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Biological synthesis of silver nanoparticles: optimization and antibacterial activity using Gymnanthemum extensum leaf extract

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Abstract*— The biosynthesis of silver nanoparticles (AgNPs) has been widely studied in several lines of research. Herbal and plant extracts have been commonly used for biosynthesis in green nanotechnology due to their lower toxicity. Gymnanthemum extensum, known as Nan Chao Woei in Thailand, is one of the traditional plants that possess various biological activities. Therefore, this research aims to synthesize AgNPs using G. extensum leaf extract. Optimal conditions were determined using UV-Vis spectrometric analysis. Results demonstrated a proper concentration of the plant extract at 20 µg/L. A silver nitrate concentration of 10 mM, a reaction time of 24 hr, pH 11, and a temperature of 80°C were found to be optimal for the formation of AgNPs. A characteristic of localized surface plasmon resonance in AgNPs was observed at a maximum wavelength of around 398-421 nm. The synthesized AgNPs displayed a size distribution of approximately 62.85-112.1 nm. Additionally, the antibacterial activity of AgNPs showed a minimum inhibitory concentration against Escherichia coli and Staphylococcus aureus of 8.86 and 17.72 µg/mL, respectively. In conclusion, the AgNPs were successfully synthesized by using G. extensum leaf extract. It could also be a potential one-step method for further studies and applications, providing a simple, rapid synthesis and an eco-friendly system.

Keywords: silver nanoparticles, antibacterial activity, Gymnanthemum extensum, biological synthesis.

I. INTRODUCTION

In nanotechnology, currently, a synthesis of nanoparticles can be carried out by physical, chemical and biological methods [1]. Biosynthesis of nanoparticles such as silver [1], copper [2], and gold [3] using some micro-organisms and plant extract has been widely studied due to less toxicity, environmentally friendly process and various applications. The plant extracts were normally used for the biosynthesis of silver nanoparticles (AgNPs) for instance *Curcuma longa* [4], *Cassia fistula* [5], *and Garcinia mangostana* [6]. *Gymnanthemum extensum* (*G. extensum*) is one of the native shrubs primarily grown in China, Nepal, Myanmar and Northeastern Thailand [7]. It is a bitter leaf tree, known as a traditional herbal plant in Thailand, called Nan Chao Woei. In Thai folk medicine, it possesses anti-diabetic effects to reduce blood lipid, blood pressure and blood sugar [8]. Other biological activities such as anti-bacterial, anti-inflammatory, and anticancer activities have also been reported [9],[10]. There are several bioactive compounds including flavonoids, steroids, triterpenes, and lactones that have been studied by using crude leaf extracts [11]. These extracted phytochemicals potentially act as reducing, capping, and stabilizing agents for a reduction of silver ions (Ag^+) to silver atoms (Ag^0) in the biosynthesis of AgNPs [12]. Therefore, this research aims to study the biological synthesis of AgNPs by using *G. extensum* leaf extract. Optimization for the biosynthesis of AgNPs and their bacterial activities were also investigated.

II. MATERIALS AND METHODOLOGY

A. Chemicals and materials

Silver nitrate ($AgNO_3$) was purchased from Avantor Performance Materials (Gliwice, Poland). Fresh leaves of *G. Extensum* were collected from Kiat Kajon Witthaya School, Chiang Yuen, Maha Sarakham, Thailand. Distilled water was used in all experiment (Department of Chemistry, Faculty of Engineering, RMUTI- KKC).

B. Biosynthesis of silver nanoparticles

Firstly, the fresh leaves of *G. extensum* were washed and dried under the sunlight for seven days. After that the dried leaves were ground by a blender, followed by consequently weighted (0.25, 0.5, and 1.0 g) in an Erlenmeyer flask. Then distilled water (50 mL) was poured into each Erlenmeyer flask. The dried plant was boiled for 1 hour. Aqueous plant extracts were filtered using Whatman no.1 filter paper. These extracts were used as reducing and stabilizing agents to reduce silver ions (Ag^+) from $AgNO_3$ to silver atoms (Ag^0). The optimized parameters were divided into six different conditions including a concentration of plant extract (5-20 μgL^{-1}), a pH (4-11), a ratio of plant extract to silver nitrate (1:1, 2:1, 1:2), a concentration of $AgNO_3$ (0.1-10 mM), a reaction time (5 min-24 hr), and a temperature (28-80 °C). A color change was also observed from colorless to a dark brown colloidal indicating a completely successful AgNPs formation. All optimization parameters were then analyzed by UV-visible spectroscopy and the optimum conditions

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were evaluated for further experiments including particle size analysis and antibacterial activity.

