Antibacterial potential of silver nanoparticles (SP-AgNPs) synthesized from *Syzygium polyanthum* (Wight) Walp. against selected foodborne pathogens

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A B S T R A C T

Foodborne diseases continue to pose a significant global health concern, causing a considerable number of deaths worldwide. In response to concerns surrounding bacterial resistance and the limitations of traditional antibiotics, there is a growing interest in exploring natural antibacterial agents as potential alternatives for addressing foodborne pathogens. Nowadays efforts are being made on exploring the potential of natural antibacterial agents against foodborne pathogens. In this study, the antibacterial efficacy of silver nanoparticles synthesized using *S. polyanthum* leaves extract (SP-AgNPs) against selected Gram-negative and Gram-positive foodborne pathogens was investigated by using multiple assays, including the well diffusion assay (WDA), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill assay. The well diffusion assay demonstrated the inhibitory potential of SP-AgNPs against all tested foodborne pathogens, with inhibition zones ranging from $10.16 + 1.25$ to $13.16 + 1.52$ mm. Furthermore, the MIC values ranged from 0.008 to 0.125 mg/mL, indicating the potent antibacterial activity of SP-AgNPs across a broad spectrum of foodborne pathogens. The MBC values, ranging from 0.008 to 0.250 mg/mL, further confirming the bactericidal ability of SP-AgNPs against these pathogens. In the time-kill experiment, most foodborne pathogens were entirely killed after 4 h of incubation at $4 \times$ MIC concentration. However, only *E. coli*, *K. pneumoniae*, and *S.* Typhimurium showed a reduction in population to 3 Log_{10} CFU/mL, indicating a strong bactericidal effect of SP-AgNPs on most tested pathogens. In conclusion, this study provides compelling evidence that SP-AgNPs exhibit potent antibacterial activity against selected foodborne pathogens. The findings suggest that SP-AgNPs synthesized using *S. polyanthum* leaves extract hold great promise as a novel antibacterial agent for effectively controlling foodborne pathogens. These findings contribute to continuing efforts in developing alternative strategies to prevent foodborne diseases and enhance food safety.

1. Introduction

Foodborne diseases, mainly caused by bacterial contamination, represent a significant and ongoing threat to global public health. Food poisoning is among the leading causes of death and infections in developing nations [1,2]. In recent years there has been an

increase in foodborne diseases and their effective prevention and treatment have become global concerns. The most common food-borne pathogens associated with foodborne illnesses are *Bacillus* sp., *Staphylococcus aureus*, *Listeria monocytogenes*, and *Escherichia coli* [3]. The rise of antibiotic resistance has further complicated this issue, emphasizing the need for specialized antibacterial agents to counter foodborne diseases [4]. Furthermore, because of the overuse of antibiotics, bacterial resistance has increased, diminishing their effectiveness [4,5]. Thus, there is an urgent need to develop innovative antibacterial agents.

Plant extracts have emerged as promising alternatives to synthetic antibiotics, with *Syzygium polyanthum* leaves gaining attention for their pharmacological properties [6,7]. However, the efficacy of these natural extracts has limited effectiveness compared to synthetic antimicrobial compounds [8,9]. Recent advancements in nanotechnology have sparked significant scientific interest with its applications in all fields. Nanoparticles are particles with sizes ranging from 1 to 100 nm. Among all the different nanomaterials, silver nanoparticles (AgNPs) are particularly interesting due to their unique physicochemical properties [10]. Plant mediated silver nano-particles offer advantages as compared to other synthesis methods because they are easy to synthesize, cost-effective, and environ-mentally friendly. Numerous studies on plant-mediated silver nanoparticles have been reported [11].

AgNPs offer a compelling solution to the challenges posed by foodborne pathogens as they have demonstrated strong antimicrobial activity against a broad spectrum of foodborne pathogens [11,12]. Unlike traditional antibiotics, their mechanism of action involves multiple pathways, making it difficult for bacteria to develop resistance [13]. AgNPs can disrupt bacterial cell membranes, interfere with cellular functions, and induce oxidative stress, contributing to their efficacy against a wide range of bacteria. By combining AgNPs with plant extracts, the goal was to enhance their antibacterial effects and offer a potential solution for combating bacterial infections more effectively [14]. However, while the antimicrobial efficacy of AgNPs is appealing, there is a growing concern surrounding their safety profile, especially their toxicity toward mammalian cells. This concern arises from the potential impact of AgNPs on human health and the environment [15]. Future studies should explore the cytotoxic effects of AgNPs across various mammalian cell types to gain a comprehensive understanding of their toxicity [16]. The combination of AgNPs with plant extracts, like *S. polyanthum* leaf extract offers an eco-friendly approach to nanoparticle synthesis, enhancing antibacterial effects.

The aim of this study was to determine the antibacterial efficacy of AgNPs synthesized using *S. polyanthum* leaf extract (SP-AgNPs) against selected foodborne pathogens. This research contributes new insights into the management of foodborne diseases, while assessing the potential risks and benefits of using SP-AgNPs.

2. Materials and methods

2.1. Plant sample collection and preparation

Serai kayu leaves (*Syzygium polyanthum* (Wight) Walp.) were procured from Taman Pertanian, UPM. They were cleaned, dried for 3 h at 50 °C, and pulverized using a Panasonic MK-5087 M blender (Panasonic Corporation Osaka, Japan). One hundred grams of the pulverized *S. polyanthum* were soaked in 400 mL of 99.8 % pure ethanol (R & M Marketing, Essex, UK) for 30 min. The solution was then filtered through a Whatman No. 2 filter. The resulting filtrate was concentrated for 1–2 h at 50 °C using a rotary vacuum evaporator, hence producing the crude extract [17].

