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Examine the Capabilities of Biosynthetic Silver Nanoparticles as an Agent Against A549 Lung Cancer Cells

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Abstract. Targeting cancer cells using nanoparticles is a good way to get around the drawbacks of conventional radiation and chemotherapy. Certain characteristics of nanoparticles, especially silver nanoparticles (AgNPs), include their anticancer efficacy and selective toxicity. Using probiotic bacteria like *Lactobacillus plantarum* to produce them is an economical and ecologically beneficial approach in the field of nanomedicine. This study's objectives are to create silver nanoparticles (AgNPs) from *Lactobacillus plantarum* cell filtrate, evaluate their biochemical and physicochemical characteristics, and look into how detrimental they are to lung cancer cells (A549) as opposed to healthy cells (WRL-68). The particles were generated by combining cell filtrate and a silver nitrate solution. The color changed to light brown due to surface plasmon photoreduction (SPR), indicating the creation of nanoparticles. UV-Vis spectroscopy confirmed the formation, revealing an absorption peak at 260 nm. FESEM studies revealed that the nanoparticles were spherical in form, with a diameter of around 31.08 nm. FTIR analysis revealed the presence of functional groups such as hydroxyl (-OH), alkanes (C-H), carbonyl (C=O), and amide (N-H), which help to reduce and stabilize the particles. The MTT test demonstrated that the silver nanoparticles had concentration-dependent toxicity on malignant A549 cells but limited effect on normal WRL-68 cells, indicating potential selectivity. The findings indicate that silver nanoparticles produced by *Lactobacillus plantarum* are a promising and generally safe approach for anticancer therapeutic applications, emphasizing the need for further research into their molecular mechanisms of action.

Highlights:

1. Silver nanoparticles biosynthesized using Lactobacillus plantarum showed spherical shape (31.08 nm) with functional groups aiding stability.
2. AgNPs exhibited concentration-dependent cytotoxicity against A549 lung cancer cells with IC_{50} = 81.69 µg/mL.
3. Minimal toxicity was observed on normal WRL-68 cells, confirming selective anticancer potential.

Key words: Biosynthesized AgNPs, *L. plantarum*, A549 Lung Cells, Anticancer Activity.

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Introduction

Lung cancer is the leading cause of cancer-related death and the second most common kind of cancer diagnosed in the United States. Many other risk factors are unintentionally connected to the development of lung cancer, despite the fact that tobacco smoking is the predominant risk factor, accounting for 80% to 90% of all lung cancer diagnoses. However, among nonsmokers, there are few causally related risk factors for lung cancer [1]. Lung cancer is one of the most common cancers in Iraq, particularly in the Middle Euphrates region and among men. According to data from the Iraqi Cancer Registry and regional research, lung cancer incidence has gradually increased. These trends are most likely caused by increased tobacco use, air pollution, and exposure to environmental contaminants from industrial operations and regional conflicts [2, 3]. Lung cancer is the third most common type of cancer in Iraq, with men having the highest incidence. There were 3,020 new cases reported in 2023. Lung cancer affected 2,129 cases. Males accounted for 70.5% of the cases, while females made up 891 (29.5%) [4]. It is classified into two types: small cell lung cancer (SCLC), which accounts for around 13% of all cases, and non-small cell lung cancer (NSCLC), which accounts for approximately 84%. Even though NSCLC has a better prognosis than SCLC, it nonetheless presents significant challenges when advanced [5]. Because of better diagnostic procedures, more people registering for cancer, and evolving risk factor profiles, its incidence has drastically increased during the last three decades [6]. Conventional surgery, radiation therapy, and chemotherapy are the primary ways used to treat cancer; these treatments typically cause significant harm to patients and have no curative effect [7, 8]. To improve therapy outcomes, new drug development is required. Because of the unique physical and chemical features of nanoparticles, there has been a surge in interest in cancer treatments in recent years, resulting in the establishment of a new anticancer discipline known as cancer nanomedicine [9, 10]. Silver nanoparticles (AgNPs), an emerging metallic nanomaterial, have piqued researchers' interest due to their remarkable physical and chemical properties [11]. Silver nanoparticles are desirable materials in anticancer applications due to their physical and chemical properties, which include surface chemistry, structure, shape, size, particle size range, reactivity in solution, solubility rate, ion release efficiency, adsorption, and finally desorption [12, 13, 14, 15]. Nanoparticle production with biological entities, such as microbes, has gained popularity as an alternative to traditional methods. Microorganism-mediated nanoparticle production is an environmentally benign, easy, and low-cost method that, most importantly, uses no toxic chemicals. Bacteria are commonly utilized in nanoparticle production due to their repeatability and are regarded as a promising nanofactory for a variety of nanoparticles, including silver nanoparticles [16, 17]. *Lactobacillus,* a bacterium genus best known for its role in food fermentation, has emerged as an important player in biotechnological and therapeutic applications [18]. These non-pathogenic probiotic bacteria are vital for gut health because they

