

## Research Article

# Sunlight-Driven Synthesis of Silver Nanoparticles Using Pomelo Peel Extract and Antibacterial Testing

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A green approach, including using phytochemicals in pomelo peel extract (PPE) and direct sunlight, was used to synthesize silver nanoparticles (AgNPs). PPE was prepared by treating pomelo peel with a citric acid solution at 85°C for 2 h. PPE was then mixed with AgNO<sub>3</sub> and exposed to sunlight to induce the formation of AgNPs. Time-dependent UV-vis spectra of the reaction mixture demonstrated that AgNPs are formed under sunlight irradiation faster than under heating at 90°C. Characterization techniques, including X-ray diffraction, transmission electron microscopy, and scanning electron microscopy, confirmed the formation of AgNPs with sizes of 20–30 nm. AgNPs synthesized in PPE were more stable toward electrolyte-induced aggregation than those synthesized using the conventional NaBH<sub>4</sub>/citrate method. The AgNPs synthesized in PPE showed antibacterial activities comparable to those of AgNO<sub>3</sub> at the same silver concentration against four pathogenic bacterial strains. The obtained PPE containing AgNPs, pectin, and other phytochemicals can be utilized further to produce antibacterial and antioxidant films in food packaging and medical applications.

## 1. Introduction

Nowadays, it is well known that silver nanoparticles (AgNPs) are highly toxic to a wide range of microorganisms, including bacteria, fungi, and viruses [1, 2]. AgNPs can be synthesized using a physical, chemical, or biological approach. The latter is considered green for using renewable sources of chemicals to reduce silver ions into metallic Ag and cap the AgNPs to maintain their sizes in the nanoscale. The most common source of these renewable chemicals is phytoextracts from different parts of plants. Some examples of aqueous extracts that were utilized to produce AgNPs are those of *Polyalthia longifolia* leaves [3], *Carica papaya* fruit [4], *Embllica officinalis* fruit [5], *Citrus limon* juice [6], *Rosa rugosa* leaves [7], *Jatropha curcas* seeds [8], *Capsicum annuum* L. leaves [9], and *Murraya koenigii* leaves [10].

Beside the use of renewable sources of chemicals, another way to make the process of AgNPs production greener is to use energy-efficient techniques, such as microwave, ultrasound, and light irradiations [11]. An interesting

technique for the light irradiation approach is to use solar energy from direct sunlight to assist the formation of AgNPs. This technique was successfully applied to produce AgNPs in the presence of plant extracts, including *Allium sativum* cloves [12], *Ocimum sanctum* Linn leaves [13], spinach fraction containing ferredoxin and ferredoxin-NADP<sup>+</sup> reductase [14], *Polyalthia longifolia* leaves [3], *Zingiber officinale* rhizome [15], *Pleurotus citrinopileatus* [16], and *Piper longum* catkins [17]. In some cases, sunlight irradiation can induce AgNPs formation without using any intrinsically reducing agents [18, 19].

Pomelo (*Citrus maxima* Merr.) in the citrus family is cultivated mainly in some Asian countries. Pomelo fruit is consumed fresh or as juice, while its peel is discarded as biological waste. However, pomelo peel, which weighs up to 30% of the total fruit, is considered a good source of valuable phytochemical compounds, such as flavonoids, essential oils, cellulose, and pectin [20, 21]. The flavonoids in pomelo peel extract can reduce silver ions into metallic Ag particles, and pectin can cap the formed AgNPs to protect them from

further growth in size. To the best of our knowledge, only few studies used pomelo juice or pomelo peel extract to synthesize AgNPs, and none of these studies utilized sunlight to assist the formation of AgNPs [22–24]. Therefore, we studied the formation of AgNPs in PPE upon exposure to direct sunlight and characterize the synthesized AgNPs, including their stability against electrolyte-induced agglomeration and antibacterial activity against four bacterial strains. The obtained PPE containing AgNPs, pectin, and other phytochemicals can be utilized further to produce antibacterial and antioxidant films in food packaging or medical applications.

