ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *ELEUSINE INDICA* L. GAERTN LEAF EXTRACT ON THE BACTERIA ISOLATED FROM THE VASE SOLUTION

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Article history
Received: February 7th, 2022
Received in revised form (1st): May 5th, 2022 | Received in revised form (2nd): June 30th, 2022
Accepted: July 18th, 2022
Available online: November 24th, 2022

Abstract

Green synthesis of silver nanoparticles (SNPs) using Eleusine indica L. Gaertn leaf extract as a reducing agent is reported. SNPs were characterized through UV-Vis spectroscopy and transmission electron microscopy (TEM). The SNPs were rod like and spherical in shape with sizes from 3 to 33 nm and an average size of 16.73 nm. Seven bacterial strains were isolated from the vase water, including Bacillus cereus CA1, Alcaligenes faecalis CA2, Micrococcus luteus CA3, Pantoe agglomerans CA4, Pseudomonas aeruginosa CA5, Pseudomonas aeruginosa CA6, and Pantoe agglomerans CA7. Identifications were made according to Bergey's Manual of Systematic Bacteriology and Bergey's Manual of Determinative Bacteriology. The SNPs inhibited the growth of bacteria and exhibited significant antimicrobial activity against different isolated bacteria strains. SEM images showed that the SNPs damaged the cell membranes of bacteria, released plasmic contents, and altered the morphology of the cells. The impact of SNPs on gram-negative bacteria was more severe than on gram-positive bacteria. This study revealed that biosynthesized SNPs from Eleusine indica L. Gaertn leaf extract are potential agents in combating bacterial contamination.

Keywords: Antimicrobial; Bacteria; Biosynthesis; Goosegrass; Silver nanoparticles.

DOI: https://doi.org/10.37569/DalatUniversity.13.2.1027(2023)

Article type: (peer-reviewed) Full-length research article

1. INTRODUCTION

The vase life of cut flowers affects the commercial value of the floriculture industry. The maximum longevity of cut flowers before their senescence has attracted much interest (Maity et al., 2019). Microorganism contamination is one of the main causes of shortening the vase life of cut flowers. Microorganisms cause stem blockage and inhibit water transportation from the basal end to the leaves and flowers (Hamed Chaman et al., 2013; Maity et al., 2019). Microorganism contamination in the vase solution can also secrete extracellular virulence factors, including enzymes, hormones, and toxic compounds (Büttner & Bonas, 2010; Salmond, 1994) and produce ethylene (Williamson et al., 2002) to accelerate senescence. Strategies to inhibit microorganism development enhance the vase life and quality of cut flowers.

Nanotechnology is a field of research and work on a nanoscale. Its applications are rapidly developing in many fields, such as medicine, industry, and agriculture (Chatterjee et al., 2015; Lopez-Esparza et al., 2016; Shaikh et al., 2021; Shankar & Rhim, 2015; Siddiqi & Husen, 2016; Solgi, 2014). Metal-based nanomaterials exhibit cytotoxic activity on the cells of microorganisms (Kim & Kuk, 2007; Kon & Rai, 2013; Morones et al., 2005). Of all the nanoparticles, silver nanoparticles (SNPs) have attracted the most interest because of their unique physical, chemical, and biological properties and the ease in which they can be scaled up for large synthesis (Maity et al., 2019). SNPs possess a focal role in the development of new antimicrobial agents. While many germs have developed antibiotic resistance, SNPs exhibit outstanding antimicrobial activity at low concentrations against bacteria, viruses, and fungi without promoting the resistance mechanism (Hemlata et al., 2020; Rafique et al., 2017; Rauwel et al., 2015; Talapko et al., 2020; Thammawithan et al., 2021). These particles are promising alternatives against a wide range of microorganisms without increasing antibiotic resistance.

There are many methods to synthesize SNPs based on chemical and physical approaches. However, the drawback of these methods is the use of expensive and toxic chemicals (Huq, 2020; Nazeruddin et al., 2014). The synthesis of nanoparticles using plant extracts is a biomimetic approach that can limit environmental issues and the effects on human health (Solgi, 2014). The phytochemicals in plant extracts play a role as reducing and caping or stabilization agents and therefore induce more catalytic activity. Phytochemicals, including terpenoids, alkaloids, phenolic compounds, and enzymes, reduce positive silver ions to the zero oxidation state. Phytochemicals affect the size distribution of SNPs (Ong et al., 2017; Regmi et al., 2004). Green synthesis of SNPs from plant extracts is a cost-effective method that does not use high pressure, energy, and temperature (Ahmed et al., 2016; Solgi, 2014). Using SNPs to inhibit microorganisms in the vase solution has advantages over other chemicals, including cost effectiveness and environmental friendliness (Anandalakshmi et al., 2016; Aziz et al., 2014; Dakal et al., 2016). *Eleusine indica* L. Gaertn, goosegrass, is an adventitious grass that thrives in tropical areas. The grass contains many secondary metabolites that are used in medical remedies to treat stomach disorders, diabetes, and fever urine retention (Regmi et al., 2004). Products that use *Eleusine indica* include dried herbal powders, tea sachets, and encapsulated food supplements (Ong et al., 2017; Zakri et al., 2021). Besides the