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boost mucosal immunity and produce bioactive substances [19]. *Lactobacillus sp.,* in particular, is being investigated for its role in cancer treatment [20]. Their capacity to modulate host immune responses and produce metabolites that kill tumor cells has positioned them as potential cancer therapeutic agents [21]. This opens up new chances to incorporate *Lactobacillus* strains. Their metabolites serve as stabilizing and reducing agents for nanoparticle production in innovative therapeutic methods, such as nanoparticle-mediated treatments [22]. The green synthesis process employs non-pathogenic bacterial species such as *Lactobacillus plantarum*. To produce enzymes, proteins, and bioactive metabolites that serve as reducing and capping agents. These activities facilitate nanoparticle production, resulting in increased biocompatibility, lower toxicity, and environmental friendliness [23].

Materials and Methods

Bacterial Isolation and Identification

Lactic acid bacteria were isolated from a locally available yogurt sample and originally grown in MRS broth under the proper incubation conditions. The cultures were then streaked onto MRS agar plates to produce pure colonies. Gram staining was used for preliminary identification based on morphological traits, and molecular identification was validated using polymerase chain reaction (PCR). The PCR output was then transferred to a specialized laboratory in Korea for gene sequencing. The sequences were examined using the BLAST (Basic Local Alignment Search Tool) tool against the NCBI database, showing that the isolate belonged to the *Lactobacillus plantarum* species [24].

Preparation of cell-free supernatant of Lactobacillus plantarum

After obtaining a sample of *Lactobacillus plantarum* from local markets, the cultures were incubated in nutrient broth at 37°C for 24 hours. Centrifuge the cultures at 10,000 rpm for 10 minutes to extract the top liquid, which was subsequently used in the biosynthesis of silver nanoparticles [25].

Biosynthesis of Silver Nanoparticles (Green Manufacturing)

Silver nanoparticles were synthesized using *Lactobacillus plantarum* culture supernatant, according to Ghyadh [26]. 45 ml of *Lactobacillus plantarum* supernatant was combined with 495 ml of 10 mM silver nitrate solution and kept in a shaking incubator at 37°C for three days. The observation of the mixture's color shift served as preliminary evidence for the creation of silver nanoparticles.

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Fig (1): Color Change as an Initial Indicator of Silver Nanoparticles Biosynthesis Using Lactobacillus plantarum Supernatant

Characterization of Silver Nanoparticles

The wavelength of the reaction fluid was determined using UV-Vis spectroscopy to confirm the presence of silver nanoparticles. The spectral measurements of the silver nanoparticles ranged from 200 to 1100 nm. This assay confirms the production of silver nanoparticles [27]. The functional groups were found via Fourier transform infrared (FTIR) spectroscopy. The silver nanoparticles' infrared spectrum ranged from 450 to 4000 cm^3 [28]. The produced silver nanoparticles' nanostructure was recorded and validated using field emission scanning electron microscopy (FESEM) [29, 30].

Silver nanoparticles synthesized using *Lactobacillus* have antitumor effects against human cancer cell lines.

