 nanometers, as shown in Fig. 11.

Fourier transform infrared (FTIR) spectroscopy

FTIR analysis can help identify the biomolecules responsible for silver ion reduction and nanoparticle stabilization. The qualitative examination uses infrared laser scanning to evaluate the chemical bonds of materials. The functional groups responsible for nanoparticle formation can be identified by FTIR spectroscopy. The silver nanoparticles biosynthesized from both cold and hot extracts of *V. agnus-castus* and Soxhlet extracts showed several absorption bands that matched different functional groups in the organic materials attached to the silver surface. All of the aqueous extracts exhibited bands at 3200 cm^{-1}, which corresponded to the stretching vibration of the phenolic or alcoholic O-H bond. The bands at 2900-2800 cm□^{1} correspond to the

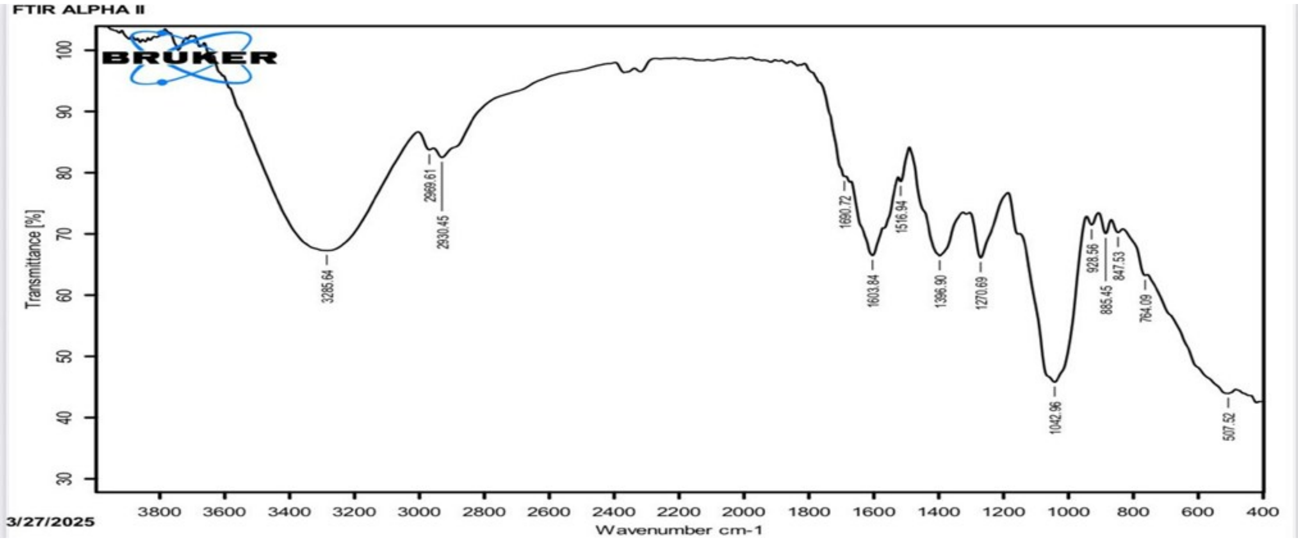

