Research Article

Antibacterial Effects of Green-Synthesized Silver Nanoparticles Using *Ferula asafoetida* against *Acinetobacter baumannii* Isolated from the Hospital Environment and Assessment of Their Cytotoxicity on the Human Cell Lines

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Acinetobacter baumannii (*A. baumannii*) is a dangerous nosocomial pathogen in intensive care units, causing fatal clinical challenges and mortality. In this study, the green synthesis of silver nanoparticles (AgNPs) using the extract of *Ferula asafoetida* and the chemical synthesis of AgNPs were carried out to evaluate their effects on *A. baumannii* bacterial strain and a human adenocarcinoma cell line. The NPs were characterized using several techniques, including field emission-scanning electron microscopy, X-ray diffraction, energy-dispersive X-ray spectrometry, UV-visible spectroscopy, and Fourier-transform infrared spectroscopy. After synthesis, the arrangement of AgNPs was confirmed based on the maximum absorption peak at 450 nm. The results showed that the AgNPs had a hexagonal structure. The antimicrobial activity of biogenic NPs significantly increased and reached a minimum inhibitory concentration of 2 µg/mL. The nanomaterials did not exhibit any toxic effects on the human cell line at certain concentrations and showed improvements compared to chemically synthesized AgNPs. However, at higher concentrations (100 µg/mL), the cytotoxicity increased. Finally, it was concluded that biosynthesized AgNPs had significant antimicrobial effects on *A. baumannii* isolated from intensive care units.

1. Introduction

The ESKAPE pathogens, including *Enterobacter* spp., *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*, have developed multidrug resistance in clinics. These pathogens are associated with high levels of lethargy and mortality, imposing significant costs on patients and healthcare systems [1]. *Acinetobacter baumannii* is a Gram-negative, nonfastidious, nonfermenting, nonmotile coccobacillus responsible for respiratory infections, pneumonia, and uri-

nary tract infections [2]. This pathogen attacks unhealthy hospitalized patients and severely damages their skin and the respiratory tract [3]. It can also proliferate over different temperatures and pH ranges and use different materials as a carbon source [1].

Ventilator-associated pneumonia caused by *A. baumannii* is responsible for high mortality rates and healthcare costs, particularly in intensive care units (ICUs). There is an urgent need to develop successful pharmaceuticals instead of beta-lactams (carbapenem) against this nosocomial pathogen [4]. Among metallic NPs, silver NPs (AgNPs)

are attractive biotic nanomaterials used for biomedical purposes [5]. These NPs have been applied in different scientific areas, such as environmental science, biomedicine, chemistry, and building industries, due to their unique properties. Besides, they play a remarkable role in nanotechnology and nanoscience, especially in nanomedicine [6]. They exhibit anti-inflammatory, antioxidant, antitumor, and antimicrobial properties, leading to their broad applications in biomedicine [7]. Overall, the biogenic synthesis of NPs is considered a valuable strategy by providing more profitable NPs with higher stability and biocompatibility [8].

There are around 130 *Ferula* species (Apiaceae) worldwide, thirty of which are found in Iran. There are several reports on the antibacterial, antidiabetic, hypotensive, antispasmodic, and antiviral (HIV, H1N1, HRV, and HSV) effects of *Ferula asafoetida* (*F. asafoetida*). One of the specific characteristics of this plant is its pungent odor produced by a nonubiquitous compound, as well as significant pharmacological properties due to the presence of volatile sulfide constituents [9]. Since the antibacterial mechanism of AgNPs appears to involve interactions with the bacterial cell membrane, producing free radicals and resulting in growth inhibition, we hypothesized that the extract of *F. asafoetida* might improve the antibacterial effects of AgNPs. Therefore, it is essential to select a proper reducing agent for biogenic AgNP synthesis.

Although several medicinal plant extracts containing different reducing agents, such as phenolic, polyphenol, and flavonoid compounds, have been widely applied for the green synthesis of AgNP [10–12], there are few reports on the use of *F. asafoetida* and its natural constituents for the biosynthesis of AgNPs. *Ferula asafoetida* contains many natural metabolites, including proteins, polysaccharides, alkaloids, and alcoholic compounds, which can act as reducing agents and exert beneficial biological effects. Therefore, the combination of AgNPs with *F. asafoetida* may lead to the development of a promising antimicrobial agent against serious infectious diseases with reduced cytotoxicity.

For the first time, the present study is aimed at investigating the potential application of the aqueous extract of *F. asafoetida* (FerEX) for the green synthesis of AgNPs, evaluating its effect on *A. baumannii*, isolated from patients admitted to the pediatric ICU (PICU), and examining its potential cytotoxic effects.