Cell cultures: The National Center for Cell Sciences (NCCS) in Pune, India, provided human lung cancer cells (A549). The Roswell Park Memorial Institute-1640 medium (RPMI), which was used to grow these cells, contained bioactives such as penicillin G, streptomycin, sodium bicarbonate, and fetal bovine serum. The medium was meticulously designed to provide optimal conditions for cell development and upkeep. To provide optimal growth conditions, the cells were maintained at 37°C in a humidified, carbon dioxide-rich atmosphere [31].

Cytotoxicity Evaluation:

The MTT test was performed to measure the inhibitory concentration (IC50). A549 lung cancer cells were planted in a 96-well plate at a density of 1×10^4-1×10^6 cells/mL and cultivated for 24 hours to 80% confluence. The cells were cultured for another 24 hours before being filled with fresh media containing serially diluted silver nanoparticles (AgNPs) at concentrations of 400, 200, 100, 50, and 25 µg/mL. After 24 hours of incubation, the cells were treated with 10 µL of MTT solution per well. The cells were then incubated for four hours at 37 degrees Celsius. After removing the top liquid, add 100 µL of DMSO to each well to dissolve the formazan crystals. Incubate for 10 minutes. A Thermo-Multiskan EX ELISA plate reader was

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used to measure optical density (OD) at a wavelength of 575 nm [32]. Using the optical density data, the following procedure was used to calculate the cell viability percentages:

$$\%\, cell\ viability = \frac{OD\ of\ treated\ samples}{OD\ of\ untreated\ sample} \times 100.$$

GraphPadPrism 9.4 (GraphPadSoftware Inc., La Jolla, CA) was used to calculate the half inhibitory concentrations (IC50) of Ag NPs.

Results

Bacterial Isolation and Identification:

Following the isolation of bacteria from a local yogurt sample, a first phenotypic diagnosis was made using Gram staining, which revealed that the isolate was a Gram-positive bacillus. To improve diagnostic accuracy, molecular analysis of the 16S rRNA gene sequence was undertaken, and genetic matching results demonstrated a strong affinity for *Lactobacillus plantarum*.

Biosynthesis of silver nanoparticles

When the supernatant of *Lactobacillus plantarum* bacteria was mixed with a silver nitrate solution, it showed that it could biosynthesize silver nanoparticles. Silver nitrate salts were reduced to silver nanoparticles, with the reaction mixture yielding approximately 0.5 grams of AgNPs (Fig 2). The nanoscale properties of the synthesised silver were confirmed using the following techniques.

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Fig (2): the resulting AgNPs from the reaction mixture were approximately 0.5 grams of AgNPs.

UV-visible spectrometer analysis

A UV-Vis spectrometer with wavelengths ranging from 200 to 1100 nm was utilized to validate the synthesis and stability of silver nanoparticles (AgNPs) when the color of the reaction mixture changed, indicating AgNP formation. As shown in Fig. 3, the absorption peak was seen at a 260 nm wavelength. This is the absorption peak for silver nanoparticles synthesized using the *Lactobacillus plantarum* bacteria supernatant. This result demonstrates that silver nanoparticles are formed.