2.2. Reagents used and the preparation of silver nanoparticles (SP-AgNPs)

To synthesize AgNPs, the procedure described by Jain and Mehata [18] and Singhal et al. [19] was followed. The reagents for the nanoparticle synthesis included silver nitrate ($AgNO_3$), extract from *S. polyanthum* leaves, and distilled water. The bio-reduction process involved mixing 5 mL of a 1 % leaf extract of *S. polyanthum* with 45 mL of a 1 mM solution containing $AgNO_3$. This facili-tated the reduction of Ag + ions to Ag0. UV–vis spectral analysis confirmed the formation of silver nanoparticles.

2.3. Bacteria strains preparation

The bacteria strains used in the study were obtained from the American Type Culture Collection (Rockville, MD, USA).

2.4. Susceptibility test

The susceptibility tests used in this research include the Well diffusion assay (WDA), Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC), and Time-kill assay. These tests were conducted to assess and analyze the inhibition of bacterial growth.

2.4.1. Well diffusion assay (WDA)

The WDA was used to test the effectiveness of SP-AgNPs against foodborne pathogens by following the Clinical and Laboratory Standard Institute protocol [20] (CLSI, 2018). Bacterial stock cultures were initially grown on Nutrient agar and subsequently transferred to either Mueller Hinton broth agar (MHA) or Mueller Hinton broth (MHB) for 24 h at 37 °C. The concentration of bacterial cells was adjusted to match a 0.5 McFarland Standard. These strains were then spread over MHA and wells were made using a 7 mm Pasteur pipette made of glass. These wells were filled with 25–50 μL of SP-AgNPS (0.1 %). The plates were subsequently incubated for 24 h at 37 °C. Following the incubation period, the inhibition zones were observed and measured in millimeters. The size of these zones served as a direct indicator of bacterial growth inhibition.

2.4.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC is the lowest concentration of an antibacterial agent capable of inhibiting bacterial growth. A 96-well polystyrene plate was used for conducting microdilution MIC testing, following the guidelines provided by (CLSI, 2018) [20]. Each well was filled with 100 μL of SP-AgNPs, followed by a two-fold dilution of the bacterial inoculum for each strain. Column 2 of the panel served as the positive control, while Column 1 served as the negative control. The microtiter plates were incubated for 24 h at 37 °C. After the incubation period, a 10 μL sample from each well was transferred onto MHA plates which were then incubated at 37 °C for 24 h. The MBC value was determined as the lowest concentration of the agent that resulted in no bacterial growth on the MHA plate [20].

2.4.3. Time kill assay

It is a broth-based method used to assess the long-term effects of antimicrobials on bacterial growth and death. This method provides a better understanding of the kinetics of bacterial growth and bactericidal activity when exposed to SP-AgNPs, which helps to determine the optimal concentration and exposure time required to kill bacterial pathogens effectively. The use of Log_{10} CFU/mL allows for easier interpretation and comparison of bacterial concentrations, as it transforms the data into a logarithmic scale. In this study, the time-kill assay was performed on selected bacteria using various concentrations of SP-AgNPs as reported in Ref. [20], with minor modifications. The SP-AgNPs were diluted in MHB medium containing the bacterial inoculum to obtain different concentrations of $0 \times MIC$, $0.5 \times MIC$, $1 \times MIC$, $2 \times MIC$, and $4 \times MIC$ for each bacterial species. At specific time intervals (0, 0.5, 1, 2, and 4 h), 10 μL samples were diluted with 1 % sterile phosphate buffer saline and spread onto MHA plates. These plates were incubated at 37 °C for 12–24 h. The total plate count (TPC) was then expressed as Log_{10} CFU/mL and plotted against time.

3. Results and discussion

Although the primary focus of this study was the antibacterial efficacy of SP-AgNPs, the basic characterization of these nanoparticles was also conducted using UV–vis, FTIR, and TEM analysis. The UV–vis examination revealed a prominent peak at 433 nm, which is a characteristic silver absorption band. Furthermore, the FTIR analysis suggested that the leaf extract of *S. polyanthum* played a dual role as both a reducing and capping agent in the AgNPs synthesis process. The TEM imaging depicted spherical SP-AgNPs with an average particle size of 27.69 nm. This analysis not only confirmed the successful synthesis of SP-AgNPs using an eco-friendly approach but also explains their primary characteristics, including size, shape, and the role of the plant extract in their formation. This primary knowledge serves as a foundation for the assessment of SP-AgNPs as a promising antibacterial agent with potential applications.

3.1. Well diffusion assay

The antibacterial efficacy of SP-AgNPs against selected foodborne pathogens was assessed using a well diffusion assay (WDA). Rulers were used to measure the diameter of the zone of inhibition, extending from the edge of the well to the boundary of the cleared area. Table 1 presents the results of the susceptibility tests, which show that SP-AgNPs effectively inhibited the growth of all examined bacteria, including both Gram-negative and Gram-positive species. The diameters of the zones of inhibition varied between 10.16 ± 1.25 mm and 13.16 ± 1.52 mm. In contrast, the negative control, DMSO, displayed no inhibitory effect on the bacterial strains.