2. Methods

2.1. Isolation of Bacterial Strains from the PICU. Samples of *A. baumannii* were collected from the blood, respiratory emissions, urine, and skin ulcers. They were collected from patients hospitalized in the PICU of Namazi Hospital, Shiraz, Iran. They were first transferred to tubes containing normal saline (0.9%) and serially diluted tenfold. The microbiological procedures were carried out using routine laboratory tests. After transferring the microbial plates to the microbiology research facility, cultures were prepared in MacConkey agar plates (Merck, Germany). Single colonies were confined and distinguished using routine laboratory bacteriological

tests based on their biochemical, culture, and microbiological features on Gram staining [13, 14].

2.2. Extraction of the Aqueous Extract of *F. asafoetida*. The leaves and other aerial parts of *F. asafoetida* were collected from the southern regions of Iran (Lar, Iran) in March 2019. The plant parts were then dried at room temperature. Next, the voucher specimens of *F. asafoetida* were deposited in the herbarium of the Department of Plant Protection of Shiraz University (No. #5237). After carefully washing the specimens with deionized water, different parts of *F. asafoetida* were dehydrated at 18–24°C for 14 days. Next, 41 g of powdered *F. asafoetida* was added to 500 mL of deionized water in a glass beaker and boiled for 16 hours at 50°C. Next, it was cooled, and the aqueous extract was filtered through a Whatman No. 1 filter paper. The black-brown bottle containing the extract of *F. asafoetida* (FerEX) was stored at 4°C until further analyses and biological experiments.

Fourier-transform infrared (FTIR) spectroscopy (Series Tensor II, Bruker, USA) was performed for characterization. Also, a gas chromatograph connected to a mass spectrometer (GC/MS, Agilent Technologies 5975C), which was equipped with an HP-5 MS capillary column (length of 30 m, inner diameter of 0.25 mm, and layer thickness of 0.25 μ m), was used to analyze the constituents of *F. asafoetida*. The oven temperature was increased from 60°C to 250°C (5°C per minute) and kept at 250°C for ten minutes. Helium gas was used as a carrier with a flow rate of 1.1 mL/min and ionization energy of 70 eV. The interface temperature was 280°C, and the mass range was 30–600 m/z. The essential oil constituents were identified based on retention indices (by injecting C9–C20 hydrocarbons under the same conditions as essential oil) and comparison with mass spectra, according to Wiley (nl7) and Adams' mass spectral libraries [15].

2.3. Green Synthesis of AgNPs. For the green synthesis of AgNPs, 5 mL of FerEX was poured into a glass vial by a sterile pipette. Next, 95 mL of silver nitrate (1 mM AgNO₃) was added and shaken at 25°C for 48 hours. Finally, the colored solution was centrifuged at 12,000 rpm for 20 minutes at 4°C and washed centrifugally three times. The obtained mass was dried using a vacuum evaporator, and biogenic *F. asafoetida* AgNP (Fer@AgNP) powder was stored in a nitrogen-filled container at 4°C.

2.4. Chemical Synthesis of AgNPs. According to a study by Abbaszadegan et al., AgNO₃ reduction by NaBH₄ was applied to synthesize chemical AgNPs. Briefly, 1 mL of AgNO₃ (0.1 mM) was slowly added to 20 mL of NaBH₄ (6.2 mM), which was previously chilled using ice and stirring [7]. This reaction was maintained in a dark room for 24 hours, and the mixture was stored at 4°C until further experiments.

2.5. Characterization. In this study, AgNPs and Fer@AgNP were characterized using FTIR spectroscopy (Tensor II, Bruker, USA), field emission-scanning electron microscopy (FE-SEM; Mira III, Tescan), energy-dispersive X-ray spectroscopy (XRD; Series S Max Finder Mira III, Tescan), and EDX mapping (Series S Max Locator Mira III, Tescan).

2.6. Antimicrobial Activity Assessment. A colony of *A. baumannii* was cultured in Luria–Bertani (LB) broth medium. A 24-hour cultured microbial strain suspension was inoculated into tubes containing 3 mL of Mueller-Hinton broth to obtain a suspension with 0.5 McFarland turbidity. The microorganism was then subjected to three types of antimicrobial tests, according to the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines, including well diffusion method, broth microdilution, and minimum bactericidal concentration (MBC) assay [16]. For preparation, the desired amounts of NPs were dispersed in a solution of double-distilled water. Next, they were exposed to ultrasonic waves for about one hour.

