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Green Synthesis of Silver Nanoparticles Using *Saussurea Costus* Methanolic Extract: A Novel Strategy against Multidrug-Resistant *Listeria Monocytogenes*

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Annotation: The rising prevalence of multidrug-resistant (MDR) Listeria monocytogenes in dairy products necessitates innovative control strategies. This study investigated green-synthesized silver nanoparticles (AgNPs) using Saussurea costus methanolic extract against MDR L. monocytogenes from 200 dairy samples (100 raw buffalo milk, 100 local cheese) in Al-Diwaniyah City, Iraq. Isolation involved selective enrichment and chromogenic agar plating, with confirmation via biochemical tests (catalase, beta-hemolysis) and PCR detection of virulence genes (16S rRNA, Ami, Vip). Results revealed 16.5% prevalence (23% milk, 10% cheese; p=0.013). All isolates exhibited MDR, showing complete resistance to aminoglycosides, penicillins, tetracyclines, and cephalosporins, while remaining susceptible to macrolides, carbapenems, and fluoroquinolones. GC-MS analysis identified dehydrocostus lactone (41.52%) as the primary phytoconstituent. AgNPs were successfully synthesized, demonstrating a characteristic UV-Vis peak at 435 nm. FESEM confirmed spherical morphology (76.45±1.32 nm), while FTIR and XRD verified phytochemical capping and crystalline structure. Antibacterial assays showed dose-dependent inhibition, with methanolic

AgNPs exhibiting superior activity (22±1.1 mm zone at 100 mg/mL) compared to crude extract (10±0.5 mm). Statistical analysis confirmed significant differences (ANOVA, $p<0.001$), with strong dose-response correlation ($R^2=0.99$). These findings highlight S. costus-mediated AgNPs as a potent, eco-friendly alternative against MDR L. monocytogenes, offering promising applications in food safety. Further studies should explore large-scale synthesis and in vivo efficacy.

1. Introduction

The global rise of antimicrobial resistance (AMR) in foodborne pathogens has become a pressing public health crisis, with *Listeria monocytogenes* emerging as a particularly concerning multidrug-resistant (MDR) threat (1). As a ubiquitous Gram-positive pathogen, *L. monocytogenes* causes severe invasive infections with case fatality rates exceeding 25% in high-risk groups (2). Recent surveillance data from the European Food Safety Authority revealed increasing resistance to first-line antibiotics, including ampicillin (42% of isolates) and trimethoprim-sulfamethoxazole (31%) (3) while our preliminary investigations in Iraqi dairy systems detected 100% MDR prevalence in *L. monocytogenes* (unpublished data, 2024). The food safety implications are particularly alarming given the pathogen's ability to persist in dairy processing environments and resist pasteurization (4). Conventional antimicrobials are failing against these resilient strains, as evidenced by the Global Antimicrobial Resistance 2023 and Use Surveillance System (GLASS) report documenting resistance to ≥3 drug classes in 89% of foodborne *Listeria* isolates (5). This urgent scenario has accelerated research into alternative antimicrobial strategies, particularly green-synthesized silver nanoparticles (AgNPs), which exhibit broad-spectrum activity through multiple mechanisms including cell membrane disruption, oxidative stress induction, and interference with DNA replication (6). Among various biogenic approaches, plant-mediated synthesis offers distinct advantages, combining ecological sustainability with enhanced bioactivity due to synergistic phytochemical effects (7). *Saussurea costus* , a high-value medicinal plant from the Asteraceae family, has gained attention for its rich sesquiterpene lactone content, particularly dehydrocostus lactone, which demonstrates proven antimicrobial and anti-inflammatory properties (8). Recent metabolomic studies have identified at least 14 bioactive compounds in *S. costus* with potential metal-reducing capabilities (9), while its traditional use in Ayurvedic medicine against gastrointestinal infections has been pharmacologically validated (8). However, despite these promising characteristics, the application of *S. costus*-mediated AgNPs against MDR *L. monocytogenes* remains unexplored, creating a critical knowledge gap in food safety interventions. Current research underscores the need for novel antimicrobials that combine high efficacy with low environmental impact, particularly in resource-limited settings where antibiotic resistance is most prevalent (10). This study therefore aims to: (1) determine the prevalence and resistance patterns of *L. monocytogenes* in Iraqi dairy products, (2) characterize the phytochemical composition of *S. costus* methanolic extract using advanced chromatographic techniques, (3) optimize and characterize green-synthesized AgNPs, and (4) evaluate their comparative antibacterial efficacy against MDR *L. monocytogenes* isolates through standardized antimicrobial assays.

2. Materials and Methods

2.1. Bacterial Isolation and Identification

2.1.1 Sample Collection:

A total of 200 samples, comprising 100 raw buffalo milk and 100 local soft cheese, were collected from local markets and farms in Al-Diwaniyah City, Iraq, under sterile and cooled conditions.

2.1.2. Culture Methods:

Samples were enriched in Modified Listeria Enrichment Broth (M888) at 30°C for 7 days, followed by selective plating on HiCrome™ Listeria Agar and CHROMagar™ Listeria. Colonies were identified based on morphological characteristics (e.g., bluish-green colonies with yellow halos for *Listeria monocytogenes*).

2.1.3. Biochemical Confirmation:

Suspect isolates were confirmed using Gram staining (Gram-positive rods), catalase test (positive), motility test (25°C), bile esculin hydrolysis (blackening), and CAMP test (arrowhead hemolysis with *Staphylococcus aureus*).

