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Antibacterial activity of silver nanoparticle LED-synthesized using *Citrus maxima* peels

Trung Nguyen Dien[a], Hong Nguyen Thi[b*]

[a] *Can Tho University, School of Education, 94000, Can Tho, Vietnam*
[b] *Can Tho University, College of Natural Sciences, 94000, Can Tho, Vietnam*

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ABSTRACT

Silver nanoparticles have garnered significant attention in research and applications due to their unique properties. The synthesis of these nanoparticles has aligned with the principles of green chemistry, utilizing environmentally-friendly materials and techniques. In this study, silver nanoparticles were synthesized using the *Citrus maxima* peel extract and blue LED irradiation. The pectin, flavonoids, and phenolic acids in the *C. maxima* extract acted as effective reductants and primary stabilizers for nanoparticle formation. The influence of different light-emitting diodes and irradiation time on nanoparticle synthesis was investigated. Ideal conditions for silver nanoparticle formation were observed with the assistance of blue LED irradiation for 120 min. Characterization techniques such as transmission electron microscopy, X-ray diffraction analysis, ultraviolet-visible spectroscopy, and Fourier-transform infrared spectroscopy confirmed the successful synthesis of spherical nanoparticles with an average size of 12.2 nm. The antibacterial activity of the silver nanoparticles was evaluated against four bacterial strains: *Lactobacillus fermentum*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Salmonella enterica*. The nanoparticles exhibited stronger inhibitory effects against gram-negative compared to gram-positive bacteria, with half-maximal inhibitory concentrations of 9.4, 18.1, 73.3, and 88.5 pM for *P. aeruginosa*, *L. fermentum*, *S. aureus*, and *S. enterica*, respectively. These findings highlight the potential of bio-synthesized nanoparticles with small size and high antibacterial activity.

1. Introduction

Silver nanoparticles (AgNPs) have been extensively studied for their distinguishing characteristics, such as electrical conductivity, catalytic, chemical stability, and antibacterial activity (Sharma et al., 2009). As a result, AgNPs are of high interest in versatile applications, including drug delivery, biosensing, and antibacterial agents (Austin et al., 2014; Liu & Huang, 2012; Mohan et al., 2015). Traditional physical and chemical methods have been the conventional approaches for AgNP synthesis (Annadhasan et al., 2012; Zhang et al., 2012). However, the use of toxic reductants in chemical methods has limited their application and led to environmental damage, while physical methods suffer from high operating costs and complicated processes (Guzman et al., 2012; Wani et al., 2011). Therefore, green synthesis methods utilizing plant extracts have emerged as a promising alternative to address these issues for AgNP synthesis. Biopolymers such as chitosan, starch, and pectin have been utilized for the green synthesis of metallic nanoparticles (Ardjoum et al., 2021; Fonseca & Prior, 2021; Kalaivani et al., 2018; Musino et al., 2021; Pascu et al., 2021; Polinarski et al., 2021; Su et al., 2019). Among those biopolymers, pectin has gained attention due to its biodegradability, non-toxicity, and affordability, making it an attractive option for reducing metallic ions and stabilizing metal nanoparticles (Devendiran et al., 2016; Ivanova et al., 2012; Nemiwal et al., 2021).

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Punita Sharma: Maharaja Agrasen University, Himachal Pradesh, Baddi, India
Fangfang Yang: Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, People Republic of China
Sobhan Mortazavi-Derazkola: Birjand University of Medical Sciences, Birjand, Iran