2.6.1. Well Diffusion Method. The well diffusion method was used to examine the antimicrobial effects in Mueller-Hinton agar plates. For this purpose, 100 μ L of the microbial suspension was added to a Mueller-Hinton agar plate and cultured using a sterile swab. A well with a dimension of 6 mm $\times$ 2 cm was prepared on the surface of each agar-containing plate, containing 50 μ L of each tested compound; the tested compounds included FerEX, AgNPs, and Fer@AgNPs. Carbapenem was also used as a standard antibiotic, and sterile refined water was used as control. Finally, the bacterial media were incubated at 37°C for 24 hours, and microbial colonies were examined for the developed inhibition zones; the hollow diameters (where no microorganisms grew) were measured and reported in millimeters.

2.6.2. Broth Microdilution. The broth microdilution method was used to determine the minimum inhibitory concentration (MIC) [17]. For this purpose, serial dilutions (0.5–250 μ g/mL) of each compound, dissolved in Mueller-Hinton broth, were added to 96-well plates, and the microbial suspensions were immediately added to each well. Microwells containing the culture medium were considered the negative controls, and the wells containing the media and microorganisms without any effective constituents were considered the positive controls (100% viability). Carbapenem was also assessed as a standard antibiotic. Finally, the microplates were transferred to a humidified incubator and maintained overnight at 37°C. The absorption of each well was read at 600 nm using a microplate reader, and the viability percentage of microorganisms in each tested well was calculated compared to the positive control. The concentration of each compound inhibiting the growth of *A. baumannii* by 90%, as compared to the control group, was considered as the MIC.

2.6.3. MBC Assay. The microorganisms were cultured overnight in a brain-heart infusion (BHI) medium, and a stock with a concentration of 10^5 – 10^6 CFU/mL was prepared. Next, 50 μ L of different compounds (concentrations of 0.5 to 250 μ g/mL) was added to a 96-well microplate, containing 40 μ L of BHI and 10 μ L of *A. baumannii* suspension. The plates were incubated overnight at 37°C. A volume of 10 μ L from each tested well (including the controls) was added to a BHI agar plate and transferred to an incubator for another 24 hours at 37°C to examine the bactericidal effect of each

compound. The concentration of each compound causing no growth of microorganisms was regarded as the MBC.

2.7. Cytotoxicity Assay (MTT Assay). The cytotoxicity of Fer@AgNP was evaluated on the MCF-7 human cell line, using the MTT assay, according to a study by Gholami et al. [18]. In this study, the MCF-7 cell line was prepared by the Cell Bank of Pasteur Institute of Iran (NCBI code: C135). The cells were maintained in DMEM medium, containing 25 mM of glucose, 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 μ g/mL). The cells were incubated at 37°C with 5% CO₂ at 95% humidity. Briefly, the cell lines were suspended in the RPMI-1640 medium and seeded into a cell culture plate. The cells were maintained under standard conditions (5% CO₂, 95% humidity, and temperature of 37°C) for 24 hours.

The RPMI-1640 medium was used to prepare different concentrations of each compound (1–500 μ g/mL), which were later added to each well, containing cells attached to the bottom after removing the previous culture medium. The cells were incubated again for 24 hours, and 25 μ L of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution was added to each well and incubated for four hours. To solubilize MTT-formazan crystals, 100 μ L of dimethyl sulfoxide (DMSO) was added to the mixture and shaken for ten minutes [19]. An ELISA plate reader (BioTek, Winooski, VT, USA) was also utilized to calculate the absorbance of each well at a wavelength of 540 nm, compared to the equivalent well of untreated cells. Cell viability was calculated using the following equations:

$$\% \text{Cell viability} = \left(\frac{\text{OD}_{\text{cells+compounds}} - \text{OD}_{\text{compounds}}}{\text{OD}_{\text{cells+media}} - \text{OD}_{\text{media}}} \right) \times 100, \quad (1)$$

where OD represents the optical density and the cell viability percentage is the percentage of living cells after the test.

2.8. Statistical Analysis. The results of biological assays were analyzed using IBM SPSS software. One-way ANOVA, followed by Tukey's post hoc test, was used to evaluate differences between the groups. All biological experiments (both antimicrobial tests and cytotoxicity assays) were performed in triplicate, and $P \leq 0.05$ was considered statistically significant.

3. Results and Discussion

For the first time, this study is aimed at evaluating the biogenic synthesis of AgNPs using the extract of *F. asafoetida* as a reducing agent. This extract contained important biological components, such as proteins and ethylene groups, and could act as a capping/stabilizing agent. Their main components were investigated using a GC/MS apparatus.

3.1. *Ferula asafoetida* Constituents. A GC/MS apparatus was used to determine the major chemical components of *F. asafoetida*. The results showed that *F. asafoetida* contained 35 chemical constituents, including propenyl butyl disulfide

TABLE 1: The principal chemical compounds of *Ferula asafoetida* essential oil.