medicinal and commercial applications, biological activities of *Eleusine indica* L. Gaertn have been revealed, including antibacterial, antifungal, antioxidant, anti-inflammatory, anthelminthic, antiviral, anti-plasmodal, antidiabetic, anti-obesity, cytotoxic, hepatoprotective, antihypertensive, anticonvulsant, anti-leishmanial, analgesic, antipyretic, and anti-trypanosomal properties (Regmi et al., 2004; Zakri et al., 2021). This study highlights the potential use of the Poaceae family species in combination with nanotechnology to create preservative solutions for cut flowers. The main aim of this study was to evaluate the bactericidal activity of SNPs "green" synthesized from *Eleusine indica* L. Gaertn leaf extract on the bacteria isolated from the vase solution of cut flowers.

2. MATERIALS AND METHODS

2.1. Plant materials and leaf extract preparation

Eleusine indica L. Gaertn belongs to the Poaceae family and can grow up to 60 cm in height. The stem is pale green with flat or folded and linear-lanceolate leaf blades (Zakri et al., 2021). *Eleusine indica* L. Gaertn leaves were washed twice with tap water and twice with distilled water. The fresh clean leaves were dried, chopped into small pieces 1 x 1 cm in size. The leaves were then ground, and 20 g of ground leaves were added to 200 ml of distilled water in a flask and heated for 30 min at 60º C. The mixture was filtered through Whatman No. 1 paper. The filtrate was collected and kept at 4º C for synthesis of SNPs (Le, 2020).

2.2. Synthesis and characterization of SNPs

Silver nitrate (1 mM) was added to the heated leaf extract and kept at 80º C under continuous stirring at 250 rpm. The formation of SNPs was monitored by observing the color change from pale yellow to yellowish brown and by UV-vis spectroscopy measurements with a Specord 200 Plus spectrophotometer (Jena, Germany). The reduction from Ag+ to Agº was confirmed by the UV-vis absorbance spectrum, which ranged from 400 nm to 700 nm with distilled water as a reference (Mussin et al., 2021; Talapko et al., 2020). The morphology of the nanoparticles was observed using a JEOL JEM-1010 scanning electron microscope operating at 100 kV. The TEM grid was prepared by placing a drop of the diluted bio-reduced solution on a carbon-coated copper grid, followed by drying under a lamp.

2.3. Isolation and screening of bacteria

Cut flower stems (carnation and gerbera) were harvested, immediately transferred to the laboratory, and cut to 20 cm under distilled water. The stems were placed in distilled water. After 10 days, 5 cm were cut from the basal end with a standard sterile knife and then further cut into small pieces. These pieces were placed in pre-sterilized tubes containing sterile distilled water and vortexed for 1 min. The mixture of extracts after vortexing and an aliquot of vase solutions were spread on nutrient agar and incubated at room temperature (22 ± 2º C) for 72 hours (Koch, 1883). Each colony was collected for identification. Biochemical tests were performed for identification of bacteria, including colony morphology, cell shape, Gram reaction, motility, growth aeration,

oxidative/fermentation test, casein hydrolysis, starch hydrolysis, gelatin hydrolysis, indole test, Vogas-Prekaure test, ammonia production, hydrogen sulfide (H_2S) production, citrate utilization test, mineral salt medium test, temperature tolerance test, NaCl tolerance test, catalase test, urease test, nitrate reduction test, and fermentation test (lactose, glucose, sucrose, galactose). Bacteria isolates were identified according to *Bergey's Manual of Systematic Bacteriology* and *Bergey's Manual of Determinative Bacteriology*.