Fig. 6. FTIR spectrum of cold-soaked extract of the plant *Vitex agnus-castus*

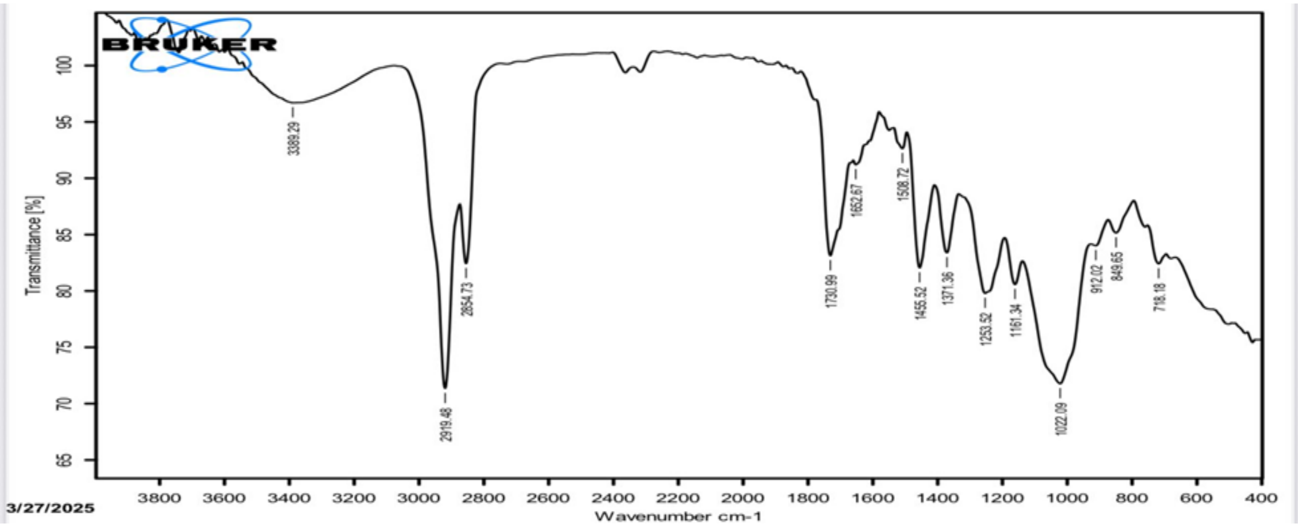

Fig. 7. *Fourier transform infrared spectrum of the acetone extract of the plant Vitex agnus-castus*

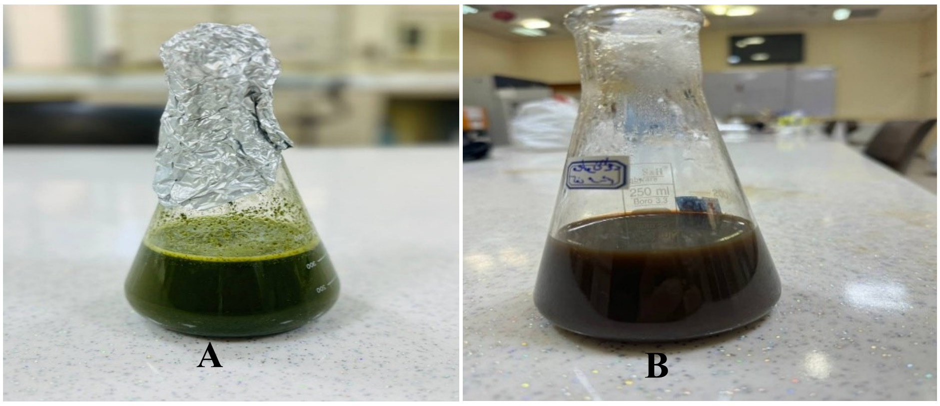

Fig. 8. *AgNPs Synthesis A: The green color of the extract before adding silver nitrate, B: the Brown color of the extract after adding silver nitrate*

stretching vibration of C-H bonds in aliphatic molecules. Furthermore, bands in the 1680-1690 cm^{-1} range correspond to the stretching vibration of carbonyl bonds in various ester and ketone compounds, as illustrated in Figure 12. The present results support previous studies in identifying the biomolecules in *Camellia sinensis* and *Prunus africana* responsible for silver ion reduction (Ssekatawa *et al.,* 2021; Alharbi *et al.,* 2022).

