GREEN SYNTHESIS OF SILVER NANOPARTICLES USING *in vitro* PLANTLETS OF *Panax vietnamensis* (NGOC LINH GINSENG) AND EVALUATION OF THEIR POTENTIAL

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Abstract. This study presents the green synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract from *in vitro*-derived plantlets of *Panax vietnamensis* (Ngoc Linh ginseng). The biosynthesis process was optimized by investigating the effects of reaction temperature and $AgNO_3$ concentration. The optimal conditions were determined to be 1.0 mM $AgNO_3$ at 80°C for 15 minutes, yielding stable AgNPs with a characteristic surface plasmon resonance peak at 417 nm and a bright reddish-brown color. Characterization using UV-Vis spectroscopy, TEM, DLS, zeta potential, and FTIR confirmed the formation of quasi-spherical AgNPs with an average hydrodynamic size of 46.7 nm, PDI of 0.429, mean core size of 35.9 ± 15.4 nm, and a highly negative zeta potential of −65.3 mV, indicating excellent colloidal stability. FTIR analysis revealed the involvement of ginsenosides, polyphenols, and other phytochemicals as both reducing and capping agents. In seed germination assays, AgNPs treatment demonstrated superior performance compared to conventional sterilizing agents (ethanol, sodium hypochlorite, and formalin). It effectively reduced contamination rates to approximately 8% while achieving the highest germination percentage (78.67- 80.67%), showing strong antimicrobial activity combined with low phytotoxicity. These results highlight the dual role of Ngoc Linh ginseng-derived AgNPs as both an efficient antimicrobial agent and a biostimulant for seed priming. The green synthesis approach offers a sustainable, cost-effective, and environmentally friendly method for producing biocompatible AgNPs with promising applications in agriculture and biomedicine.

Keywords. silver nanoparticles, green synthesis, *Panax vietnamensis*, Ngoc Linh ginseng, *in vitro* plantlets, antimicrobial activity, seed sterilization

1 INTRODUCTION

In recent years, nanotechnology and nanomaterials have attracted considerable research attention and have been widely applied in various scientific and industrial fields. Among these, biological nanotechnology has emerged as a particularly accessible and sustainable approach due to its low cost, minimal technological requirements, absence of chemical additives or stabilizing agents, and the lack of stringent synthesis conditions [1-3]. Biogenic nanomaterials have demonstrated broad applicability in medicine, materials science, environmental technology, and chemistry. Furthermore, biologically synthesized nanomaterials are generally considered safer, less toxic, and more environmentally friendly than nanoparticles produced through conventional physical or chemical methods [4]. Techno - economic analysis has further confirmed the superior cost - effectiveness of green synthesis; for instance, the yield of green - synthesized AgNPs can be more than double that of chemical methods (7.1 mg vs 3.0 mg per synthesis) with significantly lower material costs [4].

Among various nanomaterials, silver nanoparticles (AgNPs) are the most extensively synthesized owing to their high surface area - to - volume ratio, which contributes to enhanced dispersion, permeability, photocatalytic activity, biosensing capability, and antimicrobial properties [5]. The antimicrobial mechanism involves AgNPs anchoring to and penetrating bacterial cell walls, causing structural damage and modulating cellular signaling [5, 6]. Biosynthesis of AgNPs can employ diverse biological resources, including bacteria, algae, fungi, and plants [7]. Among these methods, plant extract - mediated synthesis is the most widely utilized because of its simplicity, availability, and the broad range of functional properties exhibited by the resulting nanoparticles. Plant systems are often preferred over microbial ones because they facilitate "nanofactories" that initiate rapid synthesis in minutes rather than days [8]. The use of medicinal

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plants for nanoparticle synthesis has recently emerged as a promising strategy with significant potential applications in medicine and functional foods.

Studies on Korean ginseng (*Panax ginseng*) have demonstrated that different plant parts, including roots and leaves, as well as processed products such as red ginseng and black ginseng, can be used for the biosynthesis of various gold and AgNPs [8-11]. Fresh leaves have proven particularly efficient, facilitating the rapid synthesis of gold nanoparticles in just 3 minutes and AgNPs in 45 minutes at 80°C [8, 12]. These nanoparticles have shown diverse biological activities, including Hg^{+} ion detection, protection against nicotine - induced damage in experimental animals, anti - inflammatory and antioxidant effects, and inhibitory activity against A549 lung adenocarcinoma, MCF7 breast cancer, and HepG2 liver cancer cell lines [12, 13]. The anticancer mechanism specifically involves the regulation of the EGFR/p38 MAPK/p53 pathway, resulting in inhibited cell viability [13]. In addition, several studies have reported that these nanoparticles can induce morphological alterations in cancer cell nuclei, thereby enhancing programmed cell death. The utilization of ginseng - based nano - delivery systems is crucial for medical efficacy, as raw ginsenosides naturally suffer from a very poor absorption rate of less than 5% in the gastrointestinal tract [12]. The safety and biocompatibility of ginseng - derived nanomaterials have also been emphasized, highlighting their substantial potential for biomedical applications [11].

In addition to mature plant tissues, callus cultures and *in vitro* - derived plantlets from several medicinal species have also been demonstrated to be effective biological materials for the green synthesis of AgNPs, including *Phlomis bracteosa, Costus speciosus, and Catharanthus roseus* [5]. In *C. roseus*, leaf - derived nanoparticles displayed higher toxicity than root or callus - derived ones, likely due to the presence of specific alkaloids like vinblastine and vincristine [14]. Several studies have further indicated that nanoparticles synthesized from callus and *in vitro* materials may exhibit superior physicochemical and biological properties compared with those derived from conventional plant tissues.

Ngoc Linh ginseng is an endemic medicinal herb of Vietnam recognized for its strong antioxidant, anticancer, and anti - stress properties, as well as its high economic value. The species is considered an important resource for poverty alleviation and regional economic development and is currently being conserved and cultivated in the provinces of Da Nang and Quang Ngai [15]. In addition to conventional seed propagation methods, advances in clonal propagation technologies have increasingly been applied in the research and production of vegetatively propagated Ngoc Linh ginseng plantlets [16]. These approaches are expected to reduce production costs while ensuring genetic uniformity, quality control, and disease management.