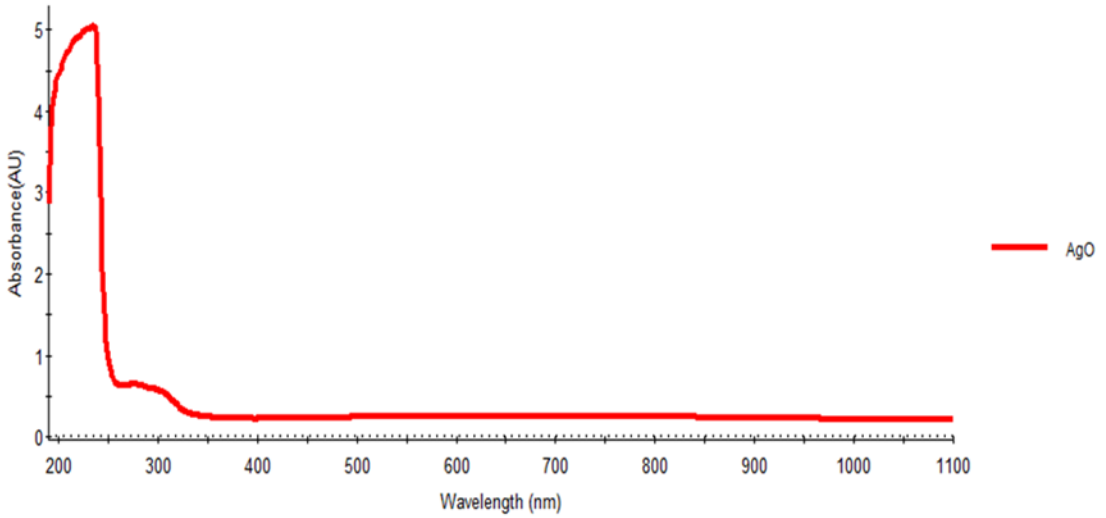

Fig (3): Absorption spectrum of silver nanoparticles synthesized using *Lactobacillus plantarum culture filtrate.*

Fourier transform infrared (FTIR) analysis

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Fourier transform infrared (FTIR) analysis was performed to determine the functional groups present in the materials under study. The FTIR spectrum of silver nanoparticles generated bio-based utilizing the cell-free supernatant of *Lactobacillus plantarum* revealed distinct peaks at wavelengths of 3882.82, 3271.85, 2924.53, 2853.96, 1743.48, 1579.54, 1385.96, 623.45, and 547.03 cm^{-1}, as shown in Fig .3. The peaks at 3882.82 and 3271.85 cm^{-1} showed the highest and broadest absorption intensity, indicating the presence of hydroxyl (O-H) stretching vibrations, reflecting the presence of phenolic or alcoholic compounds that may have contributed to the reduction of silver ions and stabilization of nanoparticles. The peaks at 2924.53 and 2853.96 cm^{-1} show C-H stretching vibrations in the alkane or alkyne groups. The peaks at 1743.48 and 1579.54 cm^{-1} represent stretching vibrations of carbonyl (C=O) and carboxyl groups, indicating the presence of acidic organic molecules. The peak at 1385.96 cm^{-1} suggests bends in the N-H bond of the amide group, suggesting the presence of proteins or peptides in the extract used. The peaks at 623.45 and 547.03 cm^{-1} indicate oscillations caused by the production of silver nanoparticles, indicating successful biosynthesis.