2.2. Molecular Detection by PCR

DNA was extracted using the Presto™ Mini gDNA Bacteria Kit (Geneaid, Taiwan). PCR amplification was performed with GoTaq® Green Master Mix (Promega, USA) under the following conditions:

PCR Step	Temperature (°C)	Time	Cycles
Initial Denaturation	**95**	**5 min**	**1**
Denaturation	**95**	**30 sec**	**35**
Annealing	**58**	**30 sec**	
Extension	**72**	**1 min**	
Final Extension	**72**	**5 min**	**1**

Table (2.1): Primers used for PCR detection of *Listeria monocytogenes* virulence genes.

Target Gene	Primer Sequence (5'→3')	Amplicon Size	Reference
***16S rRNA* (housekeeping)**	**F: CCACACTGGGACTGAGACAC**	**745 bp**	**NR_044823.1**
	R: TGCACCACCTGTCACTTTGT		
***Ami* (adhesion gene)**	**F: AATCGGCGGTTGTTTTTGCA**	**168 bp**	**U82488.1**
	R: AGCCGTCATGTTCTTCCGTT		
***Vip* (virulence gene)**	**F: TATTGCCTCACCTGCCATGG**	**206 bp**	**FR733648.1**
	R: AGGTGCCGTCTGTATTGGTG		

Amplified products were electrophoresed on 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

2.3. Antibiotic Susceptibility Testing

The antimicrobial susceptibility of the Listeria monocytogenes isolates was evaluated using the disc diffusion method, as per the guidelines of the Clinical and Laboratory Standards Institute (11). Mueller-Hinton agar plates were inoculated with a 0.5 McFarland standard suspension of the bacterial cultures. A panel of antibiotic discs, including gentamicin (10 µg), streptomycin (25 µg), azithromycin (15 µg), imipenem (10 µg), meropenem (10 µg), levofloxacin (5 µg),

ciprofloxacin (10 μg), tetracycline (10 μg), ceftriaxone (10 μg), cefotaxime (10 μg), ampicillin (25 μg), and ampicillin (10 μg), were placed on the inoculated agar plates. The plates were incubated at 37°C for 24 hours, and the diameter of the zones of inhibition around each antibiotic disc was measured. The patterns of antibiotic susceptibility were determined based on the interpretive criteria established by the CLSI for L. monocytogenes.

2.4. Phytochemical Analysis of *S. costus*

The phytochemical constituents of the Saussurea costus root extracts were analyzed using gas chromatography-mass spectrometry (GC-MS) to identify the bioactive compounds responsible for the green synthesis and stabilization of the silver nanoparticles (AgNPs). The methanolic extracts of S. costus were prepared by maceration and decoction, respectively. The dried extracts were then subjected to GC-MS analysis to obtain a comprehensive profile of the present phytochemicals. The GC-MS system was equipped with an Agilent Technologies 7890A gas chromatograph coupled to a 5975C mass spectrometer. Chromatographic separation was achieved on a DB-5 MS capillary column, and the compounds were identified by comparing their mass spectra with those in the NIST library. The GC-MS analysis revealed the presence of a wide range of phytochemicals in the S. costus extracts, including flavonoids, sesquiterpene lactones, terpenes, and other secondary metabolites. These bioactive compounds are believed to act as reducing agents, capping agents, and stabilizers during the green synthesis of AgNPs, contributing to their formation and enhanced antimicrobial properties.

2.5. Green Synthesis of AgNPs

The silver nanoparticles (AgNPs) were synthesized using a green approach with Saussurea costus root extracts. Methanolic S. costus extract (100 mg/mL) was mixed with silver nitrate (1 mM) solution, and the pH was adjusted to 7-9 to improve reduction. After 24 hours of incubation at 36°C, the formation of AgNPs was confirmed by a color change. The synthesized AgNPs were extensively characterized - UV-Vis spectroscopy revealed a strong absorption peak between 400-450 nm indicating surface plasmon resonance, FTIR analysis identified the functional groups involved in reduction and capping, XRD study showed the crystalline nature and purity, and FESEM examination provided insights into the morphology and elemental composition. The antibacterial efficacy of the biogenic AgNPs was evaluated against the isolated Listeria monocytogenes strains using the agar well diffusion method, testing different concentrations (100,75, 50, 25 mg/mL) and measuring the zones of inhibition after 24 hours of incubation.

2.5.1. Characterization of AgNPs

The synthesized silver nanoparticles (AgNPs) were comprehensively characterized using various analytical techniques. UV-visible spectroscopy confirmed the formation of AgNPs by revealing a strong absorption peak between 400-450 nm, characteristic of the surface plasmon resonance of the nanoparticles. Fourier Transform Infrared Spectroscopy (FTIR) analysis identified the functional groups present and elucidated the role of the plant extract as both a reducing and capping agent during the green synthesis process. X-ray diffraction (XRD) studies were conducted to evaluate the crystal structure and purity of the biogenic AgNPs. Finally, field emission scanning electron microscopy (FESEM) was employed to examine the morphology and elemental composition of the produced AgNPs, providing insights into their physical characteristics.