C. Characterization of silver nanoparticles

The synthesized AgNPs were mainly characterized to confirm the formation of AgNPs by using Jasco UV-visible spectrophotometer model V-530 (Jasco, Japan). Absorbance intensity was measured in a wavelength range of 350-700 nm (distilled water was used as a reference). Then, the obtained spectrums were sequentially compared in each optimal parameter. The particle size of AgNPs was evaluated using the dynamic light scattering (DLS) technique (Zetasizer nano ZS, Malvern) to determine the average size (Z-average), particle distribution and polydispersity index (PDI).

D. Antibacterial activity

The colloidal sample of AgNPs was diluted by distilled water. The 2-fold serial dilutions were prepared to examine the antibacterial activity against *Escherichia Coli (E. coli*) and *Staphylococcus aureus* (*S. aureus*) by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The inhibitory effects of AgNPs were carried out at the Department of Biochemistry, Faculty of Science (Khon Kaen University, Thailand).

III. RESULTS AND DISCUSSION

A. Effect of plant extraction concentration on AgNPs biosynthesis

Three different concentrations of plant extract were studied to find out the optimum condition for the biosynthesis of AgNPs. The dried leaves of *G. extensum* at 0.25 g, 0.5 g, and 1.0 g were boiled in 50 mL of deionized water for 1 hour resulting in the concentration of 5, 10, and 20 μgL^{-1}, respectively. The results were compared after 24 hr of AgNPs formation. UV-vis absorbance spectra of AgNPs were found at around 398-421 nm as shown in figure 1.

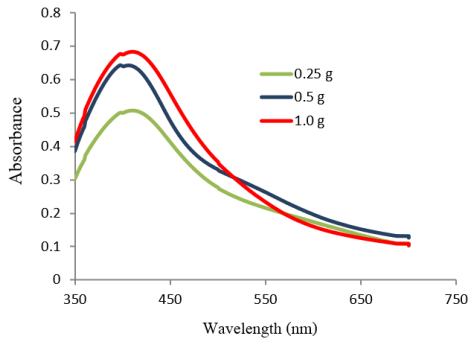

Figure 1. the effect of plant concentration against AgNPs formation showing a characteristic spectrum at 398-421 nm.

From the obtained results, the formation of AgNPs using aqueous extract of *G. extensum* increased in proportion to the concentration of plant extract. These results indicated that various phytochemicals in plant extract might play an important role as reducing agents in the biosynthesis of AgNPs. Therefore, a higher concentration of *G. extensum* resulted in a greater formation of AgNPs. The dark brown colloidal was finally observed as shown in figure 2.

(a) (b) (c)

Figure 2. *G. extensum* fresh leaf (a), aqueous leaf extracts of *G. extensum* (b), and AgNPs from *G. extensum* extracts (c).

B. Effect of pH on AgNPs biosynthesis

The different pH values were reported as one factor that affected the formation of AgNPs. Three pH values in the acidic, neutral, and basic medium were compared at the pH of 4, 7, and 11 for the optimum condition of biosynthesis of AgNPs. The results were displayed in figure 3.

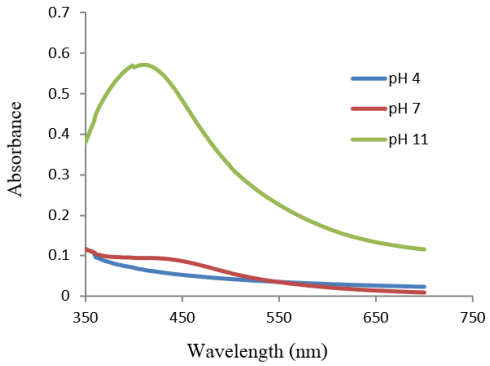

Figure 3. the effect of pH against silver nanoparticles formation using *G. extensum* extract.

The pH was considered to be one of the most important factors for the biosynthesis of AgNPs. In neutral and acidic mediums, the absorbance spectra were not observed around 400-450 nm which indicated the characteristic spectra of AgNPs. Therefore, the mixed medium between plant extract and silver nitrate was adjusted to 11 by adding 1 M of sodium hydroxide (NaOH) solution. The results confirmed that there was the formation of AgNPs in the basic condition. Therefore, the pH at 11 was kept constant for all experiments and used for further studies.

C. Effect of a ratio between plant extract and silver nitrate

The optimum conditions before the incubation time of plant extract and $AgNO_3$ were studied by varying the ratio at 1:1, 1:2, and 2:1. The results were demonstrated in figure 4.