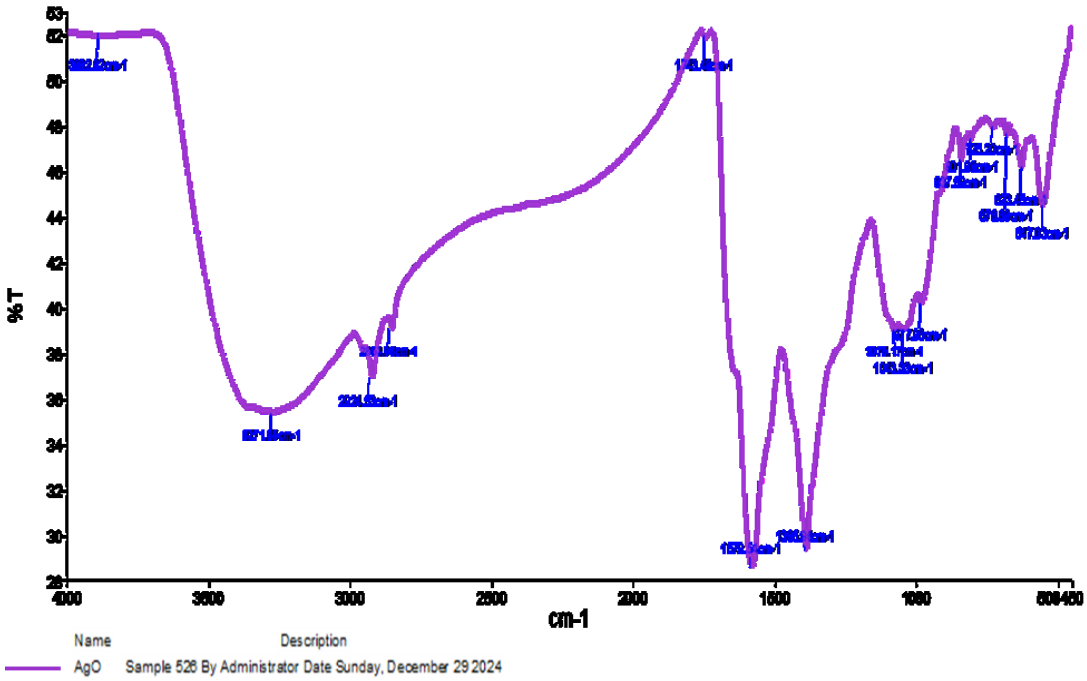

Fig (4): Fourier transform infrared (FTIR) spectrum of silver nanoparticles synthesized using *Lactobacillus plantarum* filtrate.

FESEM Analysis

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The shape of the silver nanoparticles was investigated using FESEM. The FESEM image, displayed in Fig 5, revealed that the produced silver nanoparticles were spherical in shape, and 31.08 nm in diameter.

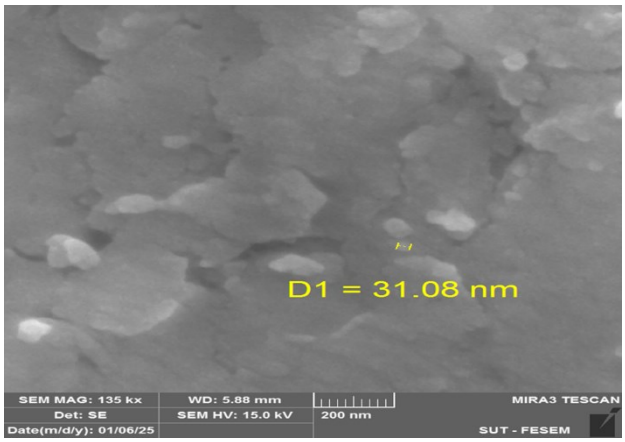

Fig (5): Scanning electron microscopic image of silver nanoparticles synthesized using *Lactobacillus plantarum* filtrate**.**

Anticancer activity:

With increasing concentrations of silver nanoparticles, cell viability declined. Fig. 6 displays the IC50 values for A549 and WRL cells, which are 81.69 and 300.4 µg/mL, respectively. The results demonstrated that adding silver nanoparticles lowered cell viability in A549 cells, and this drop was directly related to silver nanoparticle concentration.

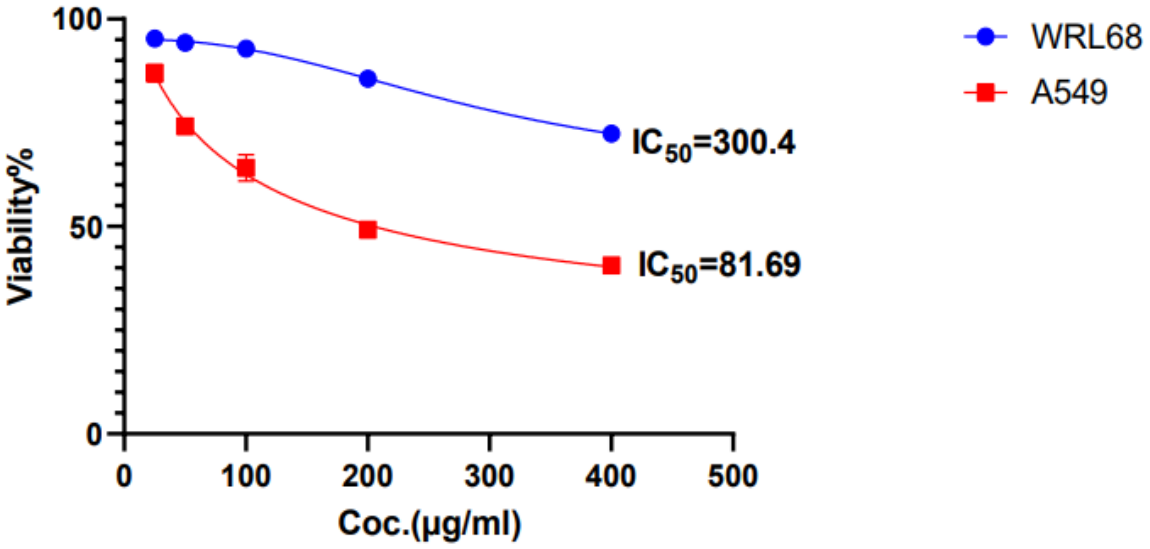

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Fig (6): Cytotoxicity of Ag NPs with A549 and normal WRL 68. Each point is the mean value of threereplicate