AgNPs have garnered attention for their remarkable bactericidal properties. The structural integrity of the bacterial cell wall is damaged by these small particles because of their ability to bind with bacterial proteins and enzymes [21]. This disruption of the bacterial cell wall and membrane is widely recognized as the fundamental mechanism behind the antimicrobial action of silver nanoparticles [22]. Moreover, the deadly effects of AgNPs extend beyond mere cell wall and membrane disruption. These nanoparticles are known for their multiple actions, involving interference with essential cellular components. This includes disruptions in the stability and functionality of vital biomolecules such as DNA and RNA, along with interference of cellular respiration [23].

Table 1
Inhibition zone of SP-AgNPs against selected foodborne pathogens.

Strains	SP-AgNPs
B. cereus ATCC33019	12.00 ± 0.50[b]
B. megaterium ATCC14581	12.66 ± 0.28[a]
B. pumilus ATCC14884	11.83 ± 0.76[a]
B. subtilis ATCC6633	13.16 ± 1.52[a]
E. coli ATCC43895	10.16 ± 1.25[a]
K. pneumoniae ATCC13773	12.83 ± 0.57[a]
L. monocytogenes ATCC19112	11.83 ± 1.60[a]
P. aeruginosa ATCC9027	12.00 ± 0.50[b]
P. mirabilis ATCC21100	11.50 ± 1.00[a]
S. aureus ATCC29737	13.00 ± 1.32[ba]
S. Typhimurium ATCC14028	12.83 ± 0.28[a]

Results were expressed as means ± standard deviation (SD); n = 3 × 1. Values with different small letters within the same columns are significantly different (p < 0.05).

3.2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Table 2 presents the MIC and MBC results for SP-AgNPs against a variety of foodborne pathogens. The data reveal that SP-AgNPs exhibit both bacteriostatic and bactericidal effects, with MIC and MBC values ranging from 0.008 mg/mL to 0.250 mg/mL. Among the tested organisms, *S*. Typhimurium, *P. mirabilis,* and *S. aureus* exhibited the lowest MICs, suggesting high sensitivity to SP-AgNPs compared to other bacteria. This susceptibility could be attributed to differences in bacterial cell wall composition [24]. The current findings align with existing literature, indicating that Gram-negative bacteria are generally more susceptible to AgNPs compared to Gram-positive [25–27]. However, some studies suggest that AgNPs derived from natural sources are more effective against Gram-positive bacteria [28].

The particle size was identified as a critical factor influencing the antibacterial activity of SP-AgNPs. Nanoparticles with sizes ranging from 1 to 25 nm appear to directly interact with bacterial cells, thereby inhibiting growth [29]. Agnihotri et al. [30] found that smaller particles, particularly those below 10 nm, have greater efficacy. Specifically, 5 nm particles were shown to be more effective than those sized at 7 and 10 nm. In summary, the study supports existing research on the variable susceptibilities of Gram-positive and Gram-negative bacteria to AgNPs while emphasizing the role of nanoparticle size in antibacterial effectiveness. The range of MIC and MBC values suggests that SP-AgNPs could serve as potential antibacterial agents against diverse foodborne pathogens.

3.3. Time-kill of B. Cereus

The time-kill assay of *B. cereus* against different concentrations of SP-AgNPs (0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC, 4 × MIC) is illustrated in Fig. 1. The time-kill assay of *B. cereus* was specifically selected for further study due to its prevalence in foodborne outbreaks. The inhibitory effect of SP-AgNPs became evident at 0.5 × MIC (0.008 mg/mL) after just 0.5 h of exposure. As indicated by the graph, the initial bacterial growth decreased from 7.14 Log_{10} CFU/mL to 4.5 Log_{10} CFU/mL at 1 × MIC. Bactericidal effects were observed at 2 × MIC (0.031 mg/mL) and 4 × MIC (0.062 mg/mL) during 4 and 2 h of incubation, respectively. This demonstrates that the antibacterial impact is directly related to both the duration of exposure and the concentration of SP-AgNPs. In essence, higher concentrations and longer exposure times yield stronger antibacterial effects. Sangdee et al. [30] reported the effect of AgNPs on bacterial growth inhibition is time and concentration-dependent which is consistent with our test results. Furthermore, comparing the results of this study with those of others reveals that different synthesis methods, sizes, and surface charges of AgNPs can result in varying antibacterial effects.