* Corresponding author(s):
E-mail address:
nthong43@ctu.edu.vn (H. N. Thi)
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Pectin, a heterogeneous polysaccharide found in the cell walls of plants, comprises a homogalacturonan backbone, xylogalacturonan, rhamnogalacturonan I, and rhamnogalacturonan II (Bidhendi et al., 2020). Pectin foundation comprised acetylated and methylated α-1,4-galacturonic acid units (Mohnen, 2008). Based on the gelling property of pectin, metallic nanoparticles synthesized by pectin exhibited outstanding performance in drug delivery, antimicrobial activity, and antioxidant activity (Lara-Espinoza et al., 2021; Qiu et al., 2018; Zhang et al., 2020). Recent studies have successfully synthesized AgNPs using the pectin-rich extracts from *Citrus* peels, including *C. limon*, *C. sinensis*, and *C. tangerina*. The antimicrobial activity of the synthesized AgNPs was also tested against *Escherichia coli* and *Staphylococcus aureus*. The obtained results indicated that AgNPs were more effective against *E. coli* than *S. aureus*; the synthesized AgNPs from *C. tangerina* extract showed more inhibition than others (Niluxsshun et al., 2021). *C. maxima* peel extract has been utilized for synthesizing AgNPs with applications in dye degradation and inhibition of bacterial growth. Phenolic acids and flavonoids present in the *C. maxima* peel extract act as effective reductants for AgNPs formation, and the synthesized nanomaterial demonstrated high antibacterial activity and removal capabilities of dyes such as methyl blue, rhodamine, and methyl orange (Tran et al., 2022). Additionally, the O–H functional groups present in naringin, naringenin, and hesperidin, which are constituents of the *C. maxima* peel extract, were responsible for synthesizing and stabilizing AgNPs. The resulting nanomaterial has been employed as a catalyst for removing 4-nitrophenol, an agent that inhibits bacterial growth, and a fungicide on plants (Huo et al., 2019). In contrast, commercial pectins with a high cost have been utilized for AgNPs synthesis in previous studies (Balachandran et al., 2013; Hileuskaya et al., 2020; Li et al., 2018; Pallavicini et al., 2017; Zhang et al., 2017). Therefore, inexpensive plant-based pectin serves as a promising alternative source for commercial pectin.

C. maxima is a member of the Rutaceae family and is widely cultivated in Vietnam (Quoc et al., 2014). The white peel of *C. maxima* constitutes up to 30% of the fruit's weight and is rich in pectin (Methacanon et al., 2014). Moreover, *C. maxima* peel extract is known to contain significant levels of phenolic acids and flavonoids (Khan et al., 2021). Recent investigations have focused on the creation of AgNPs using an aqueous extract of *C. maxima* peels through various methods such as heat, ultrasound, and sunshine, with subsequent evaluation of the initial antibacterial activity of generated AgNPs (Ali et al., 2020; Barbhuiya et al., 2022; Nguyen, 2020). In addition, the use of light irradiation has garnered interest in the synthesis of AgNPs, with different light sources, including laser, LED fluorescent light, and sunshine, being developed to enhance the formation of AgNPs (Khamhaengpol & Siri, 2016; Lee et al., 2016; Verma et al., 2017).

Interestingly, LED irradiation is more cost-effective and energy-efficient compared to traditional light sources (Zheng et al., 2009). Also, LED irradiation has been explored as a suitable means to stimulate the plasmon-mediated production of AgNPs (da Silva et al., 2020). However, limited research has been reported on the production of AgNPs using *C. maxima* extract under LED irradiation. Consequently, this study investigates the synthesis of AgNPs using the peel extract of *C. maxima* with the assistance of LED irradiation (referred to as AgNPs-Cm). The study examines the impact of different LED types and duration times in detail. Additionally, antibacterial tests were conducted on gram-negative bacteria such as *Pseudomonas aeruginosa* and *Salmonella enterica*, as well as gram-positive bacteria including *Lactobacillus fermentum* and *S. aureus*.

2. Materials and methods

2.1. Materials

C. maxima fruit was purchased in An Lac market, Can Tho city. The extract was prepared by drying the white *C. maxima* peel at 70 °C for 48 h and then powdering the dried peel. Silver nitrate ($AgNO_3$, Merck, 99.0%) was employed as the precursor for synthesizing AgNPs-Cm, while distilled water served as the solvent throughout the synthesis process.

2.2. Synthesizing of AgNPs-Cm

The *C. maxima* extract was prepared by adding 2.50 g powdered *C. maxima* peel extract into 100 ml deionized water. The mixture was subjected to centrifugation at a speed of 6800 rpm for 60 min to separate and eliminate the solid residues of *C. maxima* peels after heating at 50 °C for 40 min. (Nguyen et al., 2022). AgNPs-Cm were produced by mixing 8.5 mg of $AgNO_3$ in 50 ml of *C. maxima* extract at room temperature under LED irradiation while controlling factors such as LED light source and time. The process for AgNPs-Cm synthesis was illustrated in Figure 1. The formation of AgNPs-Cm was indicated by a color change of the solution from pale yellow to yellow-brown (Bano et al., 2023).