2.4. Antimicrobial activity

Disk diffusion studies: The antimicrobial activity of bio-synthesized SNPs at various concentrations was measured using the agar well diffusion method against the isolated bacteria. Distilled water and leaf extract were used as the control. The bacteria were spread uniformly using a sterile cotton swab on LB agar plates. The plates were inoculated with bacteria and 5-mm-diameter wells were filled with SNPs of 0.09, 0.18, 0.36, 0.45, 0.9, 1.8, 3.6, and 4.5 ppm and incubated at room temperature (22 ± 2º C) for 48 hours. The inhibition zones were measured in millimeters (mm). After the incubation time, the diameters of the growth inhibition zones were measured. All tests were carried out in triplicate.

Cell viability: Washed isolated bacterial cells were suspended in sterile water. The initial concentration of all treatments was 7.5 $\log_{10}$ CFU. SNPs were added to achieve final concentrations of 5, 15, 25, and 35 ppm. The mixtures were shaken on a rotary shaker at 150 rpm for 5 min. The treatments were incubated at room temperature (22 ± 2º C) for 48 hours before enumeration. An aliquot was used to determine the effect on cell viability by plate count on nutrient agar.

3. STATISTICAL ANALYSIS

One-way analysis of variance (ANOVA) and t-tests were performed to analyze the differences in bactericidal activity of the SNPs among the treatments. A p-value < 0.05 was used as a criterion for significance level.

4. RESULTS

4.1. Biosynthesis and characterization of silver nanoparticles

SNPs were synthesized successfully using *Eleusine indica* L. Gaertn leaf extract. The change in color of the leaf extract from pale yellow to brown after dropping $AgNO_3$ indicated the formation of SNPs in the mixture. A UV-vis spectrophometer was used to monitor the reduction of Ag^{2+} to Ag^{o}. The absorption was observed at 437 nm in the UV-vis spectrum. The shape of the nanoparticles included nanospheres and nanobars (Figure 2a). The TEM image revealed that the particle sizes ranged from 3 to 33 nm with an average size of 16.73 nm (Figure 2b).

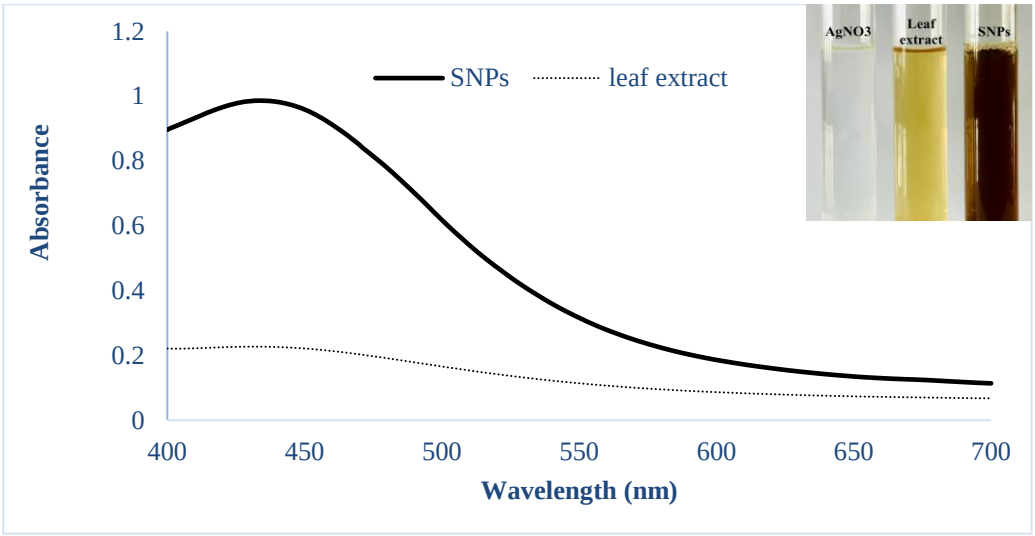
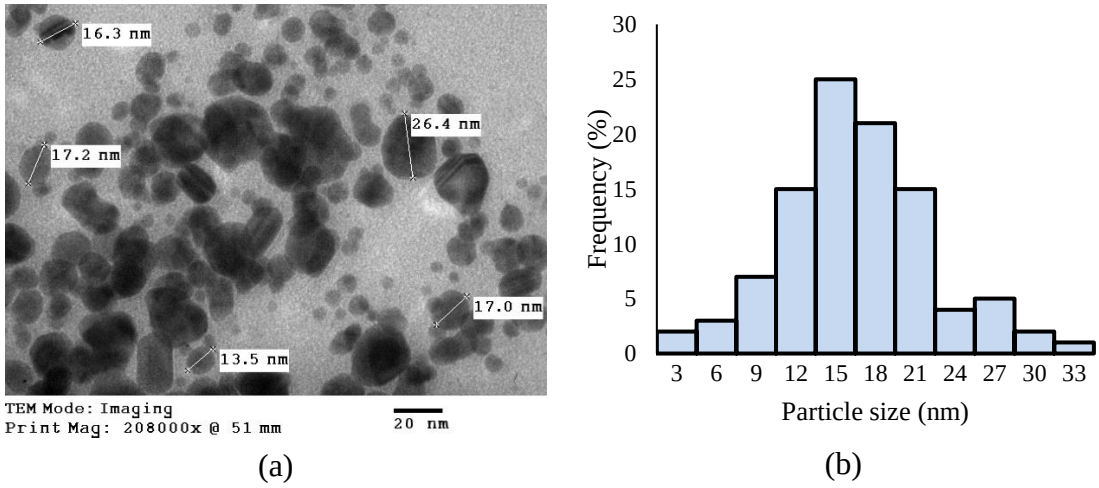