Number	Kovats index	Compounds	Area (%)	Analysis
1	905	3,4-Dimethylthiophene	0.05	GC
2	915	5,5-Dimethylpyrazolidin-3-one	0.03	GC
3	933	α -Pinene	1.1	GC
4	947	Camphene	0.16	GC
5	975	β -Pinene	15.69	GC
6	990	Myrcene	1.4	GC
7	999	Decane	1.02	GC
8	1012	2,3,4-Trimethylthiophene	0.62	GC
9	1016	α -Terpinene	0.04	GC
10	1029	Limonene	0.51	GC
11	1045	β -Ocimene	17.34	GC
12	1059	γ -Terpinene	0.06	GC
13	1088	α -Terpinolene	0.41	GC
14	1107	Dipropyl disulfide	0.16	GC
15	1118	Tetramethylthiophene	0.07	GC
16	1130	Alloocimene	0.66	GC
17	1163	n-Propyl sec-butyl disulfide	0.75	GC
18	1172	(E)-1-Propenyl sec-butyl disulfide	55.4	GC
19	1200	Dodecane	0.33	GC
20	1212	Bis(1-methyl propyl) disulfide	0.57	GC
21	1260	Carvacrol	0.59	GC
22	1300	Tridecane	0.05	GC
23	1392	(E)-3-Tetradecene	0.14	GC
24	1399	Tetradecane	0.22	GC
25	1452	Selin-4,7(11)-diene	0.13	GC
26	1462	α -Humulene	0	GC
27	1471	(+)-Epi-bicyclosquiphellandrene	0.04	GC
28	1485	β -Chamigrene	0.03	GC
29	1498	Cadina-1-4-diene	0.11	GC
30	1506	β -Dihydro agarofuran	0.11	GC
31	1513	β -Bisabolene	0.07	GC
32	1520	γ -Cadinene	0.09	GC
33	1530	δ -Cadinene	0.12	GC
34	1546	Aristolene	0.02	GC
35	1600	Hexadecane	0.03	GC
36		Unknown	1.88	GC

(55.4%), α -pinene (1.1%), camphene (0.16%), β -pinene (15.69%), myrcene (1.4%), decane (1.02%), limonene (0.51%), β -ocimene (17.34%), γ -terpinene (0.06%), α -terpinolene (0.41%), carvacrol (0.59%), and n-pentacosane (2.13%). All of the main chemical constituents of *F. asafoetida* are summarized in Table 1.

Several studies have investigated the components of *F. asafoetida*. Although there are some differences in the discovered constituents and their amounts, the main components are nearly the same [20]. Among the distinguished chemical components of *F. asafoetida*, heptane belongs to the alkane group with seven carbon particles. This colorless liquid is insoluble in polar solvents, with gasoline-like smells. Besides,

decane is a profoundly combustible natural compound from the alkene group. This compound is nonpolar and insoluble in water, similar to other alkanes [21].

Moreover, a cyclic monoterpene, namely, limonene, was found in *F. asafoetida*. It has a smell similar to orange and has been used to synthesize chemical materials in household cleansers and renewable energies. This hydrocarbon can be effectively oxidized in humid environments and may be a chiral particle [22].

Terpineol is a monoterpene derived from petitgrain oil, pine oil, and petroleum. It is commonly used in fragrances, makeup products, and flavors and has a pleasant odor similar to jasmine [23]. Thymol and carvacrol, as two well-known

FIGURE 1: The FTIR spectra of (a) FerEX and (b) Fer@AgNP.

terpenes, revealed significant antioxidant, antifungal, and antibacterial activities. Besides, numerous studies showed their potential on bacterial strains at lower concentrations [24]. These compounds and some other structures found in the plant extract can functionalize AgNPs and significantly improve their biological efficacy.

3.2. Characterization of Biogenic NPs. The FTIR analysis was performed to confirm the functional groups of biomolecules involved in capping, viable stabilization, and reduction of synthesized Fer@AgNP. The FTIR spectra of FerEX and Fer@AgNP (Figure 1) showed nine peaks at 3405, 2427, 2359, 2100, 1788, 1635, 1338, 1048, and 832 cm^{-1} , respectively. The peak at 3405 cm^{-1} in the chemically synthesized AgNPs was related to the N-H bond of amide and O-H of hydroxy groups, extending to phenols/alcohols or bending/stretching hydrogen-bonded phenols/alcohols in the extract. The peak at 2427 cm^{-1} was related to the C-H bond of alkanes. Also, the peaks at 2359 cm^{-1} to 2100 cm^{-1} were related to C $\equiv$ N and C $\equiv$ C stretching in the aromatic/aliphatic compounds.