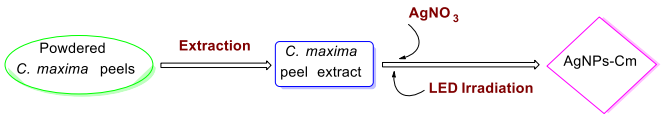

Figure 1. The synthesis process of AgNPs-Cm using *C. maxima* peels

2.3. Characteristics of AgNPs-Cm

The AgNPs-Cm formation was confirmed by analyzing the Ultraviolet-visible (UV-Vis) spectra in a wavelength range from 300 to 700 nm using a Jasco V730 spectrophotometer. The mean morphology of AgNPs-Cm was observed using transmission electron microscopy (TEM, Jem-1400, Jeol), while the crystalline phase was determined from the X-ray diffraction (XRD) pattern recorded on a Malvern Panalytical Empyrean instrument at 2θ = 10-80˚. The size distribution of AgNPs-Cm was determined from the TEM image using ImageJ software. The functional groups in the bio-synthesized AgNPs-Cm and *C. maxima* extract were characterized using Fourier transform infrared (FTIR) spectra recorded on a Thermo Nicolet 6700 apparatus.

2.4. Antibacterial activity of AgNPs-Cm

The inhibitory activity of the produced AgNPs-Cm against *P. aeruginosa* ATCC 15442, *S. enterica* ATCC 35664, *L. fermentum* ATCC 14931, and *S. aureus* ATCC 13709 were evaluated using a dilution approach. Bacterial growth was assessed using tryptic soy broth and agar. The pathogenic cultures were cultivated and incubated at 37 °C for 24 h to achieve a concentration of 5×10^5 CFU/ml, as per the MacFarland criterion. Gram-positive and gram-negative bacteria were treated with ampicillin and cefotaxime, respectively. The absorbance of the produced AgNPs-Cm at 630 nm was measured in 96-well microplates to determine the 50% inhibitory concentration IC_{50} (Kamiloglu et al., 2020). The antibacterial activity of AgNPs-Cm was assessed at a concentration of 90.0 pM.

3. Results and discussion

3.1. Synthesizing AgNPs-Cm

The synthesis of AgNPs-Cm using *C. maxima* peel extract was conducted under various LED irradiation wavelengths, including ultraviolet (LED-UV, λ = 350-400 nm), blue (LED-B, λ = 400-450 nm), green (LED-G, λ = 450-500 nm), and yellow (LED-Y, λ = 585-600 nm). To compare the synthesis efficiency of AgNPs-Cm in the presence and absence of LED irradiation, a sample without LED light (No-LED) was also prepared under identical conditions. The absorbance of green-synthesized AgNPs-Cm with and without LED irradiation is shown in Figure 2. The presence of LED lights enhanced the reduction of Ag^+ compared to the absence of LED irradiation. The results also revealed that the production of AgNPs-Cm was significantly influenced by the wavelength values. Specifically, higher AgNPs-Cm concentrations were obtained at lower exposure wavelengths, such as LED-UV and LED-B. According to da da Silva et al. (2020), the maximum absorption of AgNPs-Cm coincided with the emission peak of blue LED, greatly facilitating AgNPs-Cm formation. Furthermore, the increased absorbance observed in the LED-UV sample can be attributed to its higher energy. In contrast, the formation of AgNPs-Cm decreased under green and yellow LED irradiation owing to their comparatively lower energy levels (Stamplecoskie & Scaiano, 2010). The absorbance peaks of AgNPs-Cm remained consistent at around 409 nm with different LED irradiations, indicating that the morphology of synthesized AgNPs-Cm was unaffected by the LED treatment. Based on these findings, blue LED was determined as the optimal condition for producing AgNPs-Cm.