As a high - value medicinal species, Ngoc Linh ginseng represents a promising biological source for nanoparticle biosynthesis. The utilization of *in vitro* - derived materials for the green synthesis of nanoparticles may therefore provide a novel and highly applicable approach for the development of functional nanomaterials. Although several studies have reported the green synthesis of silver and gold nanoparticles using different parts of Korean ginseng, the use of *in vitro*-derived plantlets of the endemic Vietnamese species Ngoc Linh ginseng has not yet been explored. Moreover, previous works mainly focused on biomedical applications, whereas the dual function of these ginseng-derived AgNPs as both an effective antimicrobial agent and a biostimulant for seed sterilization and germination enhancement remains largely uninvestigated. The present study therefore aims to optimize the green synthesis of AgNPs using aqueous leaf extract (ALF) from *in vitro* plantlets of *P. vietnamensis*, comprehensively characterize their physicochemical properties, and evaluate their potential as an eco-friendly alternative to conventional chemical sterilants in seed priming of Ngoc Linh ginseng.

2 MATERIALS AND METHODS

2.1 Preparation of aqueous leaf extract

in vitro - derived Ngoc Linh ginseng plantlets were used as the biological material for nanoparticle synthesis. Approximately 15 g of dried leaf material was cut into small pieces and boiled in 100 mL of distilled water for 20 min. After extraction, distilled water was added to restore the final volume to 100 mL. The obtained extract was centrifuged at 10,000 rpm for 10 min to remove suspended particles and insoluble residues. The supernatant, referred to as ALF, was collected and stored at 4°C until further use.

2.2 Effect of temperature on silver nanoparticle synthesis

AgNPs were synthesized using the ALF as a reducing and stabilizing agent. Briefly, 5 mL of ALF was mixed with 25 mL of distilled water, followed by the addition of $AgNO_3$ solution to obtain final silver ion concentrations of 0.25, 0.5, 1.0, and 2.0 mM. The reaction mixtures were incubated at different temperatures (60, 70, 80, and 90°C) for 30 min in a temperature - controlled incubator. The formation of AgNPs was preliminarily monitored based on the color change of the reaction mixture.

2.3 Effect of $AgNO_3$ concentration and reaction time on silver nanoparticle synthesis

To optimize AgNP synthesis conditions, a 1 mM $AgNO_3$ stock solution was prepared. Subsequently, 5 mL of ALF was mixed with 25 mL of distilled water, and $AgNO_3$ was added to obtain final concentrations of 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 mM. The reaction mixtures were incubated at 50, 60, 70, 80, and 90°C. Ultraviolet - visible (UV - Vis) spectroscopy was performed at 10 min intervals throughout the reaction process until the maximum absorbance value was reached, indicating completion of nanoparticle formation.

2.4 Characterization of AgNPs

The synthesized AgNPs were characterized using multiple analytical techniques. UV - Vis spectroscopy was employed to confirm nanoparticle formation and determine the characteristic surface plasmon resonance peak of AgNPs. Transmission electron microscopy (TEM) was used to evaluate nanoparticle morphology and particle size distribution. Dynamic light scattering (DLS) analysis was performed to determine hydrodynamic particle size and nanoparticle dispersion characteristics. Particle size was determined from TEM images using ImageJ software (National Institutes of Health, USA). After calibration with the image scale bar, non - overlapping individual nanoparticles were analyzed using the Analyze Particles function. Particle size was expressed as the Equivalent Circular Diameter (ECD), calculated from the projected area of each nanoparticle [17].

2.5 Evaluation of seed protection and germination - promoting effects of AgNPs

The effects of AgNPs treatment on seed sterilization and germination were evaluated using different chemical and nanoparticle treatments. Each treatment consisted of three replicates with 50 seeds per replicate. The experimental treatments included: untreated control; 70% ethanol treatment for 10, 15, and 20 min; 2% sodium hypochlorite (Javen) treatment for 10, 15, and 20 min; 2% formalin treatment for 10, 15, and 20 min; and AgNPs treatment for 10, 15, and 20 min. Following treatment, seeds were rinsed with sterile distilled water and cultured under suitable germination conditions. Germination percentage and contamination rate were recorded and compared among treatments. Germination was assessed by the percentage of seeds showing seed coat cracking, which is a reliable early indicator of germination in *P. vietnamensis*. Contamination rate was recorded as the percentage of seeds showing microbial growth.

3 RESULTS

3.1 Effect of temperature on the biosynthesis of AgNPs from *in vitro* - derived Ngoc Linh ginseng

The synthesis of AgNPs from ALF of *in vitro* - derived Ngoc Linh ginseng was strongly influenced by both reaction temperature and $AgNO_3$ concentration. The formation of AgNPs was initially indicated by a gradual color change of the reaction mixtures from pale yellow to orange - yellow, yellowish - brown, reddish - brown, and finally dark brown or black at higher silver ion concentrations. The intensity of color increased with increasing temperature and $AgNO_3$ concentration, suggesting enhanced nanoparticle formation under elevated thermal conditions.

UV - Vis analysis confirmed the successful biosynthesis of AgNPs through the appearance of characteristic surface plasmon resonance (SPR) peaks within the range of 408 - 418 nm. At 0.25 mM $AgNO_3$, absorbance values gradually increased from 0.25 at 60°C to 0.55 at 90°C, accompanied by a color transition from pale yellow to orange - yellow. Similar trends were observed at 0.5 mM $AgNO_3$, where absorbance increased from 0.42 to 0.95 as temperature increased from 60°C to 90°C, and the solution color changed from light orange - yellow to light reddish - brown.

The highest nanoparticle formation efficiency was observed at 1.0 mM $AgNO_3$. Under this condition, absorbance values increased from 0.48 at 60°C to 1.14 at both 80°C and 90°C, while the reaction mixture developed a bright reddish - brown coloration characteristic of stable silver nanoparticle formation. The SPR peak also shifted from 412 nm at 60°C to 418 nm at 90°C, indicating changes in nanoparticle size distribution and concentration during synthesis.

In contrast, reactions containing 2.0 mM $AgNO_3$ showed signs of instability at elevated temperatures. Although the absorbance reached 0.65 at 60°C with a turbid brown appearance, the mixtures incubated at 70 - 90°C rapidly turned black and formed visible precipitates. This observation suggests excessive silver ion concentration exceeded the reducing and stabilizing capacity of phytochemical compounds in the extract, leading to nanoparticle aggregation and sedimentation.