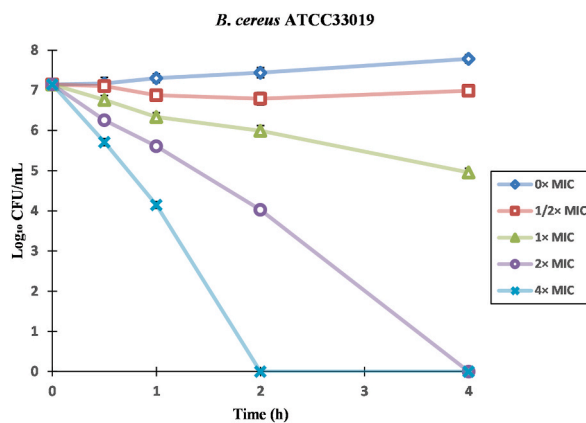## 3.4. Time-kill of B. megaterium

The graph (Fig. 2) shows the effect of different concentrations of SP-AgNPs on the growth of *B. megaterium* bacteria at different concentrations (0.000, 0.031, 0.062, 0.124, and 0.250 mg/mL) and time intervals (0, 0.5, 1, 2 & 4 h). At 1 × MIC (0.062 mg/mL), the bacterial count was reduced from 7.21 Log_{10} CFU/mL to 5.3 Log_{10} CFU/mL during 4 h of incubation, implying that SP-AgNPs have a bacteriostatic effect, which means they inhibit the growth of bacteria but do not necessarily kill them. However, SP-AgNPs showed bactericidal effect at the highest concentrations (2 × MIC and 4 × MIC, 0.124 and 0.250 mg/mL) respectively at 2 and 4 h of incubation, emphasizing the importance of optimizing nanoparticle concentrations for different bacterial strains. SP-AgNPs at highest concentrations (2 × MIC and 4 × MIC) were able to kill the bacteria, while lower concentrations only inhibited their growth. This suggests that the bactericidal effect of SP-AgNPs is concentration-dependent. Overall, the study concludes that SP-AgNPs have both bacteriostatic and bactericidal activities against *B. megaterium*, and their effectiveness is dependent on the concentration and duration of exposure.

3.5. Time-kill of B. pumilus

Fig. 3 depicts the influence of varying SP-AgNP concentrations on the survival of *B. pumilus* over time. The results indicate that

Table 2
MIC and MBC of SP-AgNPs against selected foodborne pathogens.

Tested Microorganisms	SP-AgNPs	
	MIC (mg/mL)	MBC (mg/mL)
B. cereus ATCC33019	0.015	0.015
B. megaterium ATCC14581	0.062	0.125
B. pumilus ATCC14884	0.031	0.062
B. subtilis ATCC6633	0.062	0.125
E. coli ATCC43895	0.125	0.250
K. pneumoniae ATCC13773	0.015	0.031
L. monocytogenes ATCC19112	0.031	0.062
P. aeruginosa ATCC9027	0.015	0.015
P. mirabilis ATCC21100	0.031	0.031
S. aureus ATCC29737	0.008	0.008
Salmonella Typhimurium ATCC14028	0.008	0.015

Fig. 1. Time-kill plot for *B. cereus* ATCC33019 after exposure to SP-AgNPs (0.000, 0.008, 0.015, 0.031 and 0.062 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.
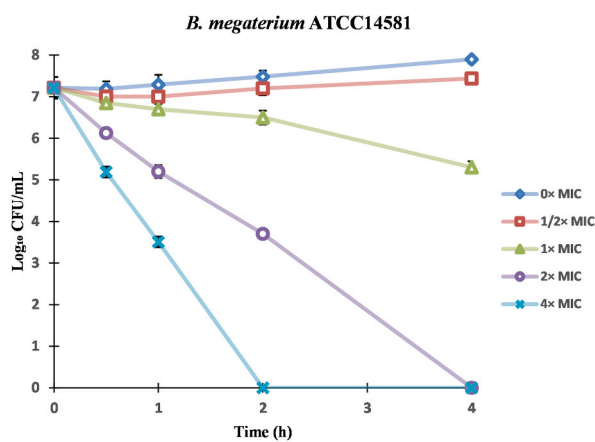
Fig. 2. Time-kill plot for *B. megaterium* ATCC14581 after exposure to SP-AgNPs (0.000, 0.031, 0.062, 0.124 and 0.250 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.
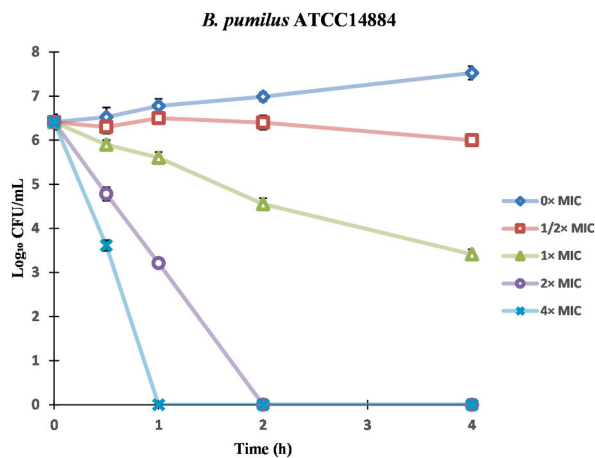
Fig. 3. Time-kill plot for *B. pumilus* ATCC14884 after exposure to SP-AgNPs (0.000, 0.015, 0.031, 0.062 and 0.124 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.

higher concentrations of SP-AgNPs lead to a more rapid and pronounced killing effect on the bacteria. All the bacterial cells were entirely killed after 2 h of incubation at 2 × MIC (0.062 mg/mL). SP-AgNPs proved to be bactericidal within short exposure duration (1–2 h) at higher concentrations of 4 × MIC (0.124 mg/mL) and 2 × MIC (0.062 mg/mL) while at lower concentrations 1 × MIC (0.031 mg/mL), SP-AgNPs were found to be bacteriostatic, reducing the bacterial population to (≥3-Log$_{10}$). *P. cubeba* L. extract demonstrated a bactericidal effect against *B. subtilis* after 4 h of incubation at a concentration of 2.5 mg/mL [31] while Muniandy et al. [32] (2015) reported that *E. polyantha* extract at a concentration of 2.5 mg/mL showed bacteriostatic effect against *B. subtilis,* SP-AgNPs, when compared to other plant extracts such as *P. cubeba* L. extract and *E. polyantha* extract, demonstrated remarkable antibacterial activity against *B. pumilus*.