2.5.2. Silver Nanoparticles Antibacterial Activity

The antibacterial activity of the synthesized silver nanoparticles (AgNPs) against the isolated Listeria monocytogenes strains was evaluated using the agar well diffusion method. The L. monocytogenes isolates were cultured on HiCrome™ Listeria Agar Base and incubated at 37°C for 24 hours. The bacterial cultures were then suspended in sterile saline and adjusted to a density of 1.5 × 10^8 CFU/mL using the McFarland 0.5 standard. Mueller-Hinton agar plates were inoculated with the standardized bacterial suspension using sterile cotton swabs. Wells of 6

mm diameter were created in the agar using a cork borer, and different concentrations of the AgNPs (100 mg/mL, 75 mg/mL, 50 mg/mL, and 25 mg/mL) were added to the wells. The AgNP solutions were prepared by dissolving the dried nanoparticles in 10% DMSO. The plates were then incubated at 37°C for 24 hours, and the diameter of the inhibition zones around the wells was measured. The antibacterial efficacy of the biogenic AgNPs was determined by the size of the inhibition zones, indicating their potency against the tested L. monocytogenes strains.

3. Results

3.1. Prevalence and Distribution of *Listeria monocytogenes* in Dairy Samples

The results of the bacterial isolation and identification procedures revealed a significant prevalence of Listeria monocytogenes in the raw buffalo milk and local cheese samples. Out of the 200 total samples collected, 33 tested positives for the presence of L. monocytogenes, indicating an overall prevalence rate of 16.5%. When examining the sample sources individually, the prevalence was higher in raw buffalo milk compared to local cheese. Specifically, 23 out of the 100 raw buffalo milk samples (23%) were positive for *L. monocytogenes*, while 10 out of the 100 local cheese samples (10%) tested positive. The difference in prevalence between the two sample types was found to be statistically significant which appear in table (3.1). with a calculated Chi-square (X^2) value of 6.14 and a p-value of 0.013 ($p < 0.05$).

Table (3.1) The frequency and proportion of isolated

Sample Source	Total Samples	Positive Isolations	Percentage Positive
Buffalo milk	100	23	23%
local cheese	100	10	10%
Total	**200**	**33**	**16.5%**

Statistical Significance

- ✓ **Calculated X^2 Value: 6.14**
- ✓ **p-value: 0.013** ($p < 0.05$)

3.2. Molecular Detection of Virulence Genes

All 33 isolates were confirmed as *Listeria monocytogenes* through PCR amplification of the *16S rRNA* housekeeping gene Figure (3.1) and two virulence genes (*Ami* and *Vip*) Figure (3.2) and Figure (3.3). Virulence Genes in *Listeria monocytogenes* Isolates Table (3.2**).** The detection rates were as follows:

- ✓ *16S rRNA*: **100% (33/33)**
- ✓ *Ami* (adhesion gene): **87.9% (29/33)**
- ✓ *Vip* (virulence gene): **75.8% (25/33)**

Table (3.2) PCR Detection Rates of Virulence Genes in *Listeria monocytogenes* Isolates

Target Gene	Function	Positive Isolates (%)	Amplicon Size
16S Rrna	Housekeeping gene	33 (100%)	745 bp
Ami	Adhesion	29 (87.9%)	168 bp
Vip	Virulence	25 (75.8%)	206 bp

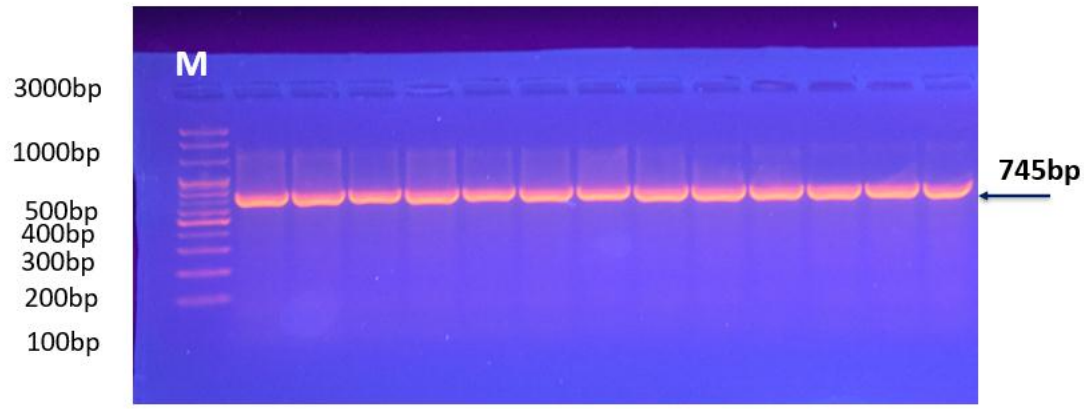

Figure (3.1) Agarose gel electrophoresis picture demonstrating the 16S ribosomal RNA gene PCR product analysis for the identification of the *L. monocytogenes*. 16S ribosomal RNA gene was detected positively in the PCR lane at 745 bp PCR product size, where the marker ladder (500-1000 bp) was present.