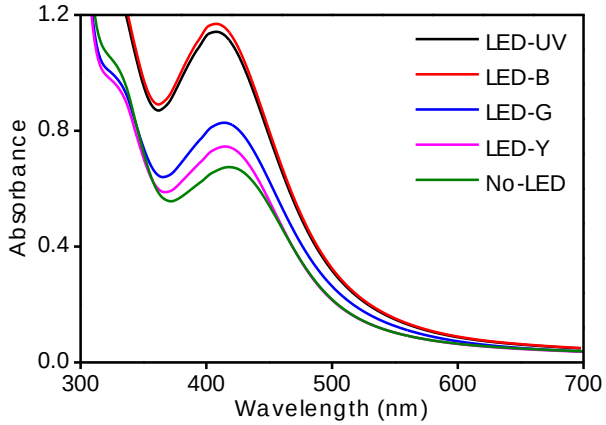

Figure 2. Impact of various LEDs on the creation of AgNPs-Cm under the following experimental conditions: 1 mM $AgNO_3$, 2.50 g of powdered peel extracted over 100 ml of water at 50 °C within 40 min, and LED irradiation (UV-ultraviolet, B-blue, G-green, and Y-yellow) within 90 min

The effect of synthesis duration on the formation of AgNPs-Cm under blue LED radiation was investigated within a time range of 0 to 150 minutes. The acquired outcomes were depicted in Figure 3. The absorption spectra of synthesized AgNPs-Cm exhibited a peak at a wavelength of 409 nm. The reduction of Ag^+ ions commenced after a 20-minute reaction and gradually increased in intensity with prolonged reaction time. However, after a 120-minute reaction, there was a negligible increase in absorbance as a consequence of the depletion of the precursor (Nagar et al., 2016). These findings have indicated that the duration of irradiation influenced the conversion of Ag^+ ions into AgNPs-Cm, with a 120-minute reaction time being identified as the optimal timeframe. Relied on the obtained from Figure 2 and Figure 3, the suitable conditions for AgNPs-Cm formation were determined as blue LED irradiation for 120 min.

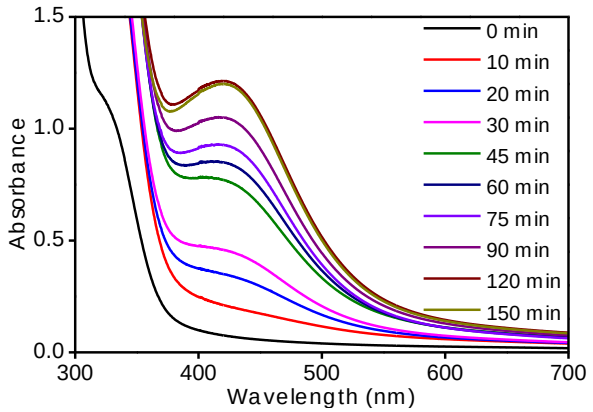

Figure 3. Impact of synthesis duration on the creation of AgNPs-Cm under the following experimental conditions: 1 mM $AgNO_3$, 2.50 g of powdered peel extracted over 100 ml of water at 50 °C within 40 min, and blue-LED irradiation within 0-150 min

3.2. Characteristics of AgNPs-Cm

Figure 4 depicts the XRD pattern of the produced AgNPs-Cm using *C. maxima* peel extract under blue LED irradiation, employing the optimal conditions. The XRD analysis confirmed the absence of a silver oxide phase, indicating the formation of pure silver crystalline structure in the produced AgNPs-Cm. The characteristic peaks observed at 2θ angles of 38.1, 44.3, 64.5, and 77.3˚ can be attributed to the (111), (200), (220), and (311) lattice planes of silver, respectively, in accordance with the diffraction standards JCPDS 04-0783 (Pozdnyakov et al., 2019). The Debye Scherrer formula *(1)* was used to calculate D as the AgNPs-Cm particle size.

$$D = \frac{0.9\,x\,\lambda}{\beta\,x\,cos} \qquad (1)$$

where λ = 0.1541 nm corresponds to the X-ray length, β represents the full width of the peak at half-maximum (FWHM), and θ denotes the Braggs angle, expressed in radians (Holzwarth & Gibson, 2011). Estimating FWHM, the Gaussian function fitted four peaks of silver by the software Origin 9.0.