3.6. Time-kill of B. subtilis

Fig. 4 illustrates the time-kill assay of SP-AgNPs against *B. subtilis*. This assay revealed two distinct bactericidal endpoints: one at 2 × MIC (0.124 mg/mL) within 2 h and another at 4 × MIC (0.250 mg/mL) within 4 h. Even at a lower concentration of 1 × MIC (0.062 mg/mL), SP-AgNPs significantly reduced viable bacterial counts, dropping from 7.04 Log$_{10}$ CFU/mL to 5.15 Log$_{10}$ CFU/mL, thus highlighting their effectiveness even at lower concentrations. The trend observed in the bacterial curve indicated that as the concentration of SP-AgNPs increased, bacterial growth was increasingly inhibited, eventually leading to complete bacterial eradication at higher concentrations. This suggests that SP-AgNPs possess both bacteriostatic and bactericidal activity against *B. subtilis*, with their effectiveness influenced by both time and concentration. The choice of evaluating bactericidal activity at 2 × MIC and 4 × MIC concentrations was based on the need to assess their performance at higher concentrations, given the rising challenge of bacterial resistance. In a study conducted by Loo et al. [8] (2018), tea mediated AgNPs achieved a bactericidal effect against *B. subtilis* subsp. spizizenii after 2 h of incubation. Additionally, another research conducted by Alqadeeri et al. [33] demonstrated that *P. cubeba* L. extract reached a bactericidal endpoint against *B. subtilis* at a concentration of 8 × MIC (5 mg/mL) during a 4 h incubation period. Similarly, Lau et al. [34] reported that *E. polyantha* extract achieved a bactericidal endpoint against *B. subtilis* after 4 h of incubation at a concentration of 5 mg/mL.

3.7. Time-kill of L. monocytogenes

The bactericidal effect of higher concentrations of SP-AgNPs at 4 × MIC was bactericidal against *L. monocytogenes* after 4 h of incubation, as shown in Fig. 5. As the concentration increased, there was a marked reduction in bacterial count. On the other hand, at 0.5 × MIC (0.015 mg/mL), bacterial growth appeared to increase in the presence of SP-AgNPs. However, at 1 × MIC (0.031 mg/mL) and 2 × MIC (0.062 mg/mL), the bacterial count noticeably decreased, from 6.95 Log$_{10}$ CFU/mL to 5.10 Log$_{10}$ CFU/mL and 3.45 Log$_{10}$ CFU/mL, respectively. It was observed that higher SP-AgNPs concentrations at 4 × MIC exhibited bactericidal activity against *L. monocytogenes* after 4 h of incubation, resulting in a substantial reduction in bacterial counts. In another study, Loo et al. [8] reported that *L. monocytogenes* was effectively eliminated when treated with tea-mediated silver nanoparticles at a concentration of 2 × MIC (7.8 µg/mL) after 4 h of incubation. Similarly, Ramli et al. [34] found that *L. monocytogenes* treated with *S. polyanthum* leaf extract was completely eradicated at 4 × MIC (2.52 mg/mL) within just 1 h of incubation. These results emphasize the strong antibacterial activity of SP-AgNPs, particularly at higher concentrations, against *L. monocytogenes*. These effects are consistent with or higher than those observed in prior studies involving different antibacterial agents.

3.8. Time-kill of S. aureus

The time-kill assay depicted in Fig. 6 illustrates the remarkable efficacy of SP-AgNPs against *S. aureus*. Remarkably, complete
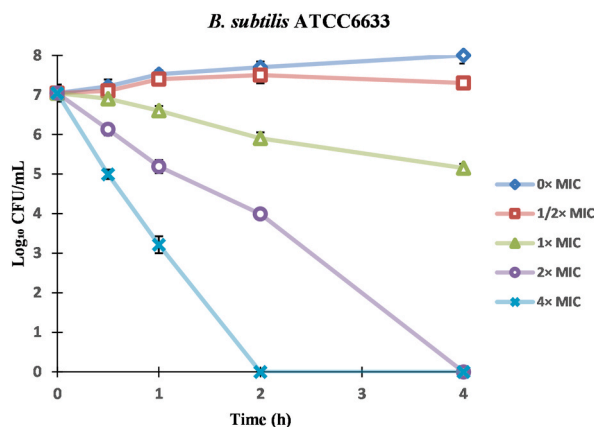
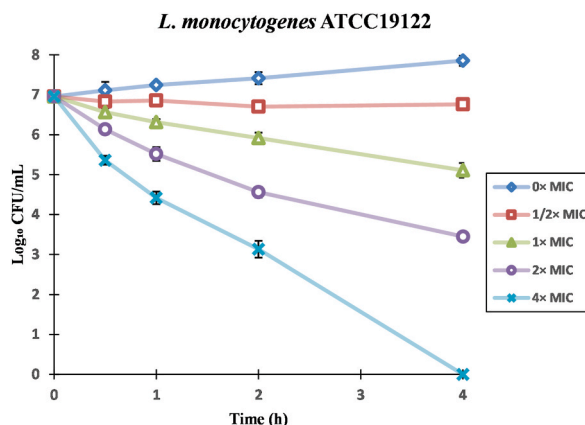

Fig. 4. Time-kill plot for *B. subtilis* ATCC6633 after exposure to SP-AgNPs (0.000, 0.031, 0.062, 0.124 and 0.250 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.