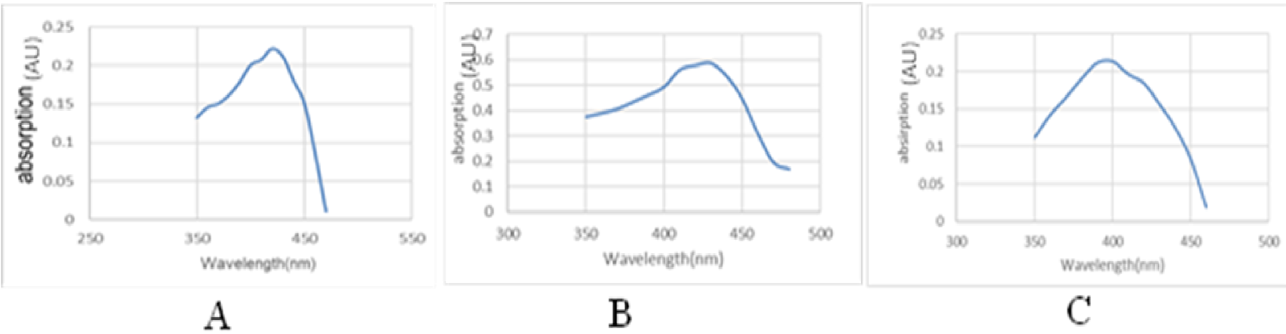

Fig. 9. *A: UV-Vis spectrum of silver nanoparticles biosynthesized from cold soak extract; B: The UV-Vis spectrum of silver nanoparticles biosynthesized from hot soak extract at 50 ^{0}C; C: The UV-Vis spectrum of silver nanoparticles biosynthesized from Soxhlet extraction*

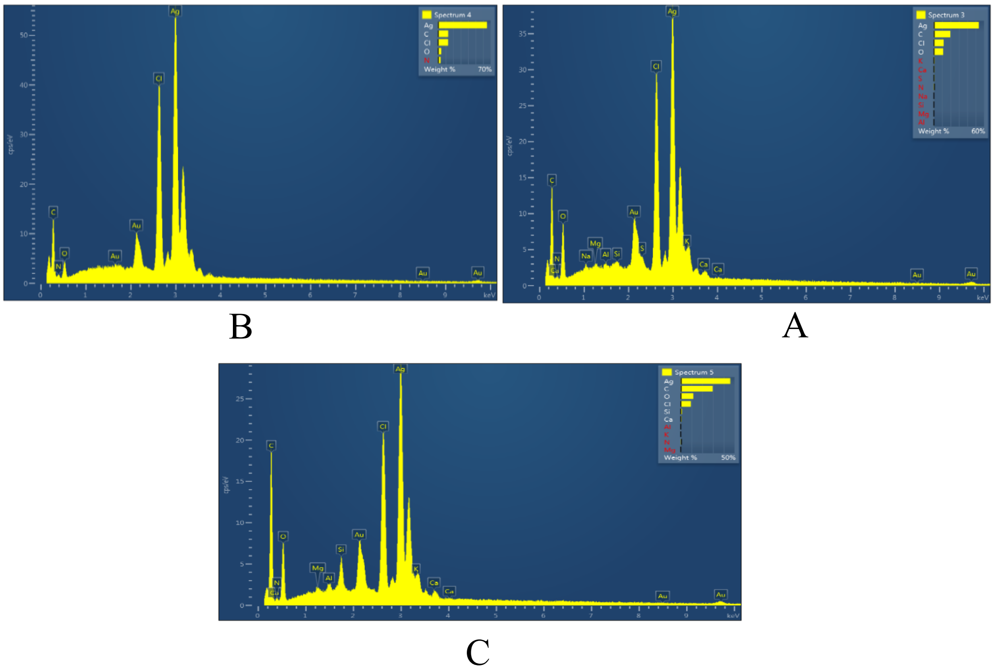

Fig. 10. *EDX spectra of AgNPs biosynthesized from the Vitex agnus-castus plant, A: Cold extract, B: Hot extract, C: Soxhlet extract*