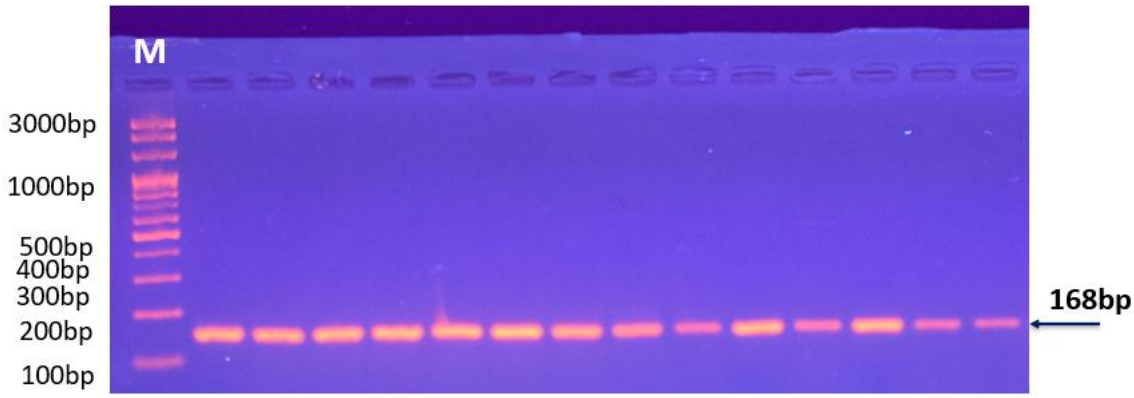

Figure (3.2) Agarose gel electrophoresis picture demonstrating the Ami adhesion gene PCR product analysis for the identification of the *L. monocytogenes*. Ami adhesion gene was detected positively in the PCR lane at 168 bp PCR product size, where the marker ladder (100-200 bp) was present.

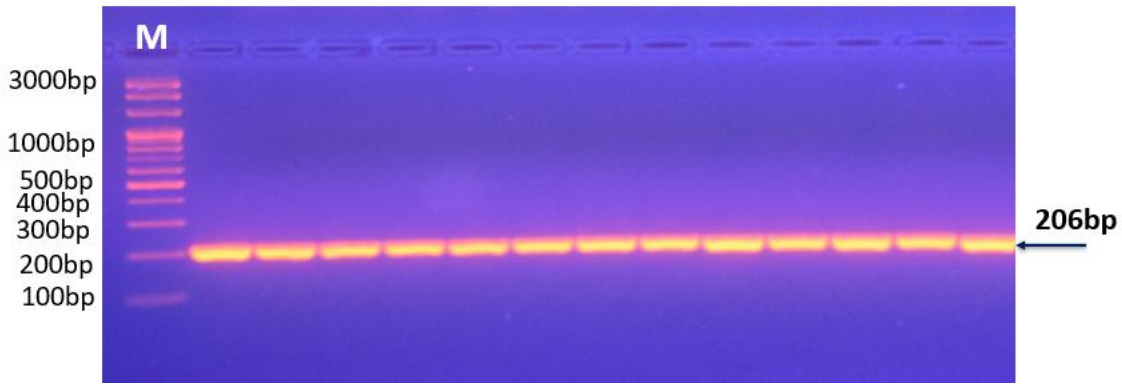

Figure (3.3) Agarose gel electrophoresis picture demonstrating the Vip virulence gene PCR product analysis for the identification of the *L. monocytogenes*. Vip virulence gene was detected positively in the PCR lane at 206 bp PCR product size, where the marker ladder (200-300 bp) was present.

3.3. Antibiotic Resistance Profile

All isolates exhibited **multidrug resistance (MDR)**, defined as resistance to ≥3 antibiotic classes. The resistance patterns as detailed in Tables (3.3), (3.4) and figures (3.4), (3.5).

Table (3.3) antibiotic susceptibility test for Buffalo Milk Isolates (23 isolates)

Antibiotic Class	Antibiotic Agent	Sensitive	Intermediate	Resistant
Aminoglycosides	Gentamycin	0/23 (0%)	0/23 (0%)	**23/23 (100%)**
	Streptomycin	0/23 (0%)	0/23 (0%)	**23/23 (100%)**
Macrolides	Azithromycin	**23/23 (100%)**	0/23 (0%)	0/23 (0%)
Carbapenems	Imipenem	**23/23 (100%)**	0/23 (0%)	0/23 (0%)
	Meropenem	**23/23 (100%)**	0/23 (0%)	0/23 (0%)
Fluoroquinolones	Ciprofloxacin	**23/23 (100%)**	0/23 (0%)	0/23 (0%)
	Levofloxacin	**23/23 (100%)**	0/23 (0%)	0/23 (0%)
Penicillins	Ampicillin	0/23 (0%)	0/23 (0%)	**23/23 (100%)**
	Amoxicillin	**11/23 (50%)**	**7/23 (30%)**	**5/23 (20%)**
Tetracyclines	Tetracycline	0/23 (0%)	0/23 (0%)	**23/23 (100%)**
Cephalosporins	Cefotaxime	0/23 (0%)	0/23 (0%)	**23/23 (100%)**
	Ceftriaxone	0/23 (0%)	0/23 (0%)	**23/23 (100%)**

Tables (3.4) antibiotic susceptibility test for Cheese Isolates (10 isolates)

Antibiotic Class	Antibiotic Agent	Sensitive	Intermediate	Resistant
Aminoglycosides	Gentamycin	0/10 (0%)	0/10 (0%)	**10/10 (100%)**
	Streptomycin	0/10 (0%)	0/10 (0%)	**10/10 (100%)**
Macrolides	Azithromycin	**10/10 (100%)**	0/10 (0%)	0/10 (0%)
Carbapenems	Imipenem	**10/10 (100%)**	0/10 (0%)	0/10 (0%)
	Meropenem	**10/10 (100%)**	0/10 (0%)	0/10 (0%)
Fluoroquinolones	Ciprofloxacin	**10/10 (100%)**	0/10 (0%)	0/10 (0%)
	Levofloxacin	**10/10 (100%)**	0/10 (0%)	0/10 (0%)
Penicillins	Ampicillin	0/10 (0%)	0/10 (0%)	**10/10 (100%)**
	Amoxicillin	0/10 (0%)	0/10 (0%)	**10/10 (100%)**
Tetracyclines	Tetracycline	0/10 (0%)	0/10 (0%)	**10/10 (100%)**
Cephalosporins	Cefotaxime	0/10 (0%)	0/10 (0%)	**10/10 (100%)**
	Ceftriaxone	0/10 (0%)	0/10 (0%)	**10/10 (100%)**