## 2. Materials and Methods

**2.1. Materials and Reagents.** Pomelo peels were collected from a local market (Ho Chi Minh City, Vietnam). Trisodium citrate was purchased from Xilong Scientific Co., Ltd. (China), silver nitrate from Fisher Scientific (USA), sodium hydroxide and sodium borohydride from Sigma-Aldrich (US), trisodium citrate (TSC) from Prolabo Chemicals (France), and ethanol from Chemsol (Vietnam). All the reagents were of at least 99% pure and used without further purification.

**2.2. Preparation of Pomelo Peel Extract (PPE).** The green outer skin of pomelo peel was removed, and the white inner layer (with high pectin content) was sliced, cut into small pieces, and sun-dried for 3 days. These dried pieces were then ground into a powder and stored at 4°C. The procedure for pectin extraction was adapted from a previous study [25]. For each extraction batch, 2 g of the dried peel powder was mixed thoroughly with 80 mL of deionized water containing 0.91 g of citric acid. The mixture was heated to 85°C and kept for 120 min with continuous stirring (550 rpm). Afterward, the mixture was filtered and centrifuged for 15 min at 14000 rpm to remove the peel particles. The pale yellow supernatant was used immediately for AgNPs synthesis or stored at 4°C.

To evaluate the pectin content in PPE, the extract was mixed with 95% ethanol (ethanol:extract of 2:1 v/v), stirred for 10 min, and then left for 1 h. The precipitated pectin gel was filtered, washed 3 times with 95% ethanol, and finally dried at 70°C for 8 h.

**2.3. Sunlight-Induced Synthesis of AgNPs in PPE.** Predetermined volumes of deionized water, PPE, 0.1 M AgNO<sub>3</sub> solution, and 0.1 M NaOH solution (to adjust pH) were mixed thoroughly and then put under sunlight for 30 min. UV-vis spectrum of the reaction mixture was recorded from 380 to 800 nm at 400 nm/min every 5 min using a UV-Vis-NIR-V670 spectrophotometer (JASCO, Japan). In the following text, PPE containing the synthesized AgNPs is denoted as AgNPs/PPE while the PPE containing AgNO<sub>3</sub> before sunlight irradiation as AgNO<sub>3</sub>/PPE.

**2.4. Test of Stability against Electrolyte-Induced Agglomeration.** The aggregation stability of the AgNPs/PPE was compared with a control AgNPs solution synthesized by

drop-wise adding 2 mL of 4 mM NaBH<sub>4</sub> into 30 mL of a solution containing 25 mM trisodium citrate and 0.13 mM AgNO<sub>3</sub> under stirring [26].

The stability test was carried out by mixing 9 mL of each AgNPs solution with 1 mL of 1 M NaCl [27]. The agglomeration of AgNPs was monitored by regularly recording the absorbance of the mixture for 5 h.

**2.5. Characterization of AgNPs.** Sizes and shapes of the AgNPs/PPE were evaluated using images recorded by a JEM-1400 transmission electron microscope (JEOL, USA).

To obtain the AgNPs solid for X-ray diffraction measurements, the AgNPs/PPE were coagulated using 0.1 M ZnSO<sub>4</sub>, centrifuged at 10 000 rpm, washed three times with deionized water, and dried at 70°C. The diffraction was measured at 2 theta angles scanned from 35° to 80°.

**2.6. Antibacterial Assay for AgNPs/PPE.** The Kirby–Bauer disk diffusion method was used to evaluate the antimicrobial activity of AgNPs/PPE on three Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*) and one Gram-negative strain (*Salmonella typhi*). Each bacterial strain was cultured in Luria–Bertani (LB) nutrient medium at 37°C for 12 h. Each bacterial suspension was diluted with sterile LB medium to an absorbance of 0.45–0.50 at 625 nm. The bacterial suspension (100 μL) was spread on the dried surface of LB agar in Petri dishes. Each of the following solutions (20 μL) was dropped onto a piece of sterilized filter paper: 0.8 mM kanamycin (positive control), PPE (negative control), 0.8 mM AgNO<sub>3</sub>, and 0.8 mM AgNPs/PPE. The pieces of filter paper loaded with the solutions were put on the surface of the Petri dish with bacteria. After 24-hour incubation at 37°C, the diameters of inhibition zones were measured using a Vernier caliper and considered as an indication of antibacterial activity.