Figure 1. UV-vis spectra of leaf extract and SNPs



(a) (b)

Figure 2. (a) TEM image of the SNPs; (b) Particle size distribution histogram of SNPs

4.2. Isolation and screening of bacteria

Seven bacteria strains were obtained from the vase solutions and stems of cut flowers after a vase life of 10 days. Bacterial colonies appeared white, yellowish, and pink in color with different colony morphologies. The selected strains were designated as a, b, c, d, e, f, and g. The morphological, physiological, and biochemical characteristics are listed in Table 1. The investigated bacteria, a, b, c, d, e, f, and g, resemble *Bacillus sp.*, *Alcaligenes sp.*, *Micrococcus sp.*, *Pantoe sp.*, *Pseudomonas sp.*, *Pseudomonas sp.*, and *Pantoe sp.*, respectively, according to *Bergey's Manual of Systematic Bacteriology* (2nd ed., vol. 3 and vol. 2, Part B.) (Brenner et al., 2005) and *Bergey's Manual of Determinative Bacteriology* (9th ed.) (Bergey & Holt, 2000).

Table 1. The physiobiochemical characteristics of selected bacterial strains

Physiobiochemical test	Strain						
	a	b	c	d	e	f	g
Colony color	White	Light Yellow	Yellow	White	White–light pink	Creamy pink	White
Colony form	Circle	Circle	Circle	Circle	Irregular	Circle	Circle
Appearance	Dull	Glistening	Dull	Dull	Glistening	Dull	Glistening
Colony elevation	Convex	Flat	Umbonate	Raised	Convex	Raised	Pulvinate
Shape	Rod	Rod	Sphere	Rod	Rod	Rod	Rod
Gram reaction	+	-	+	-	-	-	-
Motility	+	+	-	+	+	+	+
Growth aeration	Fa	Fa	Fa	Fa	Fa	Fa	Fa
Oxidative/fermentation test	O	O	O	O/F	F	O	O/F
Casein hydrolysis	+	+	+	-	-	+	+
Starch hydrolysis	+	-	-	-	+	+	+
Gelatin hydrolysis	+	-	+	+	+	+	+
Indole test	-	-	-	-	-	-	-
Vogas-Prekaure test	+	-	+	-	-	-	+
Ammonia production	-	+	+	+	-	-	+
H_2S production	-	-	-	-	-	-	-
Citrate	+	+	-	+	+	+	+
Mineral salt medium	-	-	-	+	+	-	+
Temperature 22º C	+	+	++	+	+	++	++
Temperature 30º C	++	+	++	++	++	++	++
Temperature 37º C	+++	++	+++	+++	+++	+++	+++
Temperature 44º C	++	-	++	++	++	+++	++
Salt tolerance 0.5% NaCl	*	*	*	*	*	*	*
Salt tolerance 2% NaCl	*	*	*	*	*	*	*
Salt tolerance 4% NaCl	*	*	*	*	*	*	*
Catalase	+	+	+	+	+	+	+
Urease	+	-	+	+	-	-	-
Nitrate reduction	+	+	-	+	+	+	+
Fermentation							
Glucose	+	-	+	+	+	-	+
Lactose	-	-	-	+	+	-	+
Sucrose	+	-	-	+	+	-	+
Galactose	-	-	-	-	-	-	+

Notes: +: positive; -: negative; *: weak; Fa: facultative anaerobic; O: Oxidative; F: Fermentation.