Fig. 5. Time-kill plot for *L. monocytogenes* ATCC19122 after exposure to SP-AgNPs (0.000, 0.015, 0.031, 0.062 and 0.124 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.

bacterial elimination was accomplished with a concentration of 2 × MIC (0.016 mg/mL) within just a 4-h incubation period. Furthermore, the reduction in bacterial cell count was directly proportional to the amount of SP-AgNPs used. At both 2 × MIC (0.016 mg/mL) and 4 × MIC (0.032 mg/mL), SP-AgNPs displayed bactericidal activity, achieving complete bacterial elimination after both 4 and 2 h, respectively. In similar research, Das et al. [31] observed complete bacterial cell elimination after an 8-h treatment with *O. gratissimum* leaf extract-mediated nanoparticles against *S. aureus*. Additionally, Ramli et al. [31] (2017) reported that *S. aureus* was entirely eradicated at 4 × MIC (2.52 mg/mL) within 4 h of treatment with *S. polyanthum* leaves extract. Meanwhile, Witkowska et al. [35] discovered that sage extract demonstrated bactericidal activity against *S. aureus*, at a concentration greater than 40 mg/mL, and a 24-h incubation period.

3.9. Time-kill of E. coli

Based on Fig. 7, notable changes were observed in bacterial counts following exposure to SP-AgNPs. Initially, at a concentration of 1 × MIC (0.125 mg/mL), there was a reasonable decrease in the bacterial count, reducing from 7.07 Log_{10} CFU/mL to 6.01 Log_{10} CFU/mL after 4 h of incubation. However, the most substantial impact was observed at 4 × MIC (0.500 mg/mL), where a remarkable reduction in bacterial cells occurred within just 2 and 4 h of incubation. This reduction exceeded a 3- Log_{10} units, indicating a strong bacteriostatic effect but not a bactericidal one. In another study, *S. polyanthum* leaf extract exhibited bactericidal activity against *E. coli* at a concentration of 4 × MIC (5.00 mg/mL) after 4 h of incubation [36]. Similarly, *M. fragrans* Houtt. extract eliminated *E. coli* at a concentration of 4 × MIC (5.00 mg/mL) within 4 h [37]. SP-AgNPs had excellent antibacterial activity against *E. coli* strains, particularly at lower concentrations, when compared to other leaf extracts. While SP-AgNPs demonstrated a bacteriostatic effect rather than a bactericidal one, they showed considerable efficacy in reducing bacterial counts.

3.10. Time-kill of K. pneumoniae

The inhibitory effects of SP-AgNPs on the growth of *K. pneumoniae* is depicted in Fig. 8. During 4-h incubation period, SP-AgNPs
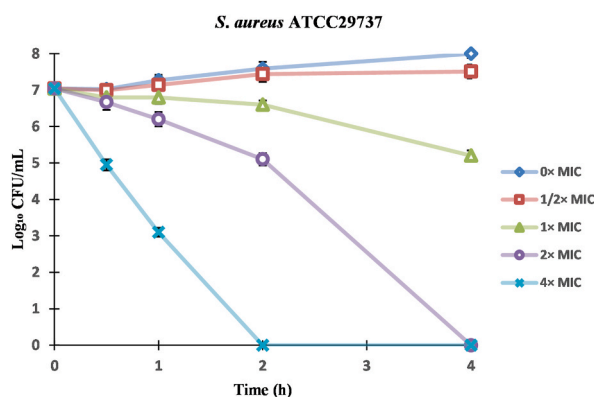
Fig. 6. Time-kill plot for *S. aureus* ATCC29737 after exposure to SP-AgNPs (0.000, 0.004, 0.008, 0.016 and 0.032 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.
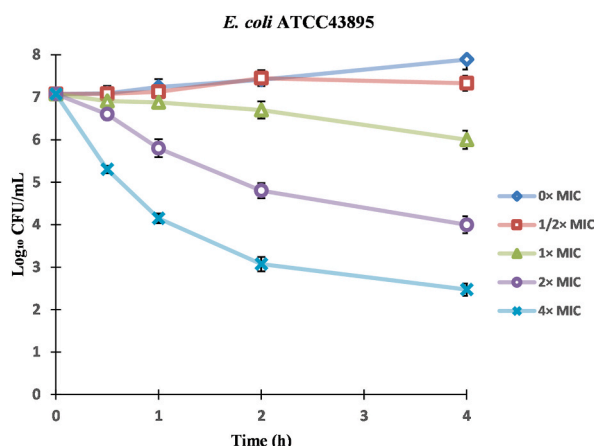
Fig. 7. Time-kill plot for *E. coli* ATCC43895 after exposure to SP-AgNPs (0.000, 0.062, 0.125, 0.250 and 0.500 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.

reduced the bacterial load from 6.55 Log_{10} CFU/mL to 5.01 Log_{10} CFU/mL at 1 × MIC (0.015 mg/mL). At 2 × MIC (0.031 mg/mL), the bacterial count decreased to 3.5 Log_{10} CFU/mL after 4 h (achieving >3-Log reduction). Remarkably, at 4 × MIC (0.062 mg/mL), the bacterial count reached 2.01 Log_{10} CFU/mL after 4 h, signifying a substantial reduction. A study by Loo et al. [8] (2018) reported that tea-mediated silver nanoparticles, at 4 × MIC (15.6 μg/mL), eliminated *K. pneumoniae* within 2 h of incubation. Similarly, *S. jambos* (L.) Alston leaves extract exhibited bactericidal properties against *K. pneumoniae* at a concentration of 2 × MIC (0.02 mg/mL) after 4 h of incubation [37]. In summary, while SP-AgNPs did demonstrate antibacterial activities against *K. pneumoniae*, the efficacy was comparatively lower than that observed with *S. jambos* (L.) Alston leaf extract. The data suggest that the antibacterial efficacy of SP-AgNPs against *K. pneumoniae* is present but varies in comparison to the results from studies using other types of leaf extracts.