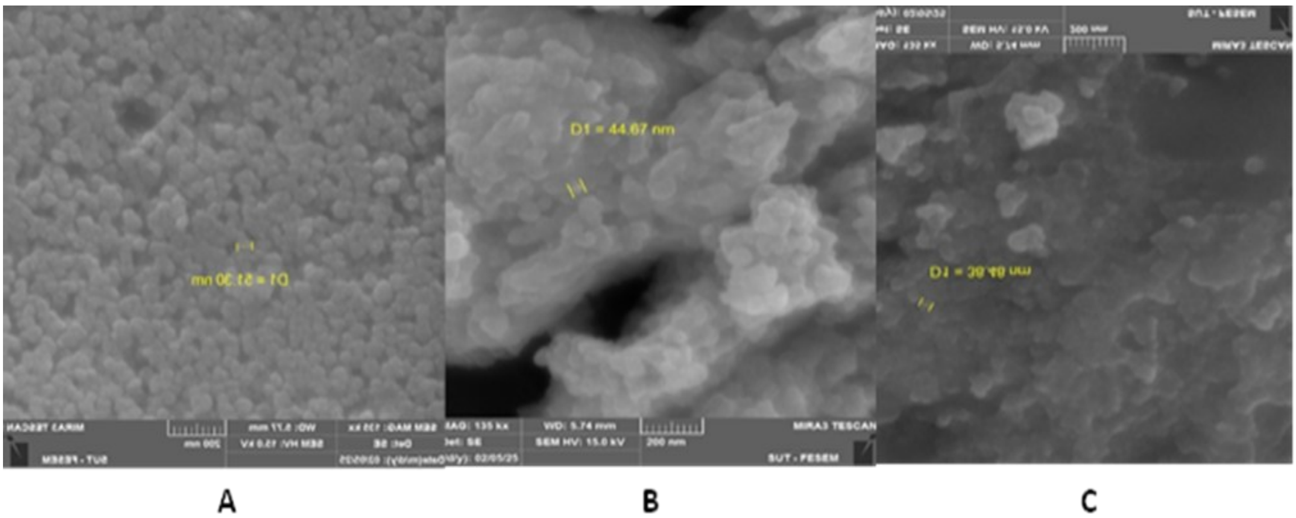

Fig. 11. *Scanning Electron Microscope (SEM) of AgNPs biosynthesized from the Vitex agnus-castus plant A: Cold extract, B: Hot extract, C: Soxhlet extract*

Table 1. Percentage of oxygen, carbon and silver in AgNPs manufactured by methods (cold soaking, hot soaking of 50 C°, Soxhlet extraction)

Type of nanoparticles	Ag	Oxygen	Carbon
Cold silver nano	46.12	11.75	29.75
50°C hot nano silver	53.80	11.26	19.73
Nano silver soxhlet	56.13	4.39	13.53

Table 2. Antibacterial activity of *V. agnus-castus* extract against Gram-positive bacteria isolated from hospital wastewater

Extract method	Concentrations mg/ml	Inhibition zone (mm)		
		Leuconostoc mesenteroides **spp cremoris**	***Kocuria rosea***	***Staphylococcus hominins***
Cold soaking extract	25	14	13	0
	35	13	13	13
	50	16	14	16
50°C hot soaking extract	25	15	14	17
	35	14	15	15
	50	18	19	14
Soxhlet water extract	25	17	12	0
	35	13	14	13
	50	15	15	14
Alcoholic soxhlet extract	25	16	13	15
	35	13	14	13
	50	16	18	21
Soxhlet acetone extract	25	15	15	16
	35	14	15	19
	50	17	20	20

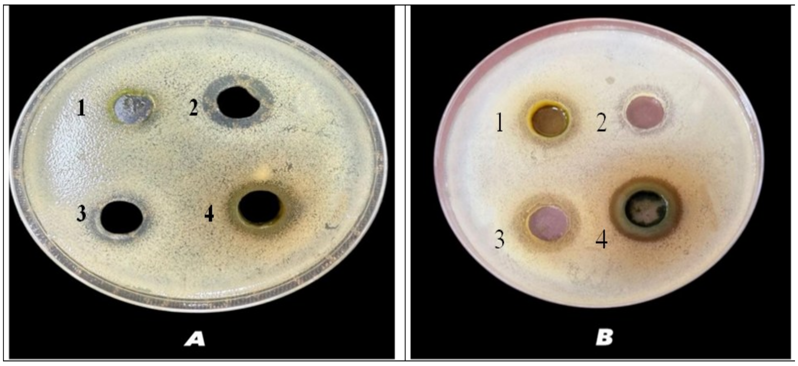

Fig. 13. *Antibacterial activity of V. agnus-castus plant extract, A: Staphylococcus hominis, B: Kocuria rosea, 1: Cold soaking extract, 2: 50°C hot soaking extract, 3: Soxhlet water extract, 4: Alcoholic soxhlet extract*