The viability rate decreased by 40.5 ± 1.8, 49.1 ± 1.1, 64.1 ± 3.2, 74.1 ± 1.8, and 86.9 ± 2.0 at concentrations of 400, 200, 100, 50, and 25. Adding the same concentration to WRL 68 cells did not significantly affect the viability rate, with percentages ranging from 72.3 ± 1.2, 85.6 ± 1.5, 92.9 ± 0.2, 94.2 ± 0.2, and 95.2 ± 0.3, as shown in Table 1.

Table (1): The cytotoxic effect of Ag-NPs on A549 and WRL68 cell line

Concentration Of Ag-NPs (µg/ml)	Mean viability (%) ± SD	
	A549	WRL-68
400	40.5±1.8	72.3±1.2
200	49.1±1.1	85.6±1.5
100	64.1±3.2	92.9±0.2
50	74.1±1.8	94.2±0.2
25	86.9±2.0	95.2±0.3

Discussion

Since it can release bioactive substances that aid in the stabilization and reduction of nanoparticles, *Lactobacillus plantarum*, a biosafe lactic acid bacterium, has been extensively employed in the manufacture of nanoparticles. The efficiency of *L. plantarum* as a green bioplatform for the synthesis of stable and eco-friendly nanoparticles has been demonstrated by a number of research studies, including [33, 34, 35]. According to Syame et al. [36], the addition of *Lactobacillus plantarum* cell filtrate to silver nitrate solution caused a color shift in the solution, which is a first visual sign of the formation of silver nanoparticles. This is because the SPR phenomenon occurs. By employing ultraviolet–visible (UV–Vis) spectroscopy, which showed an absorption peak at 260 nm, the synthesis of silver nanoparticles was verified. According to Ghyadh (26), this value is within the spectral region (246–356 nm), which is typical of bio-formed spherical silver nanoparticles and marks the beginning of the surface plasmon resonance (SPR) phenomenon.

The FTIR spectrum revealed the presence of functional groups such as hydroxyl (-OH), alkanes (C-H), carbonyl (C=O), and amide (N-H), which are most likely derived from *Lactobacillus plantarum's* secreted proteins, enzymes, and polysaccharides. According to Ogunleye et al. [37], these groups play a crucial role in the reduction of silver ions and the stability of the resulting silver nanoparticles. FESEM analysis revealed that silver nanoparticles

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biosynthesized with *Lactobacillus plantarum* cell filtrate exhibited a constant spherical shape with a diameter of around 31.08 nm. Previous research, such as [38], has shown that combining bacterial filtrate with active chemical compounds promotes the formation of stable, tiny nanoparticles by decreasing and stabilizing particles during biosynthesis.

The MTT test results showed that silver nanoparticles had a detrimental effect on malignant A549 cells that was concentration-dependent. However, only modest toxicity was observed at high concentrations, and their impact on normal WRL-68 cells was less obvious, especially at low concentrations. A study [39], which found that biosynthesized silver nanoparticles show selective toxicity towards cancer cells while having little impact on healthy cells, is in line with these findings. This selective impact is explained by the fact that cancer cells absorb the particles more readily, which causes reactive oxygen species (ROS) to be produced and oxidative toxicity to kill the cells.

Conclusion

Using *Lactobacillus plantarum* culture filtrate, silver nanoparticles were biosynthesized and showed anticancer activity against A549 lung cancer cells, with low harm to normal WRL68 cells, according to the MTT assay. These findings imply that silver nanoparticles could be a material with great promise for use in medicine, especially the treatment of cancer.

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