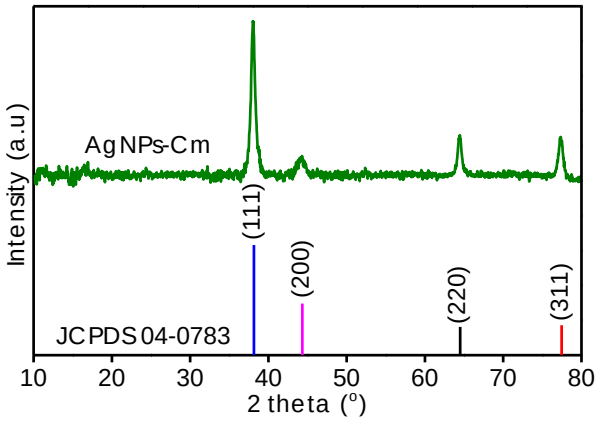

Figure 4. XRD pattern of the produced AgNPs-Cm

The size of AgNPs-Cm particles ranged from 7 to 15 nm, with an average size of 12.2 nm. The obtained XRD pattern confirmed the presence of tiny particles and pure phases in the synthesized AgNPs-Cm. The stabilization of AgNPs-Cm was effectively achieved through the presence of pectin, preventing oxidation and agglomeration (Al-

Muhanna et al., 2015; Pallavicini et al., 2017). Furthermore, the interplanar spacing (d) between two adjacent parallel planes of atoms was calculated using Bragg's Law *(2)*.

$$2\ x\ d\ x\ sin\theta = n\ x\ \lambda \quad (2)$$

where *n* is the order of the diffraction pattern. In the present case, *n* equals 1 (Le Pevelen & Lindon, 2010).

The interplanar spacing was obtained with approximate values and was given in Table 1. Lattice constant a has been estimated using the formula *(3)*.

$$a = d\ x\ \sqrt{h^2 + k^2 + l^2} \quad (3)$$

where h, k, and l are lattice planes of silver (Fan, 2012).

Table 1. The size of produced AgNPs-Cm derived from the XRD pattern

2θ,°	FWHM, rad	Particle size, nm	Interplanar spacing, Å	Lattice constant, Å
38.1	0.0126	11.7	2.36	4.09
44.2	0.0202	7.4	2.04	4.09
64.4	0.0112	14.6	1.44	4.08
77.3	0.0117	15.2	1.23	4.09

The average lattice constant, calculated from the four interplanar spacing values, was determined to be 4.09 Å. This value was in good agreement with the standard value of silver (a = 4.09 Å, JCPDS 04-0783).

Figure 5 shows the FTIR spectra of the extract derived from *C. maxima* peels and the produced AgNPs-Cm, highlighting the distinctive peaks of their functional groups. The stretching vibration of O–H and C–H exhibited strong bands at 3405 and 2934 cm^{-1}, respectively. A peak at 1745 cm^{-1} corresponded to the stretching vibration of C=O in the groups –COOH and –$COOCH_3$. Meanwhile, the asymmetric and symmetric stretching of COO– ions were observed at 1638 and 1447 cm^{-1} (Košťálová et al., 2013). Additionally, the stretching vibration of the C–OH in alcoholic groups and carboxylic acids resulted in a peak at 1051 cm^{-1} (Guibaud et al., 2003). These observed peaks in the *C. maxima* peel extract and AgNPs-Cm, assigned to the free pectin, flavonoids as well as phenolic acids, indicate that these phytochemicals were primarily responsible for the AgNPs-Cm formation and adequate stabilization of the produced AgNPs-Cm.

TEM analysis was performed to analyze the morphology of AgNPs-Cm. Figure 6 shows that the majority of the synthesized AgNPs-Cm exhibited a spherical shape, with sizes ranging from 5 to 25 nm. Notably, particles smaller than 20 nm were predominant in the obtained AgNPs-Cm, accounting for approximately 95.3% of the total. The average particle size was determined to be 12.6 nm, which was smaller than the silver nanoparticles synthesized under the same conditions without LED irradiation (AgNPs-N), measuring 17.2 nm (Nguyen et al., 2022).