Overall, the results demonstrated that increasing temperature enhanced the reduction of Ag^+ ions and promoted AgNP formation. However, excessively high precursor concentrations negatively affected colloidal stability. Among the tested conditions, 1.0 mM $AgNO_3$ at 80°C produced the most stable and efficient silver nanoparticle system, characterized by high absorbance (1.14), a distinct SPR peak at 417 nm, and a stable bright reddish - brown color without visible precipitation.

Table 1: Effect of temperature and $AgNO_3$ concentration on the biosynthesis of AgNPs from *in vitro* - derived Ngoc Linh ginseng

$AgNO_3$ concentration (mM)	60°C	70°C	80°C	90°C
0.25	0.25 (408) Pale yellow	0.38 (410) Pale yellow	0.52 (412) Light orange - yellow	0.55 (413) Orange - yellow
0.5	0.42 (410) Light orange - yellow	0.68 (413) Light yellowish - brown	0.89 (415) Yellowish - brown	0.95 (416) Light reddish - brown
1.0	0.48 (412) Orange - yellow	0.85 (415) Yellowish - brown	1.14 (417) Bright reddish - brown	1.14 (418) Bright reddish - brown
2.0	0.65 (415) Turbid brown	Black with precipitate	Black with precipitate	Black with precipitate



Figure 1: Biosynthesis of AgNPs at 80°C. (A) $AgNO_3$ concentration of 0.25 mM; (B) $AgNO_3$ concentration of 0.5 mM; (C) $AgNO_3$ concentration of 1.0 mM; (D) $AgNO_3$ concentration of 2.0 mM; (E) ALF control.

3.2 Effect of $AgNO_3$ concentration and reaction time on silver nanoparticle synthesis

The results presented in Table 2 demonstrated that both $AgNO_3$ concentration and reaction time strongly affected the biosynthesis of AgNPs from *in vitro* - derived Ngoc Linh ginseng extract. The absorbance values increased markedly during the first 15 min of reaction at all tested concentrations, indicating rapid formation of AgNPs in the reaction system. After 15 min, the absorbance values showed only negligible changes at 20 min, suggesting that the reduction reaction had nearly reached equilibrium and that nanoparticle formation was essentially completed within 15 min under the tested conditions. This result indicates that 15 min was the optimal reaction time for AgNP biosynthesis, providing high nanoparticle yield while minimizing unnecessary prolongation of the reaction process.

At lower $AgNO_3$ concentrations (0.25 - 0.75 mM), absorbance values gradually increased from 0.18 - 0.62 after 5 min to 0.79 - 1.05 after 15 min, accompanied by SPR peaks ranging from 410 to 415 nm. Increasing the $AgNO_3$ concentration to 1.0 mM significantly enhanced nanoparticle formation, resulting in an absorbance value of 1.14 at 15 min with a characteristic SPR peak at 417 nm. Notably, the absorbance at 20 min remained nearly unchanged (1.15 at 418 nm), further confirming the stabilization of the nanoparticle system after 15 min of reaction. Although higher precursor concentrations of 1.25 and 1.5 mM produced

greater absorbance values (1.27 - 1.31 at 15 min), only minimal increases were observed after 20 min, indicating saturation of the reduction process after 20 min.

Table 2: Effect of $AgNO_3$ concentration and reaction time on the biosynthesis of AgNPs from *in vitro* - derived Ngoc Linh ginseng

$AgNO_3$ concentration (mM)	**Absorbance after 5 min (λmax, nm)**	**Absorbance after 10 min (λmax, nm)**	**Absorbance after 15 min (λmax, nm)**	**Absorbance after 20 min (λmax, nm)**
0.25	0.18 (408)	0.29 (409)	0.51 (410)	0.53 (411)
0.5	0.35 (410)	0.58 (411)	0.91 (413)	0.93 (414)
0.75	0.62 (412)	0.91 (414)	1.11 (415)	1.13 (416)
1.0	0.78 (414)	1.02 (416)	1.14 (417)	1.15 (418)
1.25	0.95 (415)	1.18 (417)	1.31 (418)	1.33 (419)
1.5	1.08 (417)	1.25 (419)	1.34 (420)	1.36 (421)



Figure 2: Visual observation of silver nanoparticle synthesis at 80°C under different reaction times. (A) Samples after 5 min; (B) samples after 10 min; (C) samples after 15 min; and (D) samples after 20 min of incubation. In each panel, the tubes are arranged from left to right as follows: control, and $AgNO_3$ concentrations of 1.5, 1.25, 1.0, 0.75, 0.5, and 0.25 mM.



Figure 3: Aggregation and precipitation of silver nanoparticle samples after two weeks of storage.

The stability of the synthesized silver nanoparticle samples was evaluated by storing the colloidal solutions under dark conditions at room temperature for two weeks. After the storage period, the samples were visually assessed based on changes in color, turbidity, precipitation, and aggregation. Stable nanoparticle samples maintained a homogeneous reddish - brown coloration without visible sedimentation or phase separation, whereas unstable samples exhibited darkening, turbidity, aggregation, or precipitate formation at the bottom of the tubes. The results showed that nanoparticle samples synthesized at $AgNO_3$ concentrations higher than 1.0 mM gradually became unstable during storage, with visible aggregation and sediment formation observed after two weeks. In contrast, the sample synthesized at 1.0 mM $AgNO_3$ maintained good dispersion stability without noticeable precipitation, indicating that this concentration provided the most stable silver nanoparticle system.