Antibacterial activity of plant extracts and silver nanoparticles

The activity of *V. agnus-castus* plant extract against Gram-negative and Gram-positive bacteria isolated from hospital wastewater was determined at concentrations of 25, 35, and 50 mg/mL using the diffusion method. The results demonstrated that the plant extracts from *V. agnus-castus* L. had antibacterial activity against Gram-positive bacteria with maximum inhibition diameter was 21 mm against bacteria *Staphylococcus hominins* when the alcoholic Soxhlet extract was used at a concentration of 50 mg/ml, while the minimum inhibition diameter was 12 mm against bacteria *Kocuria rosea* when the aqueous Soxhlet extract was used at a concentration of 25 mg/ml, while some extracts did not show any effectiveness against bacteria as shown in Table 2 and Fig. 13.

The activity of the *V. agnus-castus* plant extract against Gram-negative bacteria showed a maximum inhibition diameter of 25 mm against Enterobacter cloacae complex using an acetone Soxhlet extract at a concentration of 50 mg/mL. Meanwhile, the minimum inhibition diameter was 11 mm against *Pseudomonas aeruginosa*, when an aqueous soxhlet extract was used at a concentration of 50 mg/ml. Furthermore, at a concentration of 35 mg/mL, the cold-soak extract was ineffective against *Citrobacter sedlakii*, as shown in Table 3 and Figure 11. It was found that the best inhibitory activity of the extracts was observed with the acetone Soxhlet extract against Gram-positive bacteria, due to

Table 3. The antibacterial activity of *V. agnus-castus* extract on Gram-negative bacteria isolated from hospital wastewater

Extract method	Concentrations mg/ml	Inhibition zone (mm)					
		Escherichia coli	*Enterobacter cloacae complex*	*Pseudomonas aeruginosa*	*Citrobacter sedlakii*	*Morganella morganii*	*Serratia marcesceens*
Cold soaking extract	25	16	16	19	14	16	12
	35	17	14	14	0	14	12
	50	17	19	12	16	18	18
50°C hot soaking extract	25	17	18	17	17	17	15
	35	15	16	15	16	16	15
	50	16	20	13	18	20	17
soxhlet water extract	25	17	17	20	15	16	13
	35	17	18	15	13	14	13
	50	21	22	11	15	21	17
Alcoholic soxhlet extract	25	18	16	19	18	20	14
	35	15	20	14	14	15	13
	50	19	23	15	17	18	16
Soxhlet acetone extract	25	14	18	18	15	18	18
	35	17	19	24	16	17	15
	50	19	25	14	22	17	18

its content of bioactive compounds that inhibit bacterial growth, such as phenols, heterocyclic compounds, and fluorine-substituted aromatic compounds. The effect of this extract is attributed to the fact that the cell wall structure of Gram-positive bacteria is more sensitive to plant extracts, in addition to the absence of an outer membrane, which gives it high permeability properties, destroys the bacterial membrane, inhibits biofilm formation, DNA synthesis, and protein synthesis (Jubair *et al*., 2021). This study is consistent with the study by Nigussie *et al*. (2021), which examined the effects of extracts from the leaves of *Azadirachta indica*, *Lawsonia inermis*, and *Achyranthes aspera* against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *E. coli*. Water, methanol, and ethyl acetate were used as solvents, and methanol extract was the most effective at inhibiting bacterial growth. The study by Kozłowska *et al.* (2022) demonstrated the effectiveness of plant extracts from *Betula pendula, Rubus idaeus, Morus alba, and Cistus incanus*, extracted with water and ethanol, against *Staphylococcus aureus* and *E. coli*, with the ethanol extract being more effective at inhibiting bacterial growth.