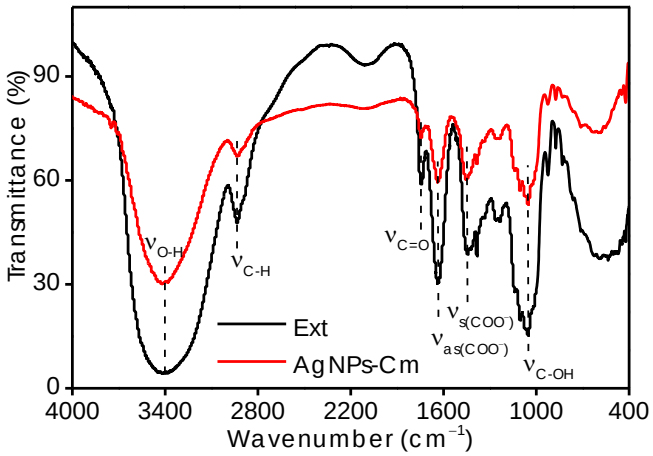

Figure 5. FTIR spectra of *C. maxima* extract (Ext) and produced AgNPs-Cm

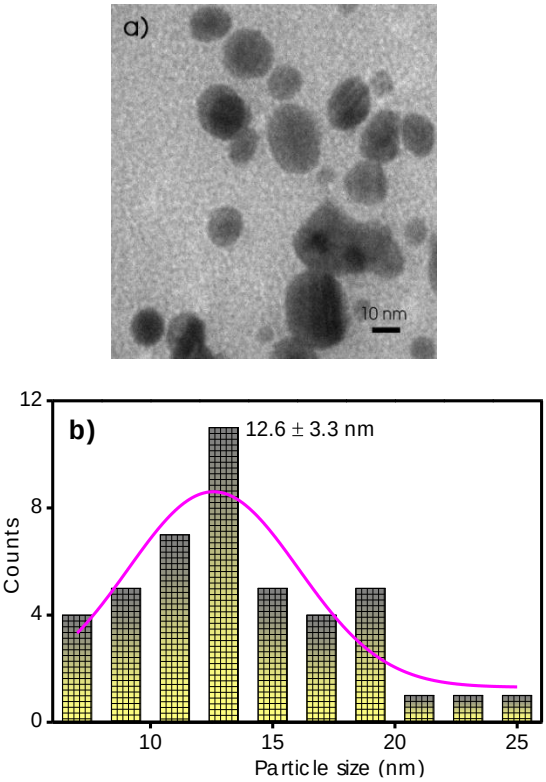

Figure 6. TEM image (a) and distribution size (b) of the produced AgNPs-Cm

3.3. Antibacterial activity of AgNPs-Cm

According to Salvioni et al. (2017), silver nanoparticles have a favorable redox potential on their surface, which can generate reactive oxygen species capable of damaging the cytoskeleton of cells and leading to the eradication of entire cell populations. In this work, the antibacterial efficacy of the synthesized AgNPs-Cm was tested against both gram-negative bacteria (*S. enterica* and *P. aeruginosa*) and gram-positive bacteria (*S. aureus* and *L. fermentum*). The concentration of prepared AgNPs-Cm was determined using the UV-Vis method (Paramelle et al., 2014), with a concentration of 90.0 pM used for the antibacterial activity testing. Figure 7 illustrates the effect of AgNPs-Cm concentration on antibacterial activity, while Table 2 presents the half-maximal inhibitory concentration (IC_{50}) of the produced AgNPs-Cm against the tested bacterial strains. Concentrations that inhibited 50% of *S. aureus*, *L. fermentum*, *S. enterica*, and *P. aeruginosa* corresponded

to values of 73.3, 60.1, 18.1, and 9.4 pM, respectively. The synthesized AgNPs-Cm exhibited significant efficacy against gram-negative bacteria at a concentration of 3.6 pM, while gram-positive bacteria were inhibited at 18.0 pM. Notably, gram-negative bacteria displayed a lower IC_{50} value for AgNPs-Cm compared to gram-positive bacteria, which can be attributed to the thicker peptidoglycan layer in the cell wall of gram-positive bacteria (Fayaz et al., 2010). Furthermore, when compared to AgNPs-N in a previous study (Nguyen et al., 2022), AgNPs-Cm demonstrated superior antibacterial activity. Specifically, AgNPs-Cm inhibited the growth of more than 50% of all tested bacteria, including *S. aureus*, *L. fermentum*, *S. enterica*, and *P. aeruginosa*, whereas AgNPs-N only exhibited inhibitory effects against the growth of *P. aeruginosa*. These results indicate that silver nanomaterials synthesized with blue LED light irradiation possess enhanced antibacterial activity compared to bio-synthesized materials without LED irradiation.