3.3 Characterization of AgNPs

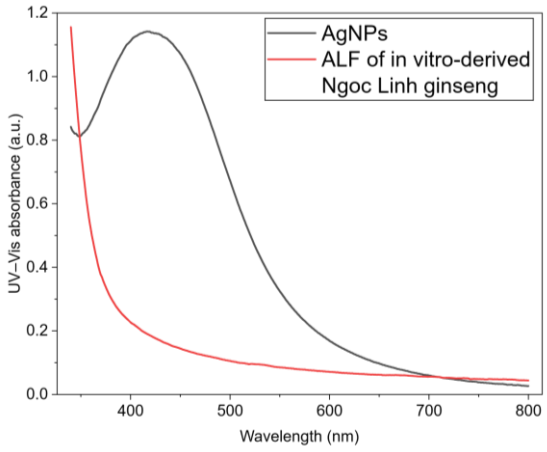


Figure 4: UV - Vis absorption spectra of green - synthesized AgNPs derived from *in vitro* cultured Ngoc Linh ginseng extract compared with the plant extract

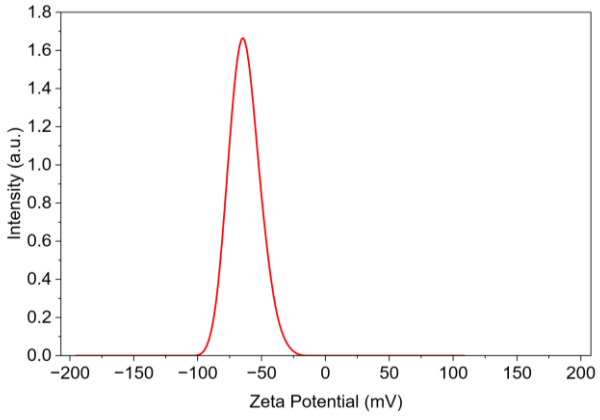


Figure 5: Zeta potential distribution of AgNPs synthesized from *in vitro* - derived Ngoc Linh ginseng extract.

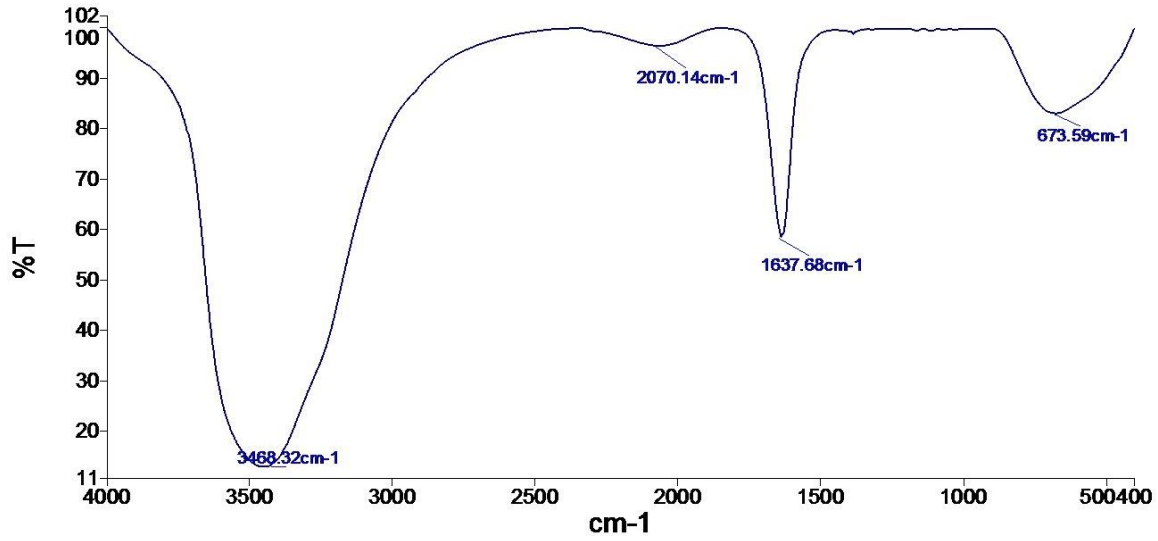


Figure 6: Fourier - transform infrared (FTIR) spectrum of AgNPs synthesized from *in vitro* - derived Ngoc Linh ginseng extract.

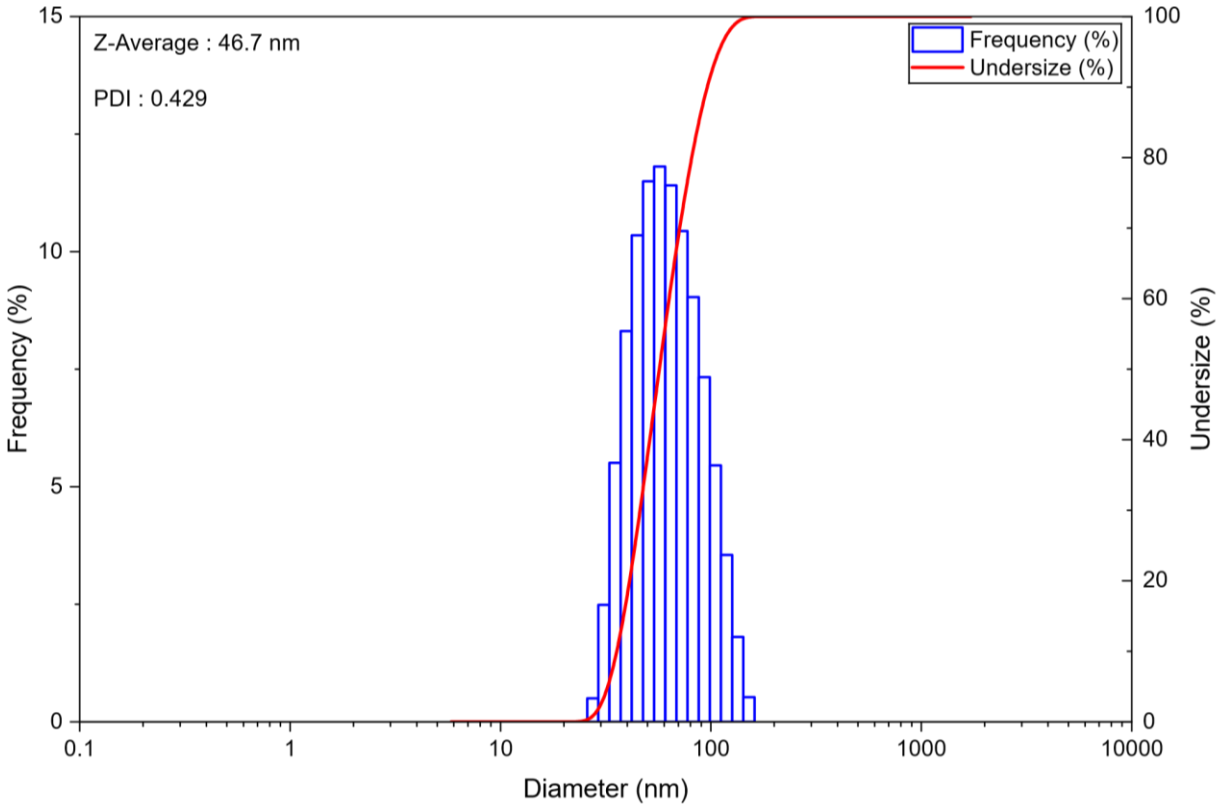


Figure 7. Particle size distribution of AgNPs synthesized from *in vitro* - derived Ngoc Linh ginseng extract determined by dynamic light scattering (DLS)