The peaks at 1788 to 1635 cm^{-1} were related to the C=C stretching of fragrant compounds. Another study reported that these peaks were related to the carbonyl (C=O) groups of proteins. Besides, the peak at 1338 cm^{-1} represented the CH₃ group of carbohydrates. Also, the peak at 1048 cm^{-1} was related to alkanes, ethers, esters, alcohols, and the C=O in-plane bending of carboxylic acids. Consistent with previous reports, the peak at 832 cm^{-1} was related to the =CH stretching of bicyclic monoterpenes [25]. In previous studies, the FTIR bands at 3405, 2100, 1788, 1635, 1048, and 832 cm^{-1} indicated the properties of AgNPs [26], and the presence of other bands confirmed the proximity of different biomolecules, which played a significant role in stabilizing Fer@AgNP. AbuDalo et al. also concluded that these bands represented the presence of aromatic compounds, alkanes, and amide bonds of proteins and amines in the structure of

biogenic AgNPs, which contributed to the production and stability of NPs [27]. In particular, for the C=O bond, a slight shift represented adsorption on the surface and suggested that this bond is related to the hydroxyl group stretching bands in biological macromolecules, such as proteins or polysaccharides found in the *F. asafotida*.

In Figure 2, the FE-SEM images of chemical AgNPs and green-synthesized biogenic Fer@AgNPs are presented. As shown in Figure 2, both chemical and biogenic NPs had circular structures, with an average size of 10 ± 2.77 nm for Fer@AgNP and 19.5 ± 5.1 for chemically synthesized AgNP (RSD < 3% for $n = 10$). Several studies have reported that most biogenic AgNPs have a spherical geometry, with a size range of 5 to 500 nm; however, different biological activities have been reported [27]. Our observations showed that Fer@AgNP had higher colloidal stability than chemically synthesized NPs after seven days of being maintained in the dark at 23–25°C with low particle agglomeration. It seems that the stability of Fer@AgNP is related to steric stabilization by natural surfactants [28] found in the FerEX, as described in Section 3.1.

The constituents of biogenic AgNPs were evaluated by the EDX analysis. The peaks were observed between 2 keV and 5 keV, confirming the presence of Ag. Figure 3 and Table 2 demonstrate the distinct presence of Ag peaks at 3 keV for Fer@AgNPs, which is a specific characteristic of AgNPs with weight and atomic percentages of 11.6% and 7.7%, respectively. The high atomic and weight percentages of organic elements, such as C, N, and O, revealed the possible presence of organic compounds, such as polysaccharides, phenols, and proteins; these compounds have also been reported in some studies [29].

X-ray diffractometry was used to confirm the crystalline structure of synthesized AgNPs. As shown in Figure 4, the hexagonal structure of Ag crystals was found, with Ag in a cubic form. The X-ray image indicated AgNPs, and a few diffraction peaks were observed at $2\theta = 38, 44, 65$, and 78

FIGURE 2: The FE-SEM image of (a) chemically synthesized and (b) biogenic AgNPs.

corresponding to the (111), (200), (220), and (311) planes, respectively. These peaks might be attributed to the natural components of *F. asafotida*, indicating the biogenic synthesis of Fer@AgNP [30]. In some studies, the crystalline structure of biogenic AgNPs synthesized using plant extracts is comparable with the crystalline structure of AgNPs synthesized using *F. asafotida* [31, 32]. Equation (2) (Debye-Scherrer equation) was used to measure the average crystalline size of nanoparticles.

$$D = \frac{k\lambda}{\beta \cos \theta}, \quad (2)$$

where D is the average size of the nanoparticles, k is the Scherrer equation (0.9), λ is the X-ray radiation wavelength, and β is the angular full width at half maximum (FWHM) of XRD peaks at diffraction point θ [33]. The average crystalline size of the biogenic Fer@AgNP using FerEX was ~ 42 nm.

As illustrated in Figure 5, a characteristic peak was observed at around λ 450 nm for AgNPs using UV-VIS spectroscopy (Figure 5(b)). The obtained range, which was consistent with previous reports, was contributed to the AgNP formation. Several studies reported the AgNP formation occurred in the wavelength of 400 to 500 nm [34]. The on-site observations and the UV-VIS spectrogram showed reducing Ag^+ ions into Ag^0 , besides the green synthesis of AgNPs.

3.3. Antibacterial Effects against *A. baumannii*. The antimicrobial effect of Fer@AgNP against *A. baumannii* was examined using the well diffusion method, and the results were compared with those obtained for AgNPs synthesized by the chemical method. The data were also evaluated based on the MICs and MBCs. The FerEX created an inhibition hollow against the microorganisms at high concentrations (50–200 $\mu\text{g/mL}$). As shown in Figure 6, the anti-*Acinetobacter* effect of all compounds was dose-dependent. The viability of bacterial strains reduced by increasing the concentration of NPs. The Fer@AgNP showed a higher antimicrobial efficacy

compared to AgNPs. The mean of three replicates for the width of the inhibition zone (in millimeter) is presented in Table 3.