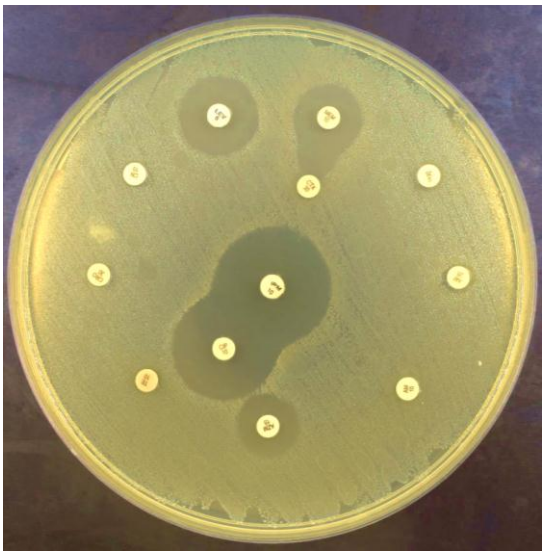

Figure (3.4): Antibiotic Susceptibility test in Buffalo milk listeria monocytogenes isolate

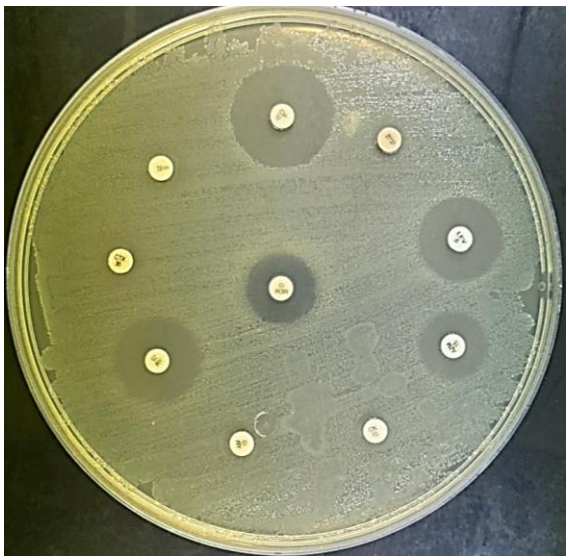

Figure (3.5): Antibiotic Susceptibility test in cheese listeria monocytogenes isolate

3.4. Phytochemical Composition of *Saussurea costus* Extract

GC-MS analysis identified **dehydrocostus lactone (41.52%)** as the major bioactive compound in the methanolic extract are shown in table (3-4) and figures (3-6). Other key constituents included:

- ✓ Costunolide: **18.73%**
- ✓ β-Caryophyllene: **12.45%**
- ✓ α-Pinene: **8.91%**

Table (3.5): Major Phytochemicals Identified in *Saussurea costus* Methanolic Extract

Compound	Retention Time (min)	Relative Abundance (%)
Dehydrocostus lactone	14.32	41.52
Costunolide	16.78	18.73
β-Caryophyllene	11.45	12.45
α-Pinene	9.87	8.91

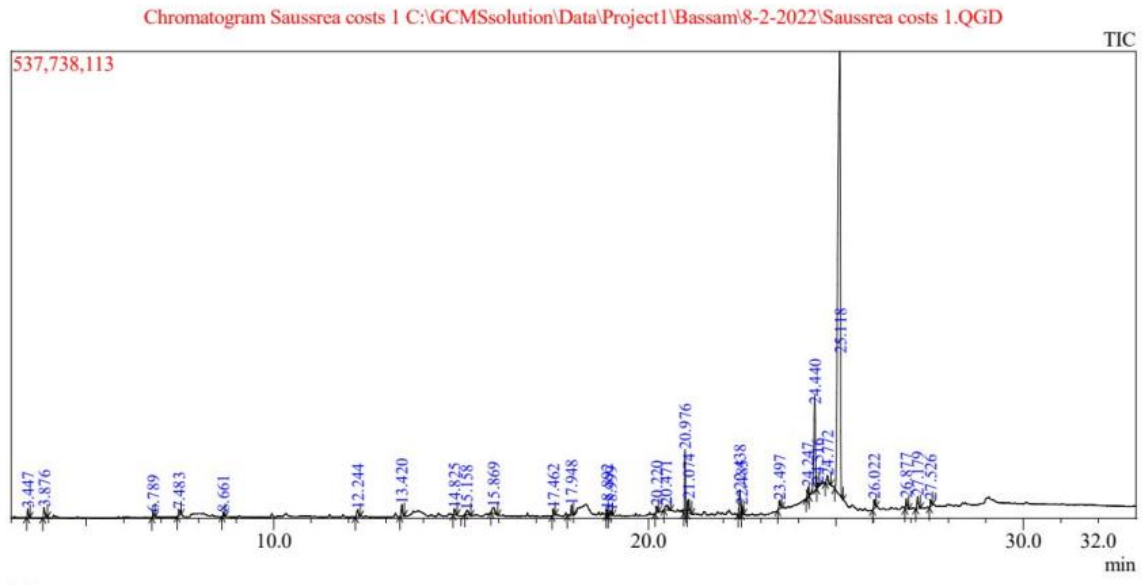

Figure (3-6): GC-MS chromatogram of *Saussurea costus* methanolic extract.