4.3. Antimicrobial activity

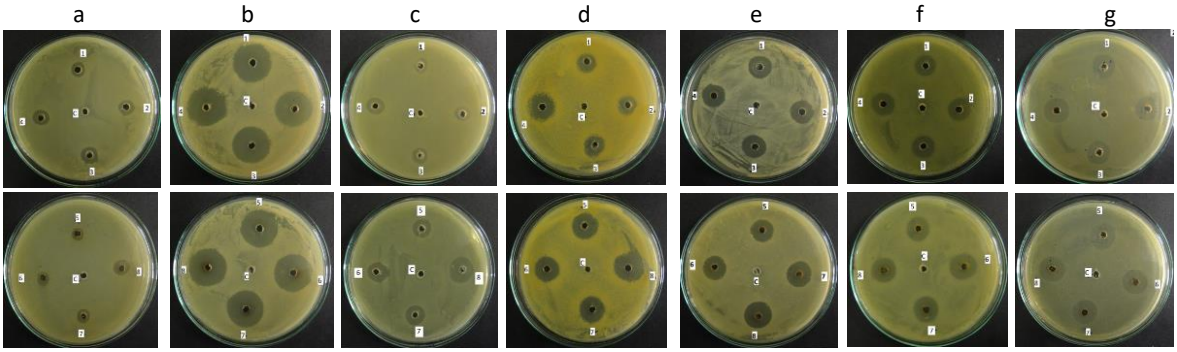


Figure 3. The antibacterial activity of the synthesized SNPs: (C) control, (1) 0.09 ppm, (2) 1.8 ppm (3) 0.45 ppm, (4) 0.9 ppm, (5) 1.8 ppm, (6) 2.7 ppm, (7) 3.6 ppm, (8) 4.5 ppm

Notes: (a) Bacillus cereus CA1; (b) Alcaligenes faecalis CA2; (c) Micrococcus luteus CA3; (d) Pantoe agglomerans CA4; (e) Pseudomonas aeruginosa CA5; (f) Pseudomonas aeruginosa CA6; (g) Pantoe agglomerans CA7.

Table 2. Zone of inhibition (in mm) for isolated bacterial strains treated with biosynthesized SNPs

NPs (ppm) / Strains	Zone of inhibition diameter (mm)							
	0.09	0.18	0.36	0.45	0.9	1.8	3.6	4.5
Bacillus cereus CA1	9.75±0.4	10.75±0.8	11.88±0.2	13.85±0.7	14.01±0.4	14.75±0.5	15.21±0.2	16.32±0.3
Alcaligenes faecalis CA2	19.65±0.7	20.73±0.8	21.91±0.9	23.01±0.7	23.95±0.5	24.88±0.4	26.05±0.6	26.99±0.3
Micrococcus luteus CA3	10.15±0.2	10.85±0.6	11.78±0.4	13.04±0.3	14.02±0.4	14.85±0.3	15.41±0.4	16.02±0.2
Pantoe agglomerans CA4	14.23±0.3	14.56±0.5	14.95±0.4	15.83±0.4	16.71±0.6	17.94±0.5	18.42±0.2	19.98±0.5
Pseudomonas aeruginosa CA5	12.75±0.9	13.13±0.2	13.19±0.2	14.09±0.4	14.81±0.3	15.82±0.6	15.74±0.4	17.29±0.2
Pseudomonas aeruginosa CA6	11.91±0.3	12.61±0.5	12.81±0.4	13.72±0.5	14.63±0.6	15.71±0.4	16.69±0.2	16.67±0.3
Pantoe agglomerans CA7	13.64±0.4	14.41±0.6	15.19±0.5	15.36±0.4	15.67±0.2	16.64±0.4	17.45±0.3	17.99±0.2

The sensitivity of the seven isolated bacterial strains to SNPs was examined using the disk diffusion assay and cell viability. The disk diffusion test data are shown in Figure 3. The antibacterial activity of the SNPs was compared with the effectiveness of the leaf extract and distilled water after the incubation period. The wells filled with leaf extract and distilled water did not show any zone of inhibition. Table 2 shows the mean diameters

of the zones of inhibition. The diameters of the inhibition zones increased with increasing SNP concentration. The bactericidal activity of SNPs was observed even at the lowest SNP concentration (0.09 ppm). The zones of inhibition differed significantly among the treatments of each bacterial strain with the exception of the 0.09 and 0.18 ppm treatments, and the 0.18 and 0.36 ppm treatments. At each concentration, *Alcaligenes faecalis* CA2 was the most sensitive to SNPs. The maximum inhibition diameter was 26.99 ± 0.3 mm for SNPs of 4.5 ppm on *Alcaligenes faecalis* CA2.