Among the tested compounds, Fer@AgNP showed a more significant inhibitory effect against *A. baumannii*. At the highest concentration (200 $\mu\text{g/mL}$), the inhibition zone was measured at 14 ± 3 mm for FerEX, 19 ± 3 mm for AgNP, and 25 ± 5 mm for Fer@AgNP. There was no significant difference between Fer@AgNP and the standard antibiotic (carbapenem). Also, smaller inhibition zones were found for the FerEX. The MIC and MBC were measured to be 31.25 and 31.25 $\mu\text{g/mL}$ for the chemically synthesized NPs and 2 and 2 $\mu\text{g/mL}$ for the biogenic NPs, respectively. These findings revealed that the antimicrobial potential of biogenic NPs was significantly higher than chemical AgNPs (Figure 6 and Table 4). The optimal MIC and MBC were obtained by carbapenem at a concentration of 1 $\mu\text{g/mL}$. Also, the antimicrobial properties of FerEX were demonstrated at high concentrations (MIC = 125 $\mu\text{g/mL}$ and MBC = 250 $\mu\text{g/mL}$).

Huang et al. synthesized biogenic AgNPs using a plant extract of *Cacumen platycladi* and evaluated their antimicrobial effects and mechanism [35]. According to their results, the MIC and MBC against *Staphylococcus aureus* and *Escherichia coli* were 5.4 and 5.4 $\mu\text{g/mL}$ and 1.4 and 27 $\mu\text{g/mL}$, respectively; the present results showed better MIC and MBC values than the study by Huang and colleagues. Another study evaluated the antimicrobial effects of biogenic AgNPs using aloe vera. The MIC of aloe vera-synthesized AgNPs against *S. epidermidis* was 10 $\mu\text{g/mL}$, weaker than our finding [36]. This significant biological effect might be due to the strong antimicrobial effects of functional groups on Fer@AgNP, such as thiol groups.

The similar MBC and MIC values of nanomaterials compared to carbapenem revealed that their antimicrobial mechanisms could be bactericidal; this argument has been proposed in several previous studies [37]. On the other hand, the differences between the MIC and MBC values of FerEX might be attributed to the bacteriostatic activity of this natural product. Several studies have shown that AgNPs, green synthesized by plant extracts, have remarkable antibacterial

(a)

(b)

FIGURE 3: The EDX results of (a) chemical (AgNP) and (b) biogenic AgNPs (Fer@AgNP).

effects [38–40]. These biogenic AgNPs significantly inhibit the growth of a wide range of microbial pathogens, such as *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Azotobacter chroococcum*, *Bacillus licheniformis*, *Staphylococcus aureus*, and *Candida albicans*, which are involved in more common hospital-acquired infections [33, 41].

Biogenic AgNPs showed the most significant antimicrobial activity against *A. baumannii*, a dangerous pathogen in ICUs and PICUs. Our results revealed the higher viability of biogenic AgNPs against this bacterial strain than chemically synthesized AgNPs. In this regard, Singh et al. measured the MIC against *A. baumannii* to be 16 $\mu\text{g/mL}$, while the

TABLE 2: Quantitative EDX results of chemically synthesized (AgNP) and biogenic NPs (Fer@AgNP).

Compounds	Elements	Intensity	Weight %	Atomic %
AgNPs	Ag	1556.5	100	100
	C	79.6	13.89	18.93
	N	64.9	18.59	21.64
	O	328.0	42.20	42.42
Fer@AgNP	Na	475.2	12.30	8.60
	Ag	173.3	11.69	7.70
	Cl	7.1	0.08	0.04
	K	27.3	0.35	0.14
	Ca	19.1	0.26	0.11
	Mg	32.4	0.63	0.42

FIGURE 4: The XRD spectra of synthesized Fer@AgNP by FerEX for determination of Ag crystals.

FIGURE 5: The UV-visible spectra of (a) the aqueous extract of FerEX and (b) biogenic AgNP.

present study found a MIC of 2 $\mu\text{g/mL}$ [41]. They proposed a synergistic relationship between bacteriogenic AgNPs and some antibiotics. They clarified the potential role of these antibiotics in combination with AgNPs in facilitating the permeabilization of AgNPs through the external cell layer by displacing Mg^{2+} or Ca^{2+} , especially in the lipopolysaccharide layer. Inhibition of UDP-3-O-(R-3-hydroxymyristoyl)-N-

acetylglucosamine deacetylase (LpxC) by natural compounds is another *in vivo* mechanism that disables *A. baumannii* and increases the opsonization of bacteria by macrophages [42]. This may suggest that functional groups on the surface of Fer@AgNP, particularly compounds containing thiol groups, help NPs to attach to lipopolysaccharides (LPS) of Gram-negative *A. baumannii* through biogenic synthesis and facilitate the integration of NPs.