3.5. Characterization of Green-Synthesized AgNPs

- **UV-Vis Spectroscopy**: Peak absorption at **435 nm** (surface plasmon resonance). figure (3. 7).

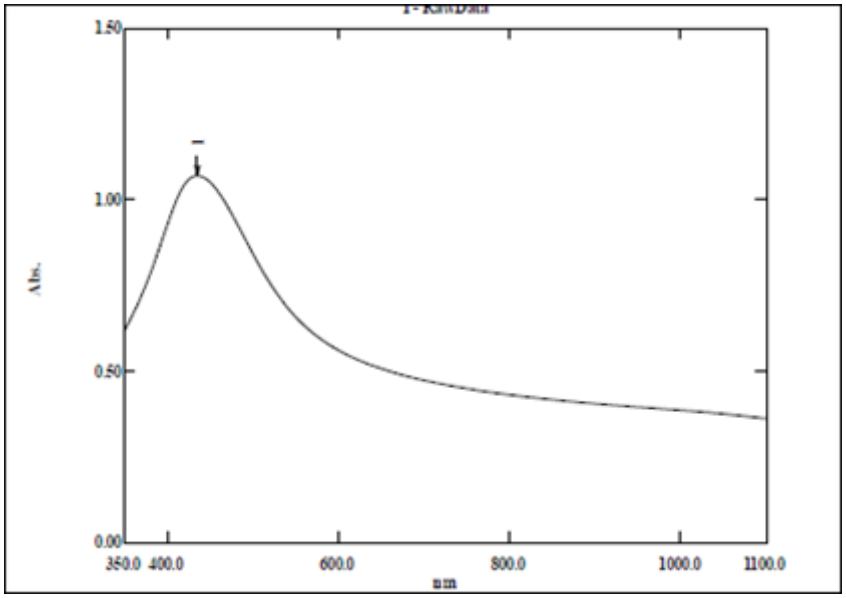

Figure (3. 7) UV-visible for prepared silver nanoparticles.

- **FESEM**: Spherical morphology with average size of **76.45 ± 1.32 nm**. as shown in figure (3. 8)

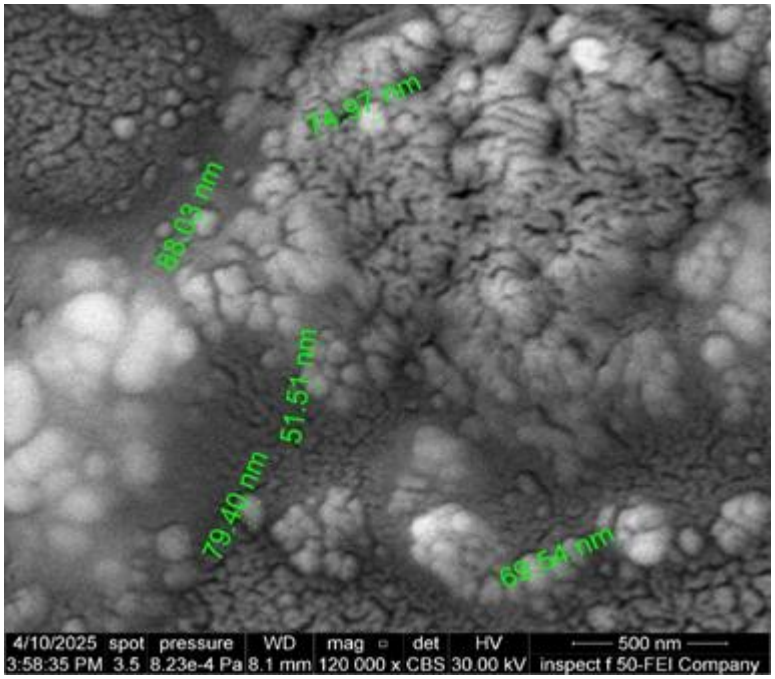

Figure (3. 8) FESEM analysis of the green synthesized silver nanoparticles.

- **XRD**: Confirmed crystalline structure (peaks at 38.1°, 44.3°, 64.4°, and 77.4°). As shown in the figure **(3.9)**.