3.11. Time-kill of P. aeruginosa

Fig. 9 illustrates the time-kill assay of SP-AgNPs against *P. aeruginosa*, indicating that SP-AgNPs can kill *P. aeruginosa* at 4 × MIC (0.062 mg/mL) within 4 h of incubation. However, the bacterial cells increased during 4 h of incubation at 0.5 × MIC (0.008 mg/mL) from 7.01 Log_{10} CFU/mL to 7.30 Log_{10} CFU/mL. Furthermore, the bacterial cell count was reduced to 5.9 Log_{10} CFU/mL and 4.21 Log_{10} CFU/mL at 1 and 2 × MIC (0.015 and 0.031 mg/mL), respectively. The number of viable cells decreased proportionally to the concentration of SP-AgNPs. The SP-AgNPs demonstrated bactericidal effects against *P. aeruginosa* at 4 × MIC and good antibacterial activity at 1 and 2 × MIC within 4 h. Thus, SP-AgNPs proved to be bactericidal at 4 × MIC and bacteriostatic at 2 × MIC and at lower concentrations of MIC concentrations. *Melastoma malabathricum* extract could completely kill *P. aeruginosa* at a concentration of 1.56 mg/mL in 8 h [38] but our study discovered that SP-AgNPs were bactericidal at a concentration of 0.062 mg/mL in 4 h. The current study's results cannot be compared to those of other studies due to concentration and incubation period differences. The findings indicated that the lethal impact of SP-AgNPs was affected by both the concentration and duration of treatment.

3.12. Time-kill of S. Typhimurium

According to Fig. 10, the SP-AgNPs demonstrated a bacteriostatic effect when tested against *S.* Typhimurium, suggesting their ability to inhibit bacterial growth. The data shows a trend toward bacteriostatic action, characterized by a decline in bacterial cell count following 1-h incubation at concentrations of 2 × and 4 × MIC. Within 4 h of incubation at these concentrations, bacterial cells were not eradicated but were reduced to levels of ≥3-Log_{10} CFU/mL, suggesting the bacteriostatic nature of SP-AgNPs. Ramli et al. [30] (2017) observed a 3-Log_{10} reduction in the *S.* Typhimurium population after a 4 h incubation with *S. polyanthum* leaf extract at a concentration of 4 × MIC (5 mg/mL). In contrast, tea-mediated silver nanoparticles achieved the complete elimination of *S.* Typhimurium within just 1 h of incubation at a concentration of 15.6 μg/mL [8]. Pal [39] reported a 3-Log_{10} CFU/mL reduction in *Salmonella* spp. after 24 h of incubation with *Camellia sinensis* extract at a concentration of 512 μg/mL. These findings demonstrate the varying degrees of antibacterial effectiveness among different agents against *S.* Typhimurium, with SP-AgNPs showing a prominent but not immediate bacteriostatic effect in this study.

3.13. Time-kill of P. mirabilis

The results from the time-kill experiment using SP-AgNPs against *P. mirabilis* is presented in Fig. 11. It was observed that SP-AgNPs exhibited bactericidal effects when used at higher concentrations. Specifically, at 4 × MIC (0.124 mg/mL), the bacterial cells were eliminated within a 4-h incubation period. At lower concentrations, SP-AgNPs displayed bacteriostatic effects, indicating that their ability to kill *P. mirabilis* depends on both concentration and time. *E. hirta* extract showed bactericidal activity against *P. mirabilis*, but at
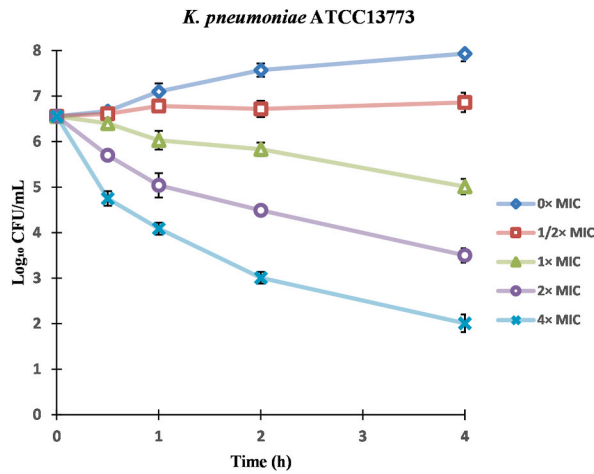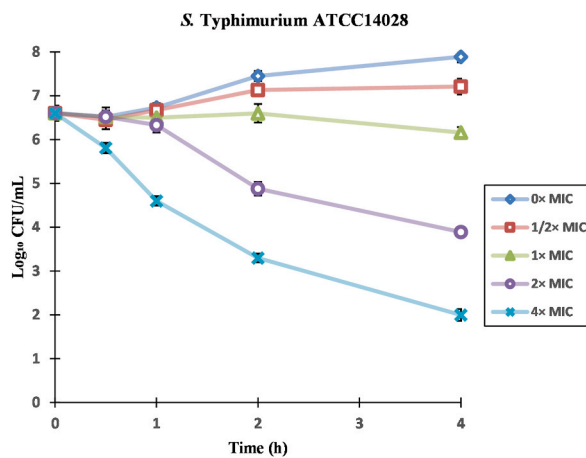
Fig. 8. Time-kill plot for *K. pneumoniae* ATCC13773 after exposure to SP-AgNPs (0.000, 0.008, 0.015, 0.031 and 0.062 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.
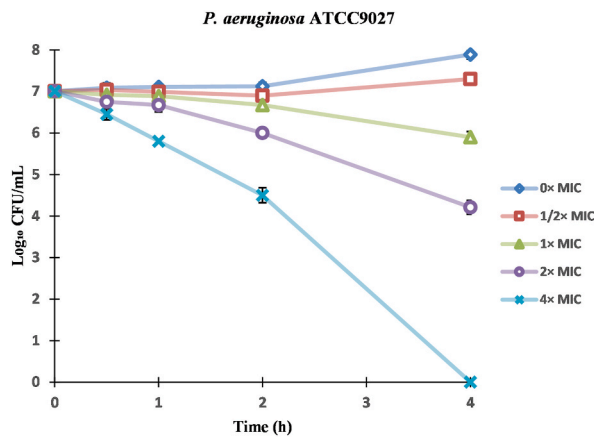
Fig. 9. Time-kill plot for *P. aeruginosa* ATCC9027 after exposure to SP-AgNPs (0.000, 0.008, 0.015, 0.031 and 0.062 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.