Bhatnager et al. studied the antibacterial activity of *F. asafetida*, extracted with water (W), hexane (H), ethanol (E), and petroleum ether (P). They used biomass to calculate the zone of inhibition (*E. coli*: 8 mm (P), 8 mm (H), 12 mm (W), and 7 mm (E); *S. aureus*: 7 mm (P), 11 mm (H), 7 mm (W), and 7 mm (E); *K. pneumoniae*: 7 mm (P), 9 mm (H), 8 mm (W), and 8 mm (E); *S. flexneri*: 13 mm (P), 15 mm (H), 7 mm (W), and 11 mm (E); and *E. faecalis*: 8 mm (P), 7 mm (H), 9 mm (W), and 7 mm (E)) [43]. Although Bhatnager et al. did not investigate the concentration dependence of the antibacterial effect of FerEXs, the antimicrobial activity was observed at the highest concentrations (up to 100 $\mu\text{g/mL}$). The anti-*Acinetobacter* effect of AgNPs was increased when they were synthesized in the presence of the aqueous extract of FerEX. The increased antimicrobial effect of biogenic AgNP compared to chemical AgNP may be due to the combination of NPs with the antibacterial constituents of FerEX extract, especially thiol-containing substances.

The MIC and MBC values of Fer@AgNP against *A. baumannii* were more compelling than those obtained in other experiments using other plant extracts or living microorganisms from different plants or living organisms (*Neurada procumbens* [44], *Xanthomonas* spp. [45], *Eucalyptus citriodora* [46], and *Acinetobacter calcoaceticus*) to synthesize biogenic AgNPs. The bacterial growth was inhibited at a concentration range from 20 $\mu\text{g/mL}$ to 500 $\mu\text{g/mL}$. Besides, the MIC and MBC values of Fer@AgNP revealed its higher antibacterial potential than chemically synthesized AgNPs. The superior antibacterial activity of Fer@AgNP compared to its chemically synthesized counterpart may be attributed to the smaller size of NPs, which offers a larger surface/volume ratio and results in their superior binding and transfer through the bacterial membrane or cellular proximity [47]. Besides, higher colloidal stability helps Fer@AgNP to be better distributed in biological environments as compared to AgNPs and to aggregate less [48–50]; this phenomenon increases the probability of NPs passing through biological membranes.

Although the antimicrobial mechanism of biogenic AgNPs and chemically synthesized AgNPs is still unknown, several mechanisms can play a role in their biological activities. When synthesized with various plant extracts, AgNPs can physically attach to bacteria and even eukaryotic cell surfaces and disrupt their integrity [51]. Internalization of the cytoplasm, interaction with cellular organelles and macromolecules, and finally production of reactive oxygen species (ROS) are the main biological mechanisms of biogenic AgNPs in the literature [52]. It is known that enhancement of ROS capacity causes membrane damage by increasing permeability, leading to the disruption of the electron transfer

FIGURE 6: Antimicrobial effects of different concentrations of *Ferula asafoetida* aqueous extract (blue), chemically synthesized AgNPs (red), biogenic AgNPs (green), and carbapenem (purple) as the standard antibiotic against *A. baumannii* using the microdilution method.

TABLE 3: Antimicrobial susceptibility well diffusion method. Zones of inhibition of the aqueous extract of *Ferula asafoetida* (FerEX), chemically synthesized AgNPs, and green synthesized AgNPs (Fer@AgNP) against *A. baumannii*.

Compounds	Hollow diameter (mm)					
	1 μg/mL	10 μg/mL	25 μg/mL	50 μg/mL	100 μg/mL	200 μg/mL
FerEX	0 ± 0	0 ± 0	7 ± 2	9 ± 2	13 ± 2	14 ± 3
AgNP	0 ± 0	0 ± 0	9 ± 2	11 ± 3	15 ± 1	19 ± 3
Fer@AgNP	0 ± 0	8 ± 2	16 ± 3	16 ± 2	21 ± 3	25 ± 5
Carbapenem	16 ± 3	21 ± 4	23 ± 4	25 ± 2	25 ± 4	27 ± 3

TABLE 4: Quantitative MBC results of biosynthesized AgNPs.