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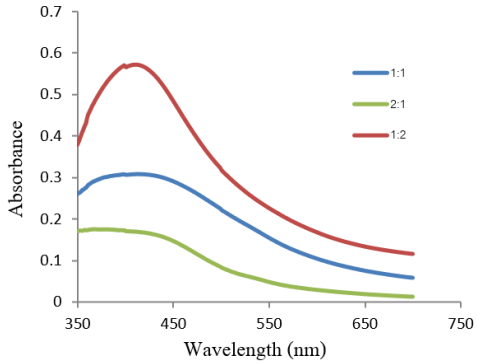

Figure 4. the effect of ratio between plant extract and silver nitrate for biosynthesis of AgNPs using *G. extensum*.

These results demonstrated that changing the ratio of plant extract and $AgNO_3$ affected the formation of AgNPs. The highest formation of AgNPs was found at the ratio of 1:2. This result suggested that the increase of $AgNO_3$ directly affected the AgNPs formation.

D. Effect of silver nitrate concentration

The concentration of silver nitrate was increased from 0.1 to 10 mM for the biosynthesis of AgNPs. The results were demonstrated in figure 5.

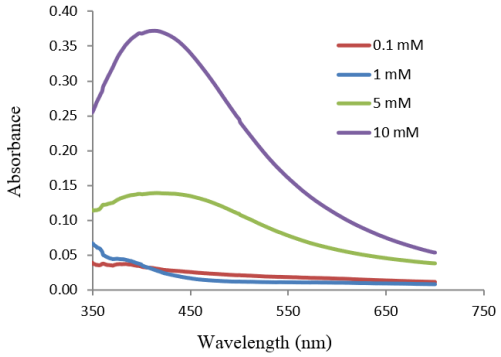

Figure 5. the effect of silver nitrate on biosynthesis of AgNPs using *G. extensum* extract.

The formation of AgNPs was directly proportional to the concentration of $AgNO_3$ as shown in figure 5. At a low concentration (0.1-1 mM) of $AgNO_3$, AgNPs were not synthesized in these conditions. Whereas, at the higher concentration (5 mM – 10 mM), the AgNPs were gradually increased. Therefore, the optimum condition for biosynthesis of AgNPs was 10 mM of $AgNO_3$. This concentration was selected and used for the next experiment.

E. Effect of reaction time for AgNPs biosynthesis

The continuous mixing between plant extract and $AgNO_3$ resulted in the reduction of Ag^+ ions to Ag^o (AgNPs). This process needed reaction time to complete the biosynthesis of AgNPs. The results were shown in figure 6.

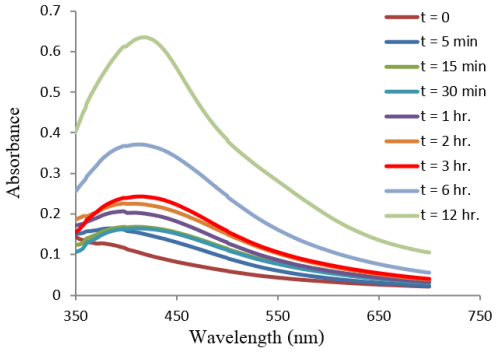

Figure 6. the effect of reaction time for the biosynthesis of AgNPs using *G. extensum* extract.

Time spent for AgNP formation was observed in 2 regions, within 1 hour (5, 15, 30, and 60 minutes) and 24 hours (1, 2, 3, 6, and 24 hr). The results showed a higher absorbance when the reaction time increased. Within 1 hour, the formation of AgNPs was slowly synthesized. In contrast, within 24 hr, it was found that the absorbance spectra between 3 and 6 hr were observed. The maximum absorbance indicated the greatest formation of AgNPs. At 24 hr after the reaction started, the absorbance was 2 times higher than the incubation time of 6 hr. Therefore, the 24 hr of reaction time was selected and further studied.

F. Effect of temperature on AgNPs biosynthesis

The last parameter to optimize was temperature. The different temperatures were examined from room temperature (28 °C) to 80°C. Since the temperature affected the formation, size, and shape of the nanoparticles [13], the effect of temperature was studied. The results were shown in figure 7.

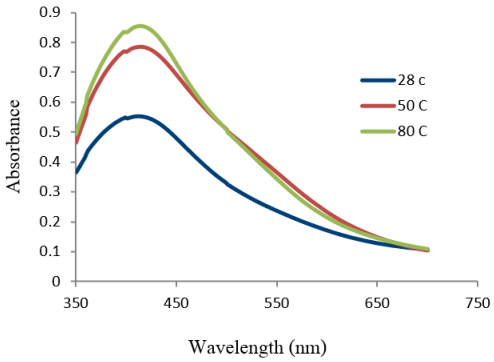

Figure 7. the effect of temperature on the biosynthesis of AgNPs using *G. extensum* extract.