Fig. 10. Time-kill plot for *S. Typhimurium* ATCC14028 after exposure to SP-AgNPs (0.000, 0.004, 0.008, 0.016 and 0.032 mg/mL). Values given in the brackets after 0 × MIC, 0.5 × MIC, 1 × MIC, 2 × MIC and 4 × MIC, respectively.
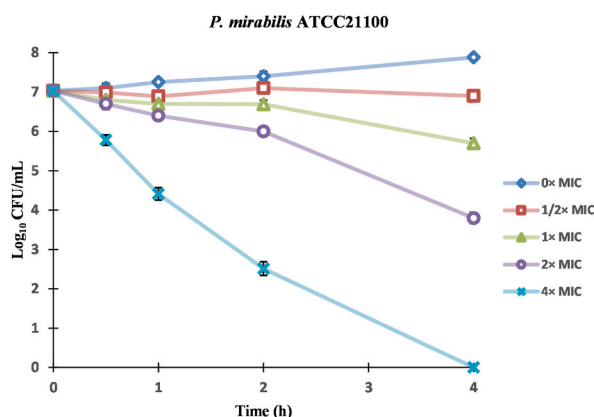
Fig. 11. Time-kill plot for *P. mirabilis* ATCC21100 after exposure to SP-AgNPs (0.000, 0.015, 0.031, 0.062 and 0.124 mg/mL). Values given in the brackets after $0 \times$ MIC, $0.5 \times$ MIC, $1 \times$ MIC, $2 \times$ MIC and $4 \times$ MIC, respectively.

a higher concentration of 50 mg/mL [40]. In a different study conducted by Ali et al. [41], *Syzygium jambos* (L.) Alston leaf extract, at a concentration of $4 \times$ MIC (5.00 mg/mL), achieved a significant 3-Log_{10} reduction in bacterial count after 4 h of incubation. In summary, this experiment concludes that SP-AgNPs exhibit a bactericidal effect against *P. mirabilis* and demonstrate notable antibacterial activity in comparison to other plant extracts.

4. Conclusion

This study provides compelling evidence for the potent antibacterial activities of AgNPs synthesized using *S. polyanthum* (SP-AgNPs) against a diverse range of foodborne pathogens. Characterization results from UV–vis spectroscopy, FTIR, and TEM affirmed the successful synthesis and primary structure of the SP-AgNPs. The susceptibility tests consistently revealed strong antibacterial activity, as indicated by WDA values that ranged from 10.16 ± 1.25 to 13.16 ± 1.52, and minimum inhibitory concentration/minimum bactericidal concentration (MIC/MBC) values that ranged from as low as 0.008 mg/mL to 0.250 mg/mL. Remarkably, SP-AgNPs achieved a substantial reduction in bacterial counts, exceeding a 3-Log_{10} CFU/mL reduction across all tested bacterial strains. These findings emphasize the potential of SP-AgNPs as a promising alternative for the development of cost-effective, environmentally friendly antibacterial agents, addressing the need for novel antibacterial agents. The study not only confirms the effectiveness of SP-AgNPs in inhibiting bacterial growth but also serves as an initial step in examining the broader potential of nanoparticles synthesized from plants. Future studies are recommended to assess the cytotoxicity of SP-AgNPs to ensure their safety and additional evaluations should also be conducted to establish a comprehensive safety profile for these nanoparticles. Hence, this study not only meets an urgent requirement but also indicates that silver nanoparticles synthesized through plant-mediated methods hold promise in antibacterial applications, while outlining actionable areas for future research.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Sadeeya Khan: Conceptualization, Data curation, Methodology, Writing – original draft. **Yaya Rukayadi:** Investigation, Project administration, Supervision, Validation, Writing – review & editing. **Ahmad Haniff Jaafar:** Supervision, Validation. **Nurul Hawa Ahmad:** Methodology, Supervision, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e22771.

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