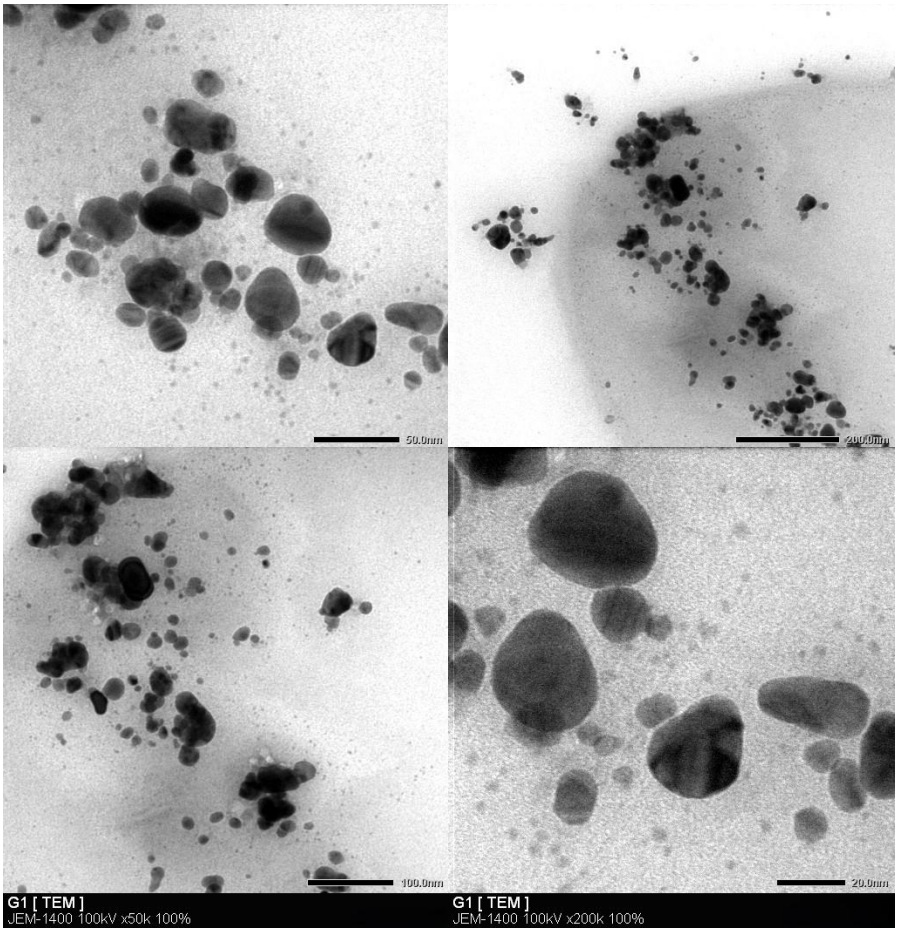


Figure 8. Transmission electron microscopy (TEM) image of silver nanoparticle colloids synthesized from ALF of *in vitro* - derived Ngoc Linh ginseng.

Table 3. Particle size statistics of AgNPs synthesized from ALF of *in vitro* - derived Ngoc Linh ginseng based on TEM analysis

Parameter	**Value**
Number of nanoparticles analyzed	100
Minimum particle size (nm)	11.8
Maximum particle size (nm)	82.4
Mean particle size (nm)	35.9
Standard deviation (SD) (nm)	15.4
Median particle size (nm)	33.9
Coefficient of variation (CV) (%)	42.9

The formation of AgNPs was confirmed by UV - Visible spectroscopy. The UV - Vis spectrum of the biosynthesized AgNPs exhibited a strong and distinct surface plasmon resonance (SPR) peak at approximately 408 - 418 nm. In contrast, the extract from *in vitro* - derived Ngoc Linh ginseng showed no characteristic absorption peak in this region, only a continuous decrease in absorbance from the ultraviolet region (Figure 4).

The zeta potential of the silver nanoparticle suspension (sample G1) was measured to be −65.3 mV, with an electrophoretic mobility of −0.000506 cm^2/Vs. The distribution profile exhibited a single sharp peak in the negative potential region, indicating uniform surface charge (Figure 5).

The Fourier transform infrared (FTIR) spectrum of the AgNPs (sample G2) displayed characteristic absorption bands at 3468 cm^{-1} (broad, strong), 2070 cm^{-1} (weak), 1637 cm^{-1} (strong), and 673 cm^{-1} (medium) (Figure 6).

The particle size and polydispersity of the AgNPs were determined by dynamic light scattering (DLS). The results showed a Z - average diameter of 46.7 nm with a polydispersity index (PDI) of 0.429 (Figure 7).

TEM analysis showed that the synthesized AgNPs were predominantly quasi-spherical with a mean particle size of 35.9 ± 15.4 nm (n = 100; Table 3 and Figure 8). Particle sizes ranged from 11.8 to 82.4 nm (median 33.9 nm, CV 42.9%), indicating a moderately broad size distribution. A small number of nanoparticle aggregates were also observed. The average particle size determined by TEM was lower than the Z-average hydrodynamic diameter (46.7 nm) obtained from DLS analysis, which is expected because DLS measures the hydrodynamic size including the organic coating and hydration layer.