The efficacy of SNPs in the solutions against bacteria is shown in Table 3. Different concentrations of SNPs were used to inoculate the isolated bacteria. The magnitude of the responses indicated that the SNPs inhibited the growth of bacteria in solution. A similar trend was observed in the diffusion assay. The bactericidal activity was influenced by the SNP concentration and the bacterial strain. In a single SNP concentration, the most SNP-resistant bacterium was *Micrococcus luteus* CA3 in terms of inhibition diameter and growth in solution. This is a strain of gram-negative bacteria.

The effect of SNPs on two strains of bacteria (*Alcaligenes faecalis* CA2 and *Bacillus cereus* CA1) was determined by transmission electron microscopy (Figure 4). The morphology of the bacteria changed while the unexposed bacterial cells remained plump. The rupture surface of gram-positive bacteria was observed and cytoplasm was seen to have diffused out. The surfaces of gram-negative cells were distorted and deformed. The SNPs were observed inside the cell, which indicated that the cellular permeability increased. The cell membranes of both gram-positive and gram-negative bacteria were damaged.

Table 3. Bacterial growth inhibition in solution when treated with biosynthesized SNPs

SNPs (ppm) / Strains	Growth inhibition compared to the control (%)						
	5	10	15	20	25	30	35
Bacillus cereus CA1	42.28±0.6	49.31±0.5	53.84±0.4	57.99±0.2	60.24±0.4	64.16±0.3	69.34±0.2
Alcaligenes faecalis CA2	49.23±0.8	63.71±0.9	66.72±0.7	69.14±0.5	73.15±0.4	75.05±0.3	79.89±0.3
Micrococcus luteus CA3	40.26±0.8	48.91±0.6	54.15±0.7	59.08±0.7	65.85±0.3	68.39±0.2	70.73±0.3
Pantoe agglomerans CA4	39.47±0.6	45.39±0.4	54.76±0.3	60.99±0.6	65.29±0.3	68.13±0.4	75.18±0.2
Pseudomonas aeruginosa CA5	42.18±0.5	50.51±0.4	58.73±0.5	63.36±0.6	67.28±0.6	71.34±0.5	74.17±0.4
Pseudomonas aeruginosa CA6	47.82±0.9	56.69±0.8	60.23±0.4	63.91±0.8	66.51±0.7	69.94±0.4	73.99±0.3
Pantoe agglomerans CA7	44.66±0.7	52.34±0.4	56.31±0.4	61.56±0.6	67.45±0.5	70.31±0.8	74.73±0.5

5. DISCUSSION

The biosynthesis of SNPs from plant extracts can be confirmed using UV-vis and the change in color of the solution. A shift in color from pale yellow to brown indicates

the formation of SNPs (Anandalakshmi et al., 2016). Similar to previous studies, UV-vis spectroscopy was used to confirm the reduction of silver ions to SNPs. The maximum peak of the surface plasmon resonance (SPR) absorption of the SNPs was at 437 nm. It was reported that surface plasmon resonance (SPS) wavelengths of SNPs range from 400 to 480 nm (Nazeruddin et al., 2014). Various phytochemicals in plant extracts influence SNP morphology and size by acting as reducing or stabilizing agents (Shaikh et al., 2021). SNPs synthesized from goosegrass leaf extract revealed sphere and bar shapes in this study, with sizes ranging from 3 nm to 33 nm. This may be the result of various phytochemicals isolated from *Eleusine indica* L. Gaertn that act as stabilizing and reducing agents (Zakri et al., 2021). The size of the SNPs is in the common range of green synthesized SNPs (Manik et al., 2020; Mussin et al., 2021).