At higher temperatures (50 and 80°C), the intensity of the absorbance was highly increased when compared with the room temperature (28 °C). These results indicated a higher formation of AgNPs in the biosynthesis due to increasing temperature and influencing reaction rate [13].

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Dynamic light scattering (DLS) analysis

The average particle size of the synthesized AgNPs was analyzed by DLS technique. Different tested temperatures were compared as shown in figure 8 and 9.

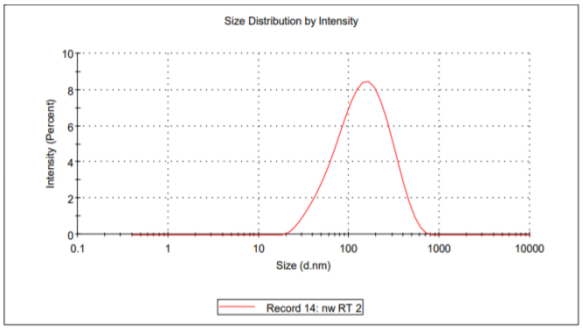


Figure 8. the size distribution of the synthesized AgNPs at room temperature (28°C)

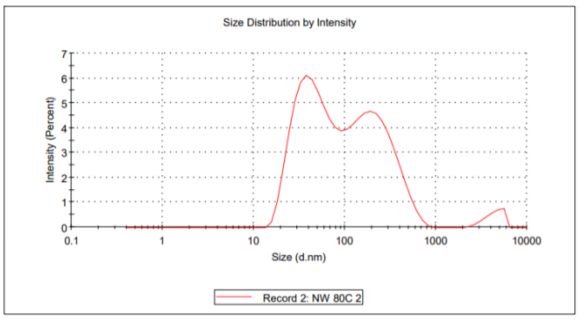
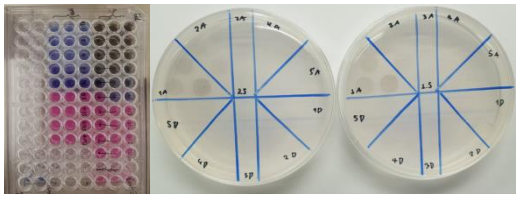


Figure 9. the size distribution of the synthesized AgNPs at the higher temperature (80°C)

The results obtained from 38 °C and 80 °C were different in size and size distribution due to temperature-dependent effects. At a higher temperature, two groups of particle size were found at around 60 nm and 200 nm (figure 9). The DLS analysis obtained in figure 8 demonstrated a z-average value of 112.1 nm with a polydispersity index (PDI) of 0.314. These values presented a low aggregation of the synthesized AgNPs since the value was close to zero and lower than 0.7 [14]. These results suggested that some bioactive compounds in the aqueous extract of *G. extensum* probably act as stabilizing agents such as tannin and flavonoids [15].

Antibacterial activity

The minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) of AgNPs were examined as shown in figure 10. The obtained results were summarized in table 1.



(a) (b)

Figure 10. the minimal inhibitory concentration (MIC) of AgNPs (a) and the minimal bactericidal concentration (MBC) against *E.coli* and *S. aureus* bacteria (b).

Table 1. antibacterial activity of the synthesized AgNPs using *G. extensum* extract

Bacteria	*Escherichia coli* O157:H7	*Staphylococcus aureus* ATCC 25923
The number of starting bacteria (CFU/mL)	3.8×10^5	8×10^5
MIC (%v/v)	1.526	3.125
MBC (%v/v)	3.125	3.125

Gram-negative and gram-positive bacteria were tested and compared using MIC and MBC values as shown in table 1. From the results, the obtained MBC value was not different when compared to the antibacterial activity against *E. coli* and *S. aureus*. However, the MBC of 3.125 (%v/v) which was found in *E. coli* showed a higher value of MIC (1.526 %v/v) indicating a potential inhibitory effect against tested bacteria. The synthesized AgNPs showed antibacterial activity against *E. coli* and *S. aureus* with MIC values of 8.86 and 17.72 µg/mL, respectively. The possible mechanisms might relate to a release of silver ions and silver nanoparticle toxicity which can inhibit bacterial growth [16]. The synthesized AgNPs can also disrupt the bacterial cell wall and membrane resulting in bacterial cell death [17].