3.4 Evaluation of seed protection and germination - promoting effects of AgNPs

The results demonstrated that AgNPs (AgNPs) exhibited superior performance compared with conventional sterilizing agents in terms of contamination control, seed viability, and treatment stability. The untreated control showed a high contamination rate of 78% with a low germination rate of only 18.67%. Treatment with 70% ethanol reduced contamination to 49.33 - 60%; however, germination remained relatively low, ranging from 12.67% to 25.33% (Table 4, Figure 9).

Among the conventional sterilizing agents, 2% sodium hypochlorite (Javen) exhibited the strongest decontamination effect, reducing contamination to 0 - 10.67%. However, prolonged exposure also negatively affected seed viability. Germination rates under Javen treatment ranged from 31.33% to 65.33%, indicating considerable phytotoxic effects associated with strong oxidizing activity. Similarly, 2% formalin reduced contamination to 16.67 - 24%, but germination rates remained moderate (32 - 50%), suggesting toxic effects on seed tissues during sterilization (Table 4, Figure 9).

In contrast, AgNP treatments produced the most balanced results between sterilization efficiency and seed viability. Contamination rates were reduced to only 8% across all exposure times (10 - 20 min), while germination rates remained exceptionally high, ranging from 78.67% to 80.67%, representing the highest values among all treatments. Furthermore, no major differences were observed among AgNP treatment durations, indicating that the nanoparticle system maintained stable sterilization efficiency and low phytotoxicity over time.

Table 4. Infection rate and seed coat dormancy rate of Ngoc Linh ginseng seeds treated with AgNPs compared with conventional chemical treatments.

Treatment	Infection rate (%)	Seed coat cracking rate (%)
Control	78 ± 4.9^{a}	18.67 ± 3.4^{a}
70% ethanol for 10 min	60 ± 4.9^{b}	12.67 ± 4.11^{a}
70% ethanol for 15 min	49.33 ± 5.25^{b}	24.67 ± 6.8^{a}
70% ethanol for 20 min	50 ± 4.9^{b}	25.33 ± 6.8^{a}
2% sodium hypochlorite (Javen) for 10 min	10.67 ± 2.49^{de}	61.33 ± 5.25^{bc}
2% sodium hypochlorite (Javen) for 15 min	0 ± 0^{e}	65.33 ± 5.25^{c}
2% sodium hypochlorite (Javen) for 20 min	0 ± 0^{e}	31.33 ± 7.36^{ab}
2% formalin for 10 min	24 ± 2.83^{c}	50 ± 4.32^{b}
2% formalin for 15 min	16.67 ± 2.49^{cd}	44 ± 6.53^{b}
2% formalin for 20 min	19.33 ± 3.4^{cd}	32 ± 7.48^{ab}
AgNPs for 10 min	8 ± 1.63^{de}	78.67 ± 2.49^{cd}
AgNPs for 15 min	8 ± 1.63^{de}	80.67 ± 2.49^{d}
AgNPs for 20 min	8 ± 3.27^{de}	78.67 ± 3.4^{cd}

Values within the same column followed by the same letter are not significantly different according to Tukey's test ($p > 0.05$).

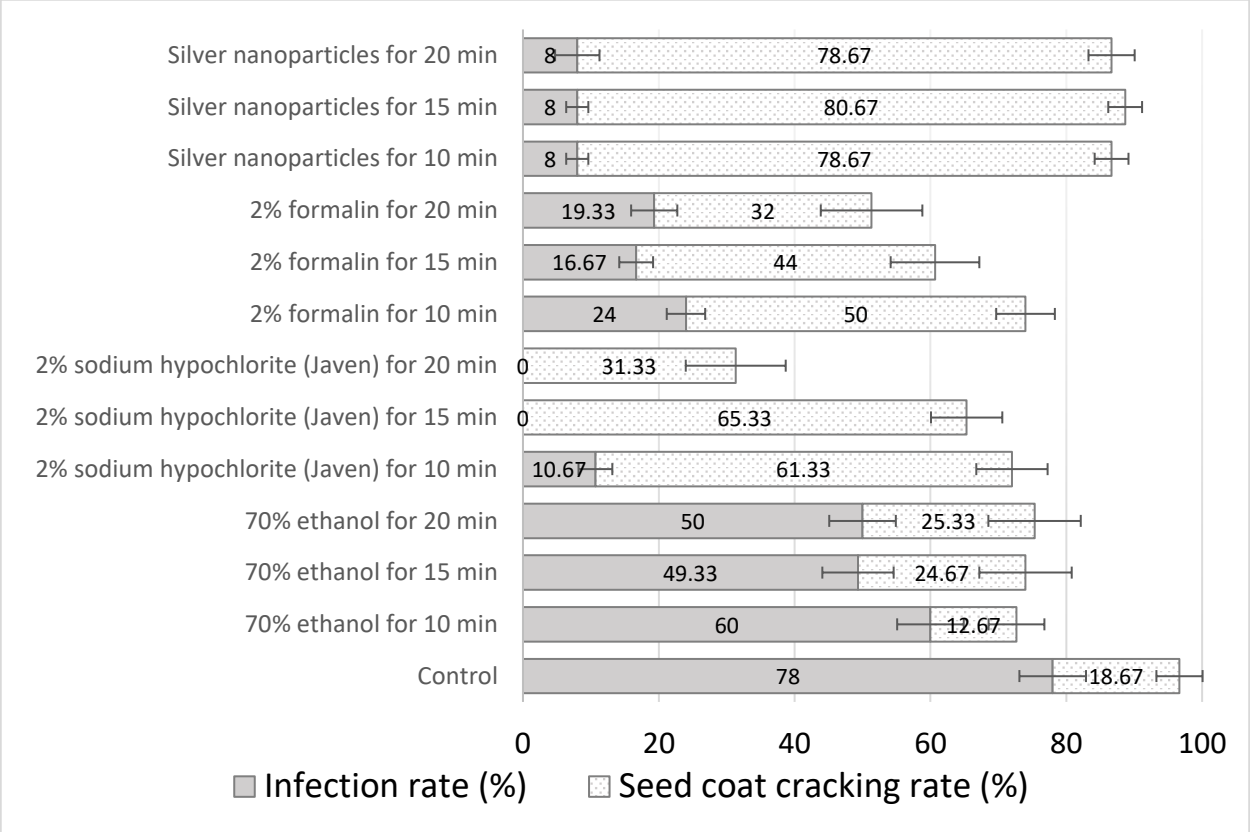


Figure 9. Contamination rate and germination rate of Ngoc Linh ginseng seeds treated with AgNPs compared with conventional chemical treatments

4 DISCUSSION

4.1 Effect of temperature and $AgNO_3$ concentration on the biosynthesis of AgNPs

The gradual color change of the reaction mixture from pale yellow to bright reddish - brown provided the first visual evidence for the occurrence of surface plasmon resonance (SPR), a characteristic physicochemical property of AgNPs [3, 8, 18]. The results demonstrated that both reaction temperature and $AgNO_3$ concentration played critical roles in determining the synthesis efficiency and colloidal stability of the biosynthesized AgNPs.