The antibacterial activity of biosynthesized silver nanoparticles from *V. agnus-castus* against Gram-positive bacteria showed a maximum inhibition diameter of 25 mm against *Kocuria rosea* when the nanoparticles were cold-soaked at 25 mg/mL. Meanwhile, the lowest inhibition diameter was 16 mm against bacteria *Kocuria rosea*, which was achieved by using biosynthesized silver nanoparticles from hot soaking at 50 °C at a concentration of 25 mg/ml and biosynthesized silver nanoparticles from an aqueous soxhlet extract at a concentration of 35 mg/ml against *Leuconostoc mesenteroides,* as shown in Table 4 and Fig. 14.

The results demonstrated the antibacterial activity of biosynthesized silver nanoparticles derived from *V. agnus-castus* against Gram-negative bacteria. The highest inhibition diameter against *Pseudomonas aeruginosa* was 25 mm, achieved with biosynthesized silver nanoparticles from an aqueous Soxhlet extract at 50 mg/ml and Soxhlet silver nanoparticles from a hot-soaking extract at 50°C, both at 50 mg/ml. Meanwhile, the lowest inhibitory diameter against *E. coli* was 15 mm, achieved using biosynthesized silver nanoparticles from an aqueous Soxhlet extract at a concentration of 35 mg/mL, as shown in Table 5.

All the biosynthesized silver nanoparticles from the cold soak extract, hot soak extract, and aqueous Soxhlet extract of the *V. agnus-castus* plant showed high activity against both Gram-positive and Gram-negative bacteria, as indicated by the inhibition diameter, which ranged between 25-15 mm among the different types of silver nanoparticles. This is attributed to direct interaction with the bacterial cell wall, inhibiting biofilm formation, triggering an innate and adaptive host immune response, producing reactive oxygen species (ROS), and inducing intracellular effects, e.g., interactions with

Table 4. Antibacterial activity of biosynthesized silver nanoparticles from *V. agnus-castus* against Gram-positive bacteria isolated from hospital wastewater

Extract method	Concentrations mg/ml	Inhibition zone (mm)		
		Leuconostoc mesenteroides* spp *cremoris	***Kocuria rosea***	***Staphylococcus hominins***
Cold nano silver	25	19	25	20
	35	18	18	19
	50	19	23	19
50°C hot nano silver	25	18	16	18
	35	17	17	18
	50	20	20	23
Nano silver soxhlet	25	17	17	17
	35	16	19	19
	50	17	22	19

Table 5. Antibacterial activity of biosynthesized silver nanoparticles from the *V. agnus-castus* L. plant against Gram-negative bacteria isolated from hospital wastewater

Extract method	Concentrations mg/ml	Inhibition zone (mm)					
		Escherichia coli	***Enterobacter cloacae complex***	***Pseudomonas aeruginosa***	***Citrobacter sedlakii***	***Morganella morganii***	***Serratia marcesceens***
Cold nano silver	25	19	19	22	20	19	22
	35	19	16	23	18	16	17
	50	17	17	23	17	19	18
50°C hot nano silver	25	19	18	21	24	17	20
	35	16	17	23	17	17	17
	50	20	20	25	19	21	18
Nano silver soxhlet	25	16	19	21	19	20	19
	35	15	19	23	19	18	18
	50	19	22	25	17	21	19

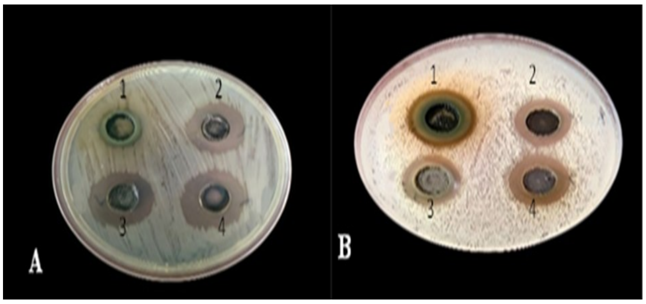

Fig. 14. *Antibacterial activity of V. agnus-castus plant extracts and biosynthesized silver nanoparticles, A: Pseudomonas aeruginosa, B: Kocuria rosea, 1: Soxhlet acetone extract, 2: Cold nano silver, 3: 50°C hot nano silver, 4: Nano silver Soxhlet*