Compounds	Concentrations (μg/mL)									
	0.5	1	2	4	8	16.1	31.25	62.5	125	250
FerEX	+	+	+	+	+	+	+	+	+	–
AgNP	+	+	+	+	+	+	–	–	–	–
Fer@AgNP	+	+	–	–	–	–	–	–	–	–
Carbapenem	+	–	–	–	–	–	–	–	–	–

chain and leakage of the cellular content. Besides, it can alternatively damage human cells and cause severe adverse effects. Therefore, in this study, we evaluated the effect of Fer@AgNPs on a cancerous human cell line as an indicator of cytotoxic effect and an anticancer agent.

3.4. Cytotoxic Effect of Fer@AgNPs. Several studies have assessed the anticancer effects of plant extracts. However, there is no report or article on the efficacy of synthesized Fer@AgNP in the selected cell lines. In this study, the effects

of FerEX, AgNP, and Fer@AgNP were assessed on the MCF-7 human cell line by the MTT assay. Figure 7 shows that both NPs (AgNP and Fer@AgNP) showed increased cytotoxic effects against the MCF-7 cell lines at concentrations from 200 to 500 μg/mL in comparison with FerEX. By decreasing the concentration, the impact of compounds is essentially reduced. Based on the findings, at concentrations below 200 μg/mL, the effect of FerEX on the human cell lines reduced.

On the other hand, for AgNP and Fer@AgNP, the cell survivability and antioxidant levels (IC₅₀) were 10 and 100 μg/mL, respectively, on the MCF-7. Therefore, Fer@AgNP had no antagonistic effects on the assessed cell lines as compared to AgNP, which exerted a significant cytotoxic effect on the MCF-7 cells. So far, many attempts have been designed to distinguish the effect of silver nanoparticles against various tumors. One of the broadly acknowledged hypotheses has ascribed the potential generation of reactive oxygen species created by nanoparticles and their ions, preventing cell growth and eventually cell apoptosis [8, 53].

FIGURE 7: The MTT assay for confirming the cytotoxic effect of the extract (SS extract, red), chemically synthesized AgNPs (control, grey), and green synthesized AgNPs (AgNPs, blue) on the MCF-7 cell lines during 48 hours.

Finally, this study showed the plausibility of the biological synthesis of AgNPs using an aqueous extract of FerEX. The presence of thiol-containing groups, alkyl halides, and other reducing agents in the extract of FerEX allows for reducing Ag particles into NPs. The green-synthesized Fer@AgNP showed high antibacterial activity against *A. baumannii*. Interestingly, these green-synthesized NPs did not initiate a significant reduction in the cell viability of human adenocarcinoma cells (MCF-7), except at elevated concentrations.

The emergence of multidrug-resistant bacteria, especially in the hospital ICUs, has limited the use of standard antibiotics and has led researchers to incorporate AgNPs and natural products. In our study, *A. baumannii* isolated from the PICUs was eliminated using biogenic AgNPs. This nanomaterial is more advantageous than chemically synthesized AgNPs and even conventional antimicrobial agents. Therefore, developing this type of NPs can be considered an alternative strategy to overcome multidrug resistance in bacteria, especially *Acinetobacter* strains in ICUs.

Future studies need to examine the long-term aspects of antimicrobial activity, such as inhibition of biofilm formation, and investigate the other potential advantages of this biogenic AgNP, especially in terms of biocompatibility and biodegradability. Overall, finding newer antimicrobial agents using biocompatible nanomaterials is essential to eradicate nosocomial infections, especially those caused by *A. baumannii*. According to the present study, Fer@AgNPs could be an excellent option to overcome these infections, possibly at lower and less toxic concentrations than what is used clinically today. Also, the combination of this biogenic nanomaterial with effective antibiotics, such as carbapenem, can be investigated in future studies.

4. Conclusion

This study is the first extensive report to demonstrate the antibacterial activity of biogenic AgNPs against a nosocomial

pathogen (*A. baumannii*) and evaluate its human cell cytotoxicity. The biogenic NPs exhibited significant antibacterial activities against this ESKAPE pathogen. The AgNPs produced by *F. asafotida* inhibited bacterial growth and showed a higher potency compared to chemically synthesized AgNPs, which might be due to disruptions in the cell wall and formation of ROS. The cytotoxic assessments showed the acceptable biocompatibility of Fer@AgNPs at lower concentrations. However, it should be noted that killing bacteria is highly specific to bacterial strains in terms of cell wall composition, growth rate, biofilm formation capacity, and type of NPs. Overall, different synthesizing procedures, sizes, and shapes can render AgNPs with variable antimicrobial properties. Moreover, the binding of NPs to any microorganism depends on interactions in the available surface area. Therefore, small-sized AgNPs with a larger surface area would exhibit a more significant microbicidal activity as compared to larger AgNPs.

Data Availability

The experimental data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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