Reaction temperature strongly influenced the kinetics of nanoparticle formation. As the temperature increased from 60°C to 90°C, UV - Vis absorbance values continuously increased at all tested $AgNO_3$ concentrations, indicating progressive enhancement in nanoparticle density [4]. This effect can be attributed to the higher activation energy provided by elevated temperatures, which accelerated the reduction of Ag^+ ions into metallic Ag^0 by phytochemical compounds such as ginsenosides, polyphenols, and polysaccharides present in Ngoc Linh ginseng extract. Higher temperatures also increased collision frequency between silver ions and reducing molecules, thereby promoting nucleation and nanoparticle growth [7]. Similar observations have been reported in studies on Korean ginseng and ginseng sprouts, in which the temperature range of 80 - 90°C was considered optimal for rapid nanoparticle synthesis while preserving the activity of natural organic capping agents [11].

A gradual redshift of the maximum absorption wavelength (λmax) from 412 nm to 418 nm was observed with increasing temperature, suggesting changes in nanoparticle size distribution and structural organization [18]. Such redshifts are commonly associated with an increase in average particle size or structural coalescence of smaller nanoparticles into energetically more stable configurations. Notably, the SPR peak observed at approximately 417 nm under the optimal condition of 1.0 mM $AgNO_3$ closely matched previously reported data for AgNPs synthesized from *in vitro* - derived Ngoc Linh ginseng, confirming the reproducibility and stability of the green synthesis process.

$AgNO_3$ concentration also played an essential role in maintaining the balance between nanoparticle formation and colloidal stability. The treatment containing 1.0 mM $AgNO_3$ at 80°C produced the highest absorbance value (1.14) together with a stable bright reddish - brown coloration, indicating that the ratio between silver ions and phytochemical compounds in the extract was optimal for the formation of a stable bio - capping layer around the nanoparticles [11]. In contrast, at 2.0 mM $AgNO_3$, visible precipitation and black coloration were observed at elevated temperatures, indicating that the silver ion concentration exceeded the reducing and stabilizing capacity of the organic compounds present in the extract. Under these conditions, the natural capping agents were insufficient to prevent Van der Waals attraction between nanoparticles, resulting in uncontrolled aggregation and sedimentation of the colloidal system.

4.2 Physicochemical Characteristics

The transformation of the reaction mixture from pale yellow to bright reddish - brown, together with the appearance of a characteristic Surface Plasmon Resonance (SPR) absorption peak at 417 - 420 nm, provided strong evidence for the successful formation of AgNPs (AgNPs) [3]. The observed SPR peaks were within the typical range of 400 - 450 nm reported for small spherical AgNPs and were consistent with previous studies on AgNPs synthesized from red ginseng and Korean ginseng extracts [12]. The gradual redshift of the SPR peak from 412 to 418 nm with increasing temperature and $AgNO_3$ concentration suggested changes in particle size distribution and nanoparticle density within the colloidal system. This phenomenon is commonly associated with the growth or structural coalescence of nanoparticles toward a lower free - energy state during the biosynthesis process.

FTIR analysis provided important chemical evidence explaining the formation and stabilization mechanism of the synthesized AgNPs. The strong absorption bands observed at 3468 cm^{-1} and 1637 cm^{-1} corresponded to O - H stretching vibrations and C=O/amide I functional groups, respectively, indicating the involvement of hydroxyl - containing compounds such as ginsenosides, polysaccharides, phenolics, and peptide - associated proteins present in Ngoc Linh ginseng extract [3, 11]. These phytochemicals played dual roles as reducing agents converting Ag^+ into metallic Ag^0 and as biological capping agents adsorbed onto the nanoparticle surface [12]. In addition, the absorption peak detected at 673 cm^{-1} was attributed to Ag - O interactions, confirming the chemical association between the metallic silver core and the surrounding organic layer. Similar FTIR signatures have been widely reported in plant - mediated green synthesis of AgNPs.

A strong correlation was observed between the particle characterization results obtained from DLS analysis and the FTIR data. The synthesized AgNPs exhibited a hydrodynamic diameter (Z - average) of 46.7 nm with a polydispersity index (PDI) of 0.429, indicating relatively good particle uniformity and stable colloidal dispersion. The larger hydrodynamic size was attributed to the presence of an organic phytochemical coating and hydration layer surrounding the silver core, as evidenced by FTIR analysis [5]. This bio - organic shell likely contributed to particle stabilization and prevented direct particle - particle contact. The relatively low PDI value further demonstrated that the synthesized nanoparticles possessed moderate monodispersity and limited aggregation, satisfying the criteria for stable nanoscale colloidal systems. The average particle size determined by TEM (35.9 ± 15.4 nm) was smaller than the DLS value because DLS measures the hydrodynamic diameter of nanoparticles in suspension, including the surface coating, hydration layer, and small aggregates, whereas TEM measures only the physical core size of dried nanoparticles. Therefore, the discrepancy between the DLS and TEM results is expected and has been widely reported for biosynthesized nanomaterials [19].