DNA and/or proteins (Anand *et al*., 2022). Furthermore, depending on the outcome, silver nanoparticles have demonstrated a modest antibacterial effect against Gram-positive bacteria compared to Gram-negative bacteria. Gram-positive bacteria contain a thick covering of peptidoglycan. This makes it difficult to disseminate silver nanoparticles over the cell wall (Sumitha *et al*., 2018), so disrupting cell function and preventing growth. In contrast to Gram-negative bacteria, the outer membrane of Gram-positive bacteria may prevent a greater amount of silver from reaching the cytoplasmic membrane. As a result, Gram-positive bacteria showed greater tolerance to the produced silver nanoparticles than Gram-negative bacteria (Samuggam *et al*., 2021). The present study is consistent with the study by Feng *et al.* (2022), which biosynthesized silver nanoparticles from the glycyrrhizin plant by increasing the inhibition diameters with the increase of reduced silver nanoparticles concentrations. They evaluated the antibacterial activities of silver nanoparticles against *E. coli* and *Staphylococcus aureus* using the Oxford cup method and the filter paper diffusion method, respectively. Moreover, with the increase in concentrations of Glycyrrhizin -reduced silver nanoparticles (20, 60, 100 μg/mL), the inhibition diameters for both types of bacteria treated with Glycyrrhizin -reduced silver nanoparticles increased. The results showed that the inhibition diameter of *E. coli* caused by reduced silver nanoparticles was at a concentration of 100 µg/ml to 22 and 16 mm using the Oxford cup method and the filter paper method, respectively. Similarly, the inhibition diameter of *Staphylococcus aureus* after treatment with reduced silver nanoparticles at 100 µg/ml using the Oxford cup method and the filter paper method was 18 and 14 mm, respectively.

The present study is consistent with the study by Abdellatif *et al*. (2022) in the activity of biosynthesized silver nanoparticles from the plants. The biosynthesized silver nanoparticles from the plants *Mentha piperita* showed strong antibacterial activity against pathogenic bacteria including *E. coli*, *Staphylococcus aureus*, *Bacillus cereus, Salmonella typhimurium* except *Proteus mirabilis.* Furthermore, AgNPs from *Zingiber officinale* showed substantial activity against *Staphylococcus aureus*, *E. coli*, and *Klebsiella pneumoniae*. While Csakvari *et al.,* (2021) evaluated the antibacterial activity of *Cannabis sativa* leaf extracts, with or without biosynthesized silver nanoparticles, on four bacterial strains, three Gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) and one Gram-positive bacterium (*Staphylococcus aureus*) using the agar disc diffusion method. Their results showed the antibacterial effect of *Cannabis* leaf extracts alone and their mixture containing prepared silver nanoparticles against both Gram-positive and Gram-negative bacterial strains.

Conclusion

The extract of *V. agnus-castus* plant and biosynthesized silver nanoparticles showed antibacterial activity against bacterial species isolated from hospital wastewater. The biosynthesised silver nanoparticles from plants exhibited strong antibacterial activity against both Gram-positive and Gram-negative bacteria (*Staphylococcus hominins* strain MK-1, *Enterobacter cloacae* strain NCTC 9394, *Pseudomonas aeruginosa* strain WPB098, *Citrobacter sedlakii* strain NWPK, and *Escherichia coli* strain S51) demonstrating the benefits of their antimicrobial properties. Therefore, silver nanoparticles can be considered promising antibacterial agents and a viable alternative to antibiotics. The extraction and biosynthesis of silver nanoparticles from *V. agnus-castus* can be an environmentally friendly, cost-effective, and safe process for their antimicrobial activities and healthcare applications.

ACKNOWLEDGEMENTS

We would like to thank the head of the Ecology department at the College of Science for their valuable support in completing this work.

Conflict of interest

The authors declare that they have no conflict of interest.

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