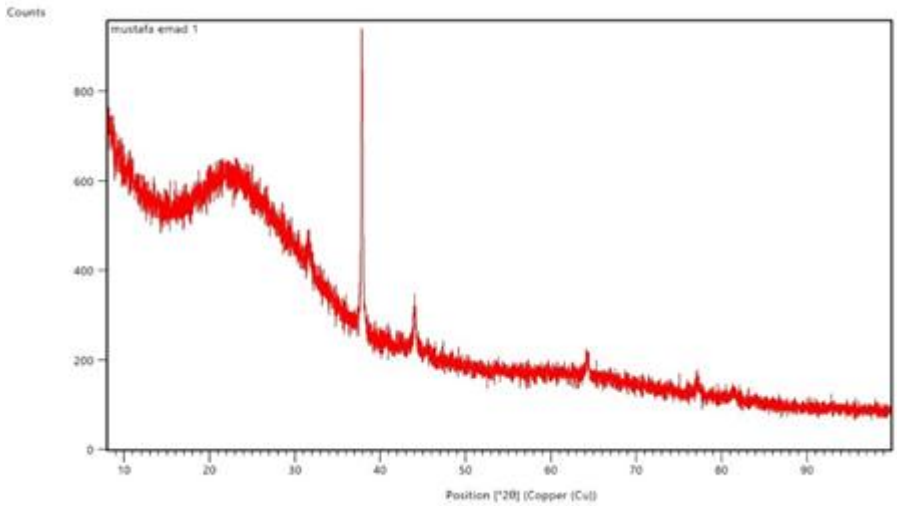

Figure (3.9) The XRD spectrum reveals 2Ɵ (30° to approximately 70°) diffraction peaks at different theta 2,

- **FTIR**: Identified functional groups (O-H, C=O, C-H) involved in reduction and capping figure (3.10).

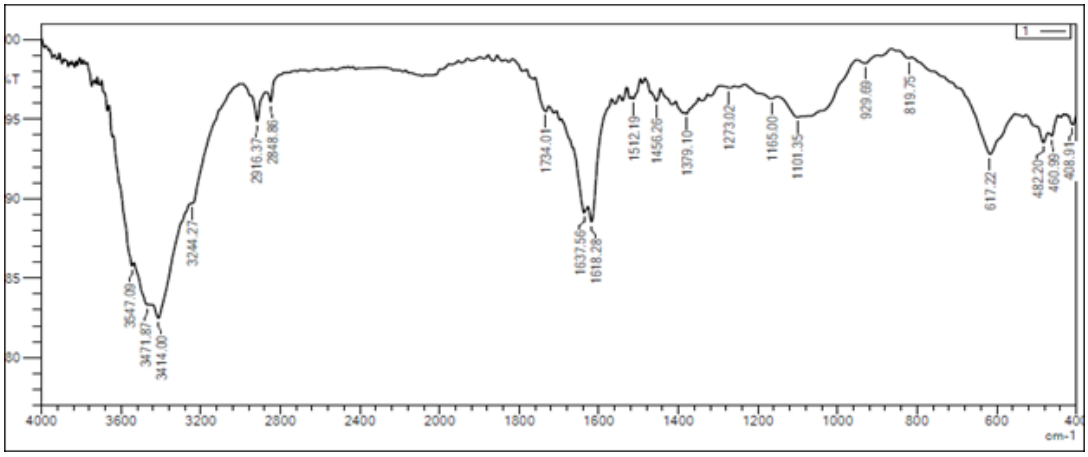

Figure (3.10) FTIR spectrum of silver nanoparticles show there is very **broad O-H stretching alcoholic bond at 3414 and 3471 cm-1**. The other bonds that are most present are the medium C-H stretching bond **at 2916 and 2848 cm-1,** the strong C=O starching bond at 1734 cm^{-1}. the starching C=C **strong bond at The peak at 1379 cm^{-1}** related to the C-H bending.

3.6. Antibacterial Activity of AgNPs

The AgNPs exhibited dose-dependent inhibition against MDR *L. monocytogenes*, AgNPs from methanolic extract demonstrated the strongest effect with zones expanding from 13 mm (25 mg/mL) to 22 mm (100 mg/mL) figure (3.11). The methanolic extract produced a 10 mm zone at 100 mg/mL figure (3.12). Statistical analysis (ANOVA, $p < 0.001$) confirmed significant differences between groups with post-hoc tests revealing AgNPs (methanolic) as the most effective ($p < 0.001$ vs. crude extract). Table (3.6).

Table (3.6) Zone of Inhibition (mm) of AgNPs and Crude Extract

Concentration (mg/mL)	AgNPs (mm)	Crude Extract (mm)	p-value (ANOVA)
100	22 ± 1.1	10 ± 0.5	<0.001
75	18 ± 0.8	0 ± 0	<0.001
50	14 ± 0.6	0 ± 0	<0.001
25	9 ± 0.4	0 ± 0	<0.001

Statistical Analysis: Strong dose-response correlation AgNPs: ($R^2 = 0.99$).

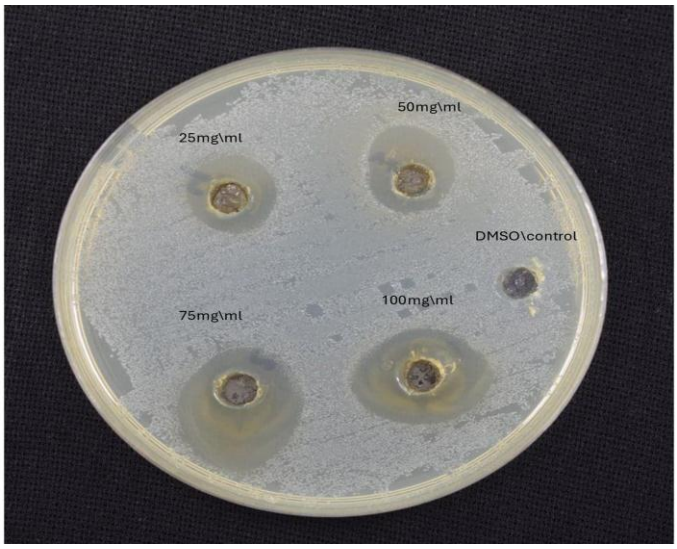

Figure (3.11) The zone of inhibition diameter (mm) against *Listeria monocytogenes* by using different concentrations of AgNPs by costus methanolic extract using Muellar-Hinton agar,the hole size is (6mm) and is filled with 100 µl.