The zeta potential value of −65.3 mV represented a critical indicator confirming the outstanding colloidal stability of the synthesized AgNP system. In general, nanoparticle suspensions with absolute zeta potential values greater than ±30 mV are considered highly stable due to strong electrostatic repulsion between particles [12]. Therefore, the obtained value of −65.3 mV indicated an exceptionally stable colloidal system, surpassing previously reported AgNPs synthesized from fresh ginseng leaves (−19.3 mV) and neem sprouts (−55.2 mV). The strong negative surface charge likely originated from negatively charged hydroxyl and carbonyl functional groups identified by FTIR analysis. These negatively charged phytochemical molecules formed a stable biological coating on the nanoparticle surface, generating sufficient electrostatic repulsion to overcome Van der Waals attractive forces and effectively prevent aggregation [12]. This mechanism explains why the optimized 1.0 mM $AgNO_3$ formulation remained stable during long - term storage without

sedimentation, whereas higher precursor concentrations (>1.0 mM) resulted in aggregation due to insufficient capping capacity and destabilization of the colloidal system.
Overall, the combined physicochemical analyses formed a coherent and complementary characterization system for the biosynthesized AgNPs. UV - Vis spectroscopy confirmed nanoparticle formation through SPR absorption; FTIR identified the phytochemical compounds responsible for reduction and stabilization; DLS revealed the hydrodynamic size and dispersion characteristics of the nanoparticles; and zeta potential analysis demonstrated the excellent colloidal stability provided by the bio - organic coating derived from Ngoc Linh ginseng extract. Together, these findings confirm the successful green synthesis of highly stable AgNPs with promising potential for biomedical and agricultural applications.

4.3 Sterilization efficiency and germination - promoting effects of AgNPs

Establishing an effective sterilization protocol is vital for plant micropropagation, where the primary objective is to eliminate microbial contaminants without compromising tissue viability [4]. The main objective of the seed treatment experiment was to compare the performance of the green-synthesized AgNPs with conventional sterilants commonly used for *P. vietnamensis* seeds (70% ethanol, 2% sodium hypochlorite, and 2% formalin). Although a pure $AgNO_3$ control was not included, AgNPs achieved a markedly better balance between low contamination (~8%) and high germination (78.67 - 80.67%) than the chemical treatments, demonstrating their practical advantage as a less phytotoxic alternative.
70% ethanol and 2% formalin only achieved moderate decontamination, AgNPs maintained a consistently low contamination rate (~8%) across all treatment durations. This capability can be attributed to the ultra - small size and high surface - area - to - volume ratio of the nanoparticles with a mean core size of 35.9 ± 15.4 nm determined by TEM analysis, which facilitates direct contact and penetration of bacterial cell walls, leading to structural membrane alterations and a subsequent loss of permeability [3, 18]. In contrast, while sodium hypochlorite (Javen) exhibited the highest bactericidal potency (0% contamination), it functions as a non - selective oxidant. This often results in protein denaturation and the destruction of plant cell membranes, leading to characteristic tissue "burning" and a marked decline in germination rates as exposure time increases [4].
A notable finding in this study was the highest germination rate observed in the AgNP treatment group (78.67 - 80.67%), representing a four - fold increase compared to the untreated control (18.67%). This phenomenon aligns with established research on nanopriming in various seeds [4]. Biogenic nanoparticles have been reported to create nanopores in the seed coat, thereby enhancing water uptake and gas exchange. Furthermore, the penetration of AgNPs into seed tissues can activate respiratory enzymes, such as dehydrogenase, and antioxidant systems, facilitating starch metabolism to provide the necessary energy for rapid and uniform embryo germination [4, 18].
The discrepancy in phytotoxicity between AgNPs and conventional chemicals is fundamentally rooted in the bio - capping layer. FTIR and zeta potential analysis (−65.3 mV) confirmed the presence of valuable phytochemicals from Ngoc Linh ginseng, such as saponins and proteins, which encapsulate the silver core [20, 21]. This biological coating acts as a "shield," improving biocompatibility and preventing the sudden release of silver ions that could otherwise induce excessive reactive oxygen species (ROS) and oxidative stress within seed cells [20, 22]. This stable bio - capping enables AgNPs to maintain effective sterilization without compromising seed viability, even during extended 15 - minute treatment periods [4, 23].
Colloidal Stability and Application Potential The ability of AgNPs to maintain an optimal balance between decontamination and embryo protection offers a promising alternative to hazardous chemicals like Javen or formalin. Utilizing green - synthesized AgNPs not only minimizes accumulated chemical toxicity in the culture environment but also serves as a bio - stimulant, significantly enhancing the success rate of micropropagation for high - value medicinal species like Ngoc Linh ginseng.

5 CONCLUSION

In conclusion, this study successfully developed an efficient and eco-friendly protocol for the green synthesis of silver nanoparticles (AgNPs) using aqueous leaf extract from *in vitro*-derived plantlets of Ngoc Linh ginseng. The optimized conditions (1.0 mM $AgNO_3$ at 80°C for 15 min) produced highly stable, quasi-spherical AgNPs with excellent physicochemical properties, including a strong SPR peak at 417 nm,

hydrodynamic size of 46.7 nm, mean TEM size of 35.9 ± 15.4 nm, and outstanding colloidal stability (−65.3 mV zeta potential). The biosynthesized AgNPs demonstrated significant potential in agricultural applications, particularly in seed sterilization and germination enhancement. Compared to traditional chemical sterilants, AgNPs achieved effective microbial control while remarkably promoting seed germination, likely due to the biocompatible capping layer derived from ginseng phytochemicals.

Despite these promising findings, several limitations should be acknowledged. First, the optimization of synthesis parameters was conducted using a one-factor-at-a-time approach rather than Response Surface Methodology (RSM), which could have provided a more comprehensive understanding of interactive effects among variables. Second, although AgNPs showed strong antimicrobial activity in seed contamination assays, quantitative antibacterial evaluations such as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), or agar well diffusion tests against specific phytopathogens were not performed. Third, a pure $AgNO_3$ control treatment was not included in the seed priming experiments, limiting the ability to fully distinguish the contribution of the phytochemical capping layer from the effect of silver ions alone. Additionally, X-ray diffraction (XRD) analysis was not conducted to confirm the crystalline structure of the synthesized AgNPs. Finally, the study was carried out at laboratory scale, and long-term stability, large-scale production feasibility, and potential ecological impacts remain to be investigated.This research not only valorizes Ngoc Linh ginseng - a precious Vietnamese medicinal plant - but also contributes to the growing field of plant-mediated green nanotechnology. Further studies addressing the above limitations, including the application of RSM for process optimization, comprehensive antimicrobial assays, and scale-up evaluations, are recommended to fully exploit the commercial potential of these ginseng - derived AgNPs in agriculture, medicine, and environmental applications.

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