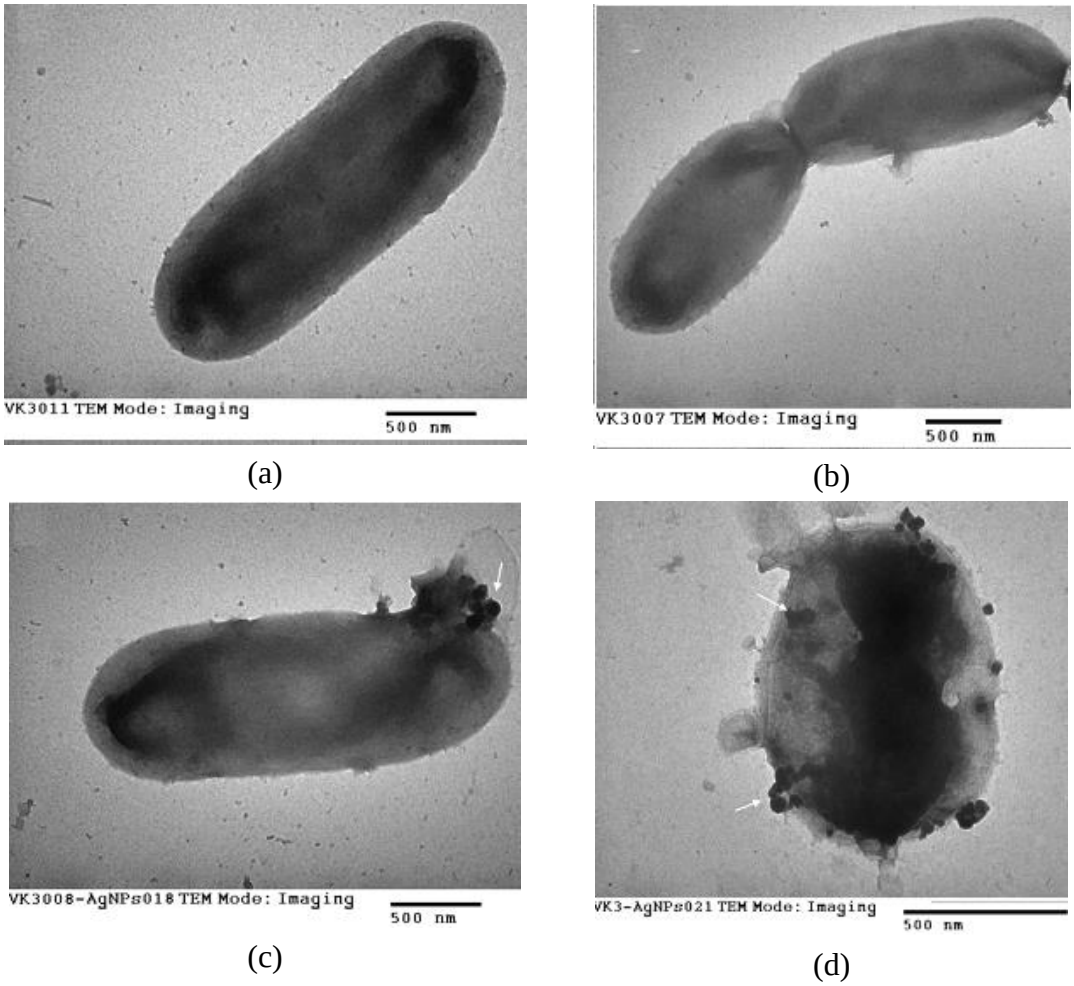


Figure 4. TEM images of Bacillus cereus CA1 and Alcaligenes faecalis CA2

Notes: (a) untreated *Bacillus cereus* CA1; (b) untreated bacterium and *Alcaligenes faecalis* CA2; (c) treated *Bacillus cereus* CA1; (d) treated *Alcaligenes faecalis* CA2.

After 10 days, seven bacterial strains were isolated from the vase water of cut flowers and identified according to *Bergey's Manual of Systematic Bacteriology* (Brenner et al., 2005) and *Bergey's Manual of Determinative Bacteriology* (Bergey & Holt, 2000).

Bacteria were identified by physiobiochemical characteristics, such as Gram reaction, morphology, motility, catalase, carbohydrate breakdown, and ability to grow at different temperatures or NaCl concentrations (Table 1). The bacteria are gram-positive and gram-negative bacteria that belong to the genera *Bacillus, Alcaligene, Micrococcus, Pantoe*, and *Pseudomonas.* The prominent isolated species are similar to those reported in previous studies (Alaey et al., 2011; Bowyer et al., 2003; Hutchinson et al., 2004; Li et al., 2012).

Silver ions and their compounds are widely known to act as antimicrobial agents (Ahmed et al., 2016; Aziz et al., 2014). SNPs exhibited antimicrobial activity against the isolated bacteria, including two gram-positive strains and five gram-negative strains. The results clearly show the different growth and inhibition zones of each strain. This broad effect is consistent with previous studies (Ibrahim, 2015; Pant et al., 2013). The impact of SNPs depends on the bacterial strain. The bactericidal effects of SNPs could be a result of their impact on the cell wall and membrane, and then on enzymes, RNA, and DNA (Dakal et al., 2016; Lopez-Esparza et al., 2016; Shockman & Barrett, 1983). The penetrating properties of SNPs and the structure of the bacterial cell wall influence antibacterial activity. The cell membranes of bacteria are negatively charged. SNPs are positively charged, which leads to the loss of permeability control of the cell membrane, resulting in cell death (Hamouda & Baker, 2000). This study clearly demonstrated that the SNPs exhibited bactericidal activity on all isolates. The results indicate that the toxicological activity depended on the dose (Tables 2 and 3). Higher SNP concentrations increase cell death. At 15 ppm, more than 50% bacterial growth inhibition was reported. We propose that these biosynthesized SNPs can be used as antibacterial agents. We speculate that higher SNP concentrations accelerate the interaction between cells and particles, with the result that decreasing cell viability and increasing inhibition zone size were observed (Figure 3 and Tables 2 and 3).