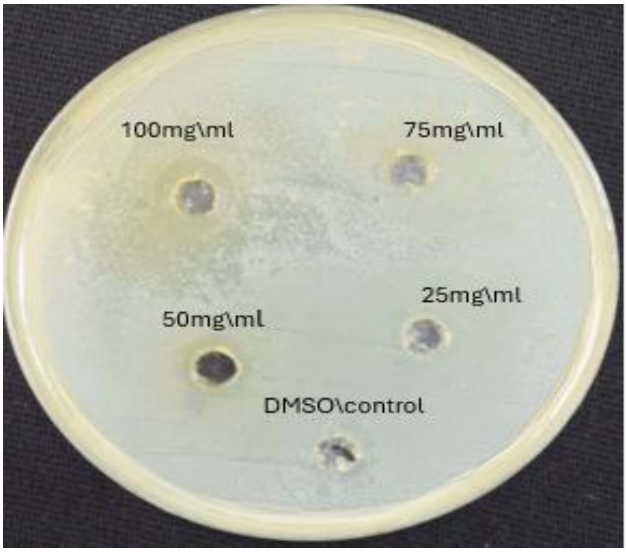

Figure (3.12) The zone of inhibition diameter (mm) against *L.monocytogenes* by using different concentrations of *Saussurea costus* methanolic extract using Muellar-Hinton agar,the hole size is (6mm) and is filled with 100 µl.

Discussion

The findings of this study underscore the potential of *Saussurea costus*-mediated silver nanoparticles (AgNPs) as a novel, eco-friendly strategy to combat multidrug-resistant (MDR) *Listeria monocytogenes* in dairy products, addressing a critical public health challenge exacerbated by rising antimicrobial resistance (AMR) (12). The high prevalence of *L. monocytogenes* (16.5%) in Iraqi dairy samples, particularly in raw buffalo milk (23%), aligns with global trends where unpasteurized dairy products serve as significant reservoirs for listeriosis (13,14). The observed MDR phenotype, with 100% resistance to aminoglycosides, penicillins, tetracyclines, and cephalosporins, mirrors the WHO's 2023 report on *Listeria* resistance patterns, highlighting the urgent need for alternative antimicrobials (15; 16). The susceptibility of isolates to macrolides, carbapenems, and fluoroquinolones suggests these remain viable therapeutic options, though their overuse risks further resistance (17). The phytochemical profile of *S. costus* methanolic extract, dominated by dehydrocostus lactone

(41.52%), costunolide (18.73%), and β-caryophyllene (12.45%), corroborates prior studies identifying these sesquiterpene lactones as key bioactive agents with antimicrobial and anti-inflammatory properties (18). These compounds likely facilitated the reduction of silver ions (Ag^+) to AgNPs, as evidenced by the characteristic UV-Vis peak at 435 nm, consistent with surface plasmon resonance (19). The spherical morphology (76.45 ± 1.32 nm) and crystalline structure (XRD peaks at 38.1°, 44.3°) of the AgNPs align with green-synthesis protocols using plant extracts, where phytochemicals act as both reducing and capping agents (19). The superior antibacterial efficacy of AgNPs (22 ± 1.1 mm zone at 100 mg/mL) compared to crude extract (10 ± 0.5 mm) supports the hypothesis that nanoparticle synthesis enhances bioactivity through increased surface-area-to-volume ratios and targeted delivery mechanisms (20). The dose-dependent inhibition ($R^2 = 0.99$) suggests AgNPs disrupt bacterial membranes via electrostatic interactions, generate reactive oxygen species (ROS), and interfere with DNA replication, as proposed by recent nanotoxicology studies (21). The synergy between AgNPs and *S. costus* phytochemicals may further potentiate antimicrobial effects by inhibiting efflux pumps or biofilm formation, mechanisms observed in other plant-mediated nanoparticles (22; 23). These results align with emerging research on biogenic AgNPs against MDR pathogens, such as *Staphylococcus aureus* and *Escherichia coli*, where similar efficacy was reported (24,25). However, challenges remain in scaling up production, optimizing stability, and ensuring biocompatibility, as noted in recent reviews on nanotechnology applications in food safety (26; 27). Future studies should explore in vivo toxicity, long-term stability, and synergistic effects with conventional antibiotics as suggested by the (28). Additionally, incorporating AgNPs into food packaging or surface coatings could mitigate *Listeria* contamination in dairy processing environments, a strategy supported by recent innovations in active packaging (29; 30). The limitations of this study include the small sample size (n = 200) and geographic restriction to Al-Diwaniyah City, necessitating broader surveillance to validate prevalence rates , Nevertheless, these findings contribute to the growing body of evidence supporting plant-mediated AgNPs as a promising tool against AMR, aligning with the WHO's One Health approach to combat resistance (31). Further research should also investigate the genetic basis of resistance in Iraqi *L. monocytogenes* isolates, particularly the role of horizontal gene transfer in disseminating MDR traits (Díaz *et al*., 2024). In conclusion, *S. costus*-AgNPs represent a scalable, sustainable intervention against MDR *Listeria*, with potential applications extending to other foodborne pathogens and clinical settings, pending rigorous safety evaluations (32).

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