IV. CONCLUSION

Biosynthesis of silver nanoparticles using *G. extensum* extract has been successfully carried out by optimization of various conditions as follows; the plant concentration was 20 μgL^{-1}, 10 mM $AgNO_3$, pH at 11, the ratio between plant and $AgNO_3$ was 1:2, reaction time was 24 hr, and the temperature was 80 °C. The optimal conditions showed a particle size of around 62-112 nm. The synthesized AgNPs exhibited antibacterial activity against *E. coli* and *S. aureus.* This current research provided a rapid biosynthesis with a simple, low cost and eco-friendly method.

V. ACKNOWLEDGEMENT

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REFERENCES

[1] Simon S, Sibuyi NRS, Fadaka AO, Meyer S, Josephs J, Onani MO, et al. Biomedical Applications of Plant Extract-Synthesized Silver Nanoparticles. Biomedicines. 2022;10(11):2792

[2] Kasana RC, Panwar NR, Kaul RK, Kumar P. Biosynthesis and effects of copper nanoparticles on plants. Environmental Chemistry Letters.

2017;15(2):233-40
[3] Muddapur UM, Alshehri S, Ghoneim MM, Mahnashi MH, Alshahrani MA, Khan AA, et al. Plant-Based Synthesis of Gold Nanoparticles and Theranostic Applications: A Review. Molecules. 2022;27(4).
[4] Maghimaa M, Alharbi SA. Green synthesis of silver nanoparticles from Curcuma longa L. and coating on the cotton fabrics for antimicrobial applications and wound healing activity. Journal of Photochemistry and Photobiology B: Biology. 2020;204:111806.
[5] Mohanta YK, Panda SK, Biswas K, Tamang A, Bandyopadhyay J, De D, et al. Biogenic synthesis of silver nanoparticles from Cassia fistula (Linn.): In vitro assessment of their antioxidant, antimicrobial and cytotoxic activities. IET Nanobiotechnol. 2016;10(6):438-44.
[6] Karthiga P. Preparation of silver nanoparticles by Garcinia mangostana stem extract and investigation of the antimicrobial properties. Biotechnology Research and Innovation. 2018;2(1):30-6.
[7] Bunwong S, Chantaranothai P, Keeley SC. Revisions and key to the Vernonieae (Compositae) of Thailand. PhytoKeys. 2014(37):25-101.
[8] Ong KW, Hsu A, Song L, Huang D, Tan BK. Polyphenols-rich Vernonia amygdalina shows anti-diabetic effects in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2011;133(2):598-607.
[9] Faisal M, Maungchanburee S, et al. Dichloromethane crude extract of Gymanthemum extensum combined with low piperine fractional Piper nigrum extract induces apoptosis on human breast cancer cells. Indian J Pharm Sci. 2021; 83(2):238-251
[10] Youn UJ, Park EJ, Kondratyuk TP, Simmons CJ, Borris RP, Tanamatayarat P, et al. Anti-inflammatory sesquiterpene lactones from the flower of Vernonia cinerea. Bioorg Med Chem Lett. 2012;22(17):5559-62.
[11] Igual M, Poleti Martucci M, Da Costa F, Gobbo-Neto L. Sesquiterpene lactones, chlorogenic acids and flavonoids from leaves of Vernonia polyanthes Less (Asteraceae). Biochemical Systematics and Ecology. 2013;51:94–7
[12] Rahman A, Kumar S, Bafana A, Dahoumane SA, Jeffryes C. Biosynthetic Conversion of Ag^{+} to highly Stable Ag^{0} Nanoparticles by Wild Type and Cell Wall Deficient Strains of Chlamydomonas reinhardtii. Molecules. 2018;24(1).
[13] Jiang XC, Chen WM, Chen CY, Xiong SX, Yu AB. Role of Temperature in the Growth of Silver Nanoparticles Through a Synergetic Reduction Approach. Nanoscale Res Lett. 2011;6(1):32.
[14] Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems. Pharmaceutics. 2018;10(2).
[15] Karuvantevida N, Razia M, Bhuvaneshwar R, Gnanasekar S, Seetharaman PK, Sivaramakrishnan S. Bioactive Flavonoid used as a Stabilizing Agent of Mono and Bimetallic Nanomaterials for Multifunctional Activities. Journal of Pure and Applied Microbiology. 2022;16.
[16] Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. Int J Nanomedicine. 2020;15:2555-62.
[17] Dakal TC, Kumar A, Majumdar RS, Yadav V. Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. Frontiers in Microbiology. 2016;7.