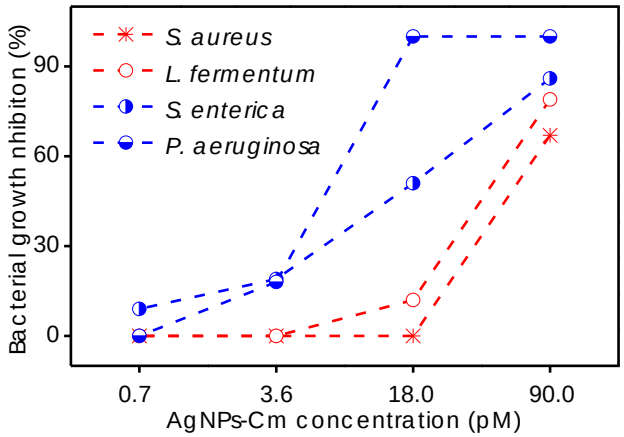

Figure 7. Various concentrations of produced AgNPs-Cm for assessing antibacterial activity

Table 2. IC_{50} of the synthesized AgNPs-Cm against bacteria

	S. aureus	*L. fermentum*	*S. enterica*	*P. aeruginosa*
IC_{50}, pM	73.3 ± 4.2	60.1 ± 3.6	18.1 ± 4.6	9.4 ± 0.5

Table 3. This work compares the synthesized AgNPs-Cm using *C. maxima* peel with other studies

Synthesis	Shape, size	Antibacterial activity	Reference
Sunlight irradiation	Spherical, 13 nm	*S. typhi, S. aureus, S. pyogenes*	(Nguyen, 2020)
Ultrasound at room temperature	Spherical, 35-40 nm	*B. cereus, E. coli*	(Barbhuiya et al., 2022)
Light irradiation at room temperature	Spherical, 3-10 nm	*B. cereus, E. coli, P. aeruginosa, S. aureus, Salmonella*	(Tran et al., 2022)
Blue LED at room temperature	Spherical, 12 nm	*S. aureus, L. fermentum, S. enterica, P. aeruginosa*	This work

In this study, the production of AgNPs-Cm through the utilization of a pectin-rich extract derived from *C. maxima* peels under blue LED irradiation offers several advantages in terms of cost, synthetic conditions, and antibacterial activity. As shown in **Table 3**, recent studies have focused on utilizing the extract of *C. maxima* peels for the synthesis of AgNPs-Cm and evaluating their antibacterial activity. The inclusion of blue LED in the present study resulted in enhanced formation of AgNPs-Cm and reduced reaction time. The utilization of LED light provides energy in specific wavelengths, offering advantages over light irradiation and ultrasonic heating methods employed in previous studies. Moreover, we successfully employed a natural pectin-rich extract from *C. maxima* peel for the effective synthesis of AgNPs-Cm, eliminating the need for commercial pectin. It is noteworthy that the synthesized AgNPs-Cm exhibited similar sizes and demonstrated potent inhibition of bacterial growth. The nanomaterials synthesized using green methods exhibited a spherical morphology with a size below 50 nm, and the light-assisted AgNPs showed smaller particle sizes compared to those synthesized using ultrasonic heating.

4. Conclusions

In this study, the utilization of blue LED irradiation proved to be instrumental in enhancing the production of AgNPs-Cm. FTIR analysis confirmed the presence of pectin, flavonoids, and phenolic acids in the extract, which played a crucial role in reducing Ag^+ ions and stabilizing the resulting AgNPs-Cm. The synthesized AgNPs-Cm exhibited purity of phase, spherical morphology, and small size of 12.2 nm. Remarkably, the AgNPs-Cm demonstrated strong antimicrobial activity against *S. aureus* (IC_{50} = 73.3 pM), *L. fermentum* (IC_{50} = 60.1 pM), *S. enterica* (IC_{50} = 18.1 pM), and *P. aeruginosa* (IC_{50} = 9.4 pM). The findings underscore the eco-friendly and energy-saving nature of the green synthesis approach used for AgNPs-Cm in this study, which effectively utilized agricultural waste sources to produce a nanomaterial with potent inhibitory properties against bacterial growth.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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CRediT authorship contribution statement

Trung Nguyen Dien: Conceptualization, Investigation, Writing
Hong Nguyen Thi: Formal analysis, Investigation, Methodology

ORCID Numbers of the Authors

Trung Nguyen Dien: 0000-0003-4497-3149
Hong Nguyen Thi: 0000-0002-3777-0629

Supplementary File

None.

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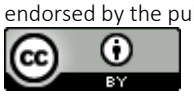

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