In terms of a single SNP concentration, the effect on the gram-negative bacteria was more severe than on the gram-positive bacteria. The most distinctive difference in the membranes of gram-positive and gram-negative bacteria is the thickness of the peptidoglycan layer. The thicker membrane may limit the effect of SNPs (Kim & Kuk, 2007). Two isolated positive bacterial strains, *Bacillus cereus* CA1 and *Micrococcus luteus* CA3, were more resistant to SNPs than the isolated gram-negative bacteria. The antimicrobial mechanism of SNPs include damaging cell membranes, altering membrane integrity, interrupting metabolic processes, and finally killing the cell (Shankar & Rhim, 2015). Increasing the SNP concentrations led to larger inhibition zones. The proton motive force on the surface of bacteria may lead to differences in inhibition zone diameter (Aziz et al., 2014). The antibacterial activity of SNPs on all seven bacteria strains depended on the SNP concentration. Increasing SNP concentration led to the formation of more pits on the cell walls of bacteria (Edrisi et al., 2012). SNPs then accumulated in the membrane and increased cellular permeability (Kim & Kuk, 2007). SNPs release ions that generate reactive oxygen species, and as a result, kill bacteria (Aboyewa et al., 2021; Patil & Muthusamy, 2020; Tyavambiza et al., 2021). The bacterial growth inhibition test was performed to test the direct effect of SNPs on bacterial viability (Table 3). A similar

trend was observed in the inhibition assay. An increase in SNPs concentration increased cell death. Viability was under the influence of the bacterial strain as well.

The morphology of bacteria cells was observed by TEM. TEM images revealed cellular membrane disruptions after exposure to SNPs. Besides releasing silver ions, SNPs can themselves kill bacteria by adhering to the cell surface, then forming pits where nanoparticles can accumulate to denature the cell membrane. The results of this study are similar to those for *E. coli* (gram-negative) and *S. aureas* (gram-positive) that were treated with SNPs synthesized from papaya (Feng et al., 2000). This suggests that one of the ways that SNPs kill bacteria is by disruption of the cell membrane. The TEM image of gram-positive cells indicated that SNPs adhered to the cell membrane while SNPs were observed inside the gram-negative cells (Figure 4c and 4d). The deformation and damage with collapsed cell membrane were observed in the gram-negative bacteria while the membranes of gram-positive bacteria were partially damaged. The different cell wall structure and components might lead to this difference (Chatterjee et al., 2015). The cell wall of gram-positive bacteria is much thicker than that of gram-negative bacteria (Erickson, 2017). The fewer peptidoglycan molecules in gram-negative bacteria lead them to be more susceptive than gram-positive cells (Feng et al., 2000).

Compared to the distilled water and leaf extract, the SNPs synthesized from *Eleusine indica* L. Gaertn leaf extract show significant antibacterial activity. The mechanism of bactericidal activity of SNPs has remained controversial and is not yet clearly understood, but the general mechanism is the ability to damage the cell membrane permeability, damage the respiration functions of the cell, and encourage the formation of free radicals or the interaction of silver ions with phosphorus moieties present in the DNA of microorganisms, resulting in the inactivation of DNA replication (Logaranjan et al., 2016). Our findings further confirmed the interaction between the cell surface and SNPs. The results suggest SNPs as possible agents to kill blockage bacteria and prolong the vase life of cut flowers by inhibiting bacteria contamination.

6. CONCLUSIONS

SNPs were synthesized successfully from *Eleusine indica* L. Gaertn leaf extract. Multiple shapes, including spheres and bars with an average size of 16.73 nm, were observed. Seven bacterial strains were isolated from vase water. They consisted of gram-positive and gram-negative bacteria that belong to the genera *Bacillus, Alcaligene, Micrococcus, Pantoe*, and *Pseudomonas.* The biosynthesized SNPs inhibited the growth of these bacteria. The bactericidal effect of SNPs on all seven bacteria strains depended on the SNP concentrations and the bacterial strains. Gram-positive bacteria exhibited greater resistance to SNPs than gram-negative bacteria.

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