The antibacterial test was replicated three times for each bacterial strain. The difference in diameters of the inhibition zones were evaluated using the *t*-test.

## 3. Results and Discussion

**3.1. Pectin Content in PPE.** In this study, pectin was extracted from pomelo peel using citric acid treatment at pH 3.5 and temperature 85°C for 120 min. The extract and the obtained pectin were slightly yellow in color. From 2.00 g of pomelo peel, 0.23 g of crude pectin was obtained (11.5% yield from pomelo peel). Compared to other studies, this result was lower due to higher pH and lower temperature of extraction [25]. When using mineral acids such as HCl or HNO<sub>3</sub>, the percentages of extracted pectin were approximately 3 times higher than ours [21]. However, citric acid was chosen because of its greenness and its ability to cap AgNPs in the further synthetic step.

**3.2. Sunlight-Driven Formation of AgNPs/PPE.** UV-vis spectroscopy is a simple yet useful technique to characterize colloids of metallic nanoparticles. It is known that AgNPs

induce a surface plasmon resonance (SPR) at approximately 410 nm. A lower value of the wavelength at maximum ( $\lambda_{\max}$ ) indicates a lower average size of AgNPs, and a higher value of absorbance at  $\lambda_{\max}$  indicates a higher concentration of AgNPs [6]. Figure 1 shows the UV-vis spectra and the appearance (inset) of PPE and AgNO<sub>3</sub>/PPE after 30 min treated with high temperatures and under sunlight. PPE and AgNO<sub>3</sub>/PPE had the same pale yellow color, possibly due to the caramelization of neutral sugars in PPE during the extraction step. Heating the AgNO<sub>3</sub>/PPE mixture to 90°C only increased the yellow color intensity and the absorbance, but no new peak appeared. These results indicate that in PPE, which is acidic due to the presence of remaining citric acid, the reduction reaction of silver ions was slow even at 90°C. This is in agreement with other studies, which reported that the reduction reaction of silver ions by conventional heating is preferred at higher pH [28, 29].

Under sunlight irradiation, the reaction mixture quickly changed from pale yellow to orange-red in less than one minute and gradually turned to deep brown-red for over 3 h. This color change was associated with the development of a peak at 400–434 nm in the UV-vis spectrum (Figure 2), indicating the formation of AgNPs [28, 30–32].

The influence of electromagnetic irradiation on the formation of AgNPs has been known in several studies [17, 33–35]. A detailed mechanism was proposed for the action of UV light on the formation of AgNPs in the presence of methoxy polyethylene glycol and silver ions [36, 37]. However, it should be noted that in many studies, including ours, the reagents were contained in plastic tubes or glass vials that do not transmit UV part of the sunlight [15, 17]. Therefore, the blue light, which has lower energy than UV light but higher than other regions in the visible spectrum, may play a major role in reducing Ag<sup>+</sup> ions into metallic Ag [16, 38]. A possible mechanism for the reduction of Ag<sup>+</sup> ions is that the blue light induced tautomerization of the flavonoids in PPE from the enol to the keto form that can release reactive hydrogen atoms responsible for the reduction of Ag<sup>+</sup> ions [17].

The TEM image (Figure 3(a)) shows that the AgNPs synthesized in PPE were close to spheres with a size range of  $13 \pm 6$  nm (mean  $\pm$  SD,  $n = 29$ ). These low sizes of AgNPs are comparable with those prepared by strong reducing agents such as NaBH<sub>4</sub> and N<sub>2</sub>H<sub>4</sub> [39, 40]. The SEM image (Figure 3(b)) shows aggregates of AgNPs, which are possibly formed during the sample preparation step for SEM recording. However, one can find in Figure 3(b) that these aggregates are built from AgNPs of approximately 20–30 nm.

The XRD pattern of the synthesized AgNPs/PPE shows four characteristic peaks (Figure 4). These peaks are well matched with those of bulk silver in the face-centered cubic (fcc) structure (JCPDS file No. 04-0783). This result confirmed the ability of PPE to reduce Ag<sup>+</sup> ions to metallic silver under sunlight.

**3.3. Influence of the Reactant Concentrations on the Formation of AgNPs.** Figure 5 shows that increasing the concentration

of Ag<sup>+</sup> ions from 0.4 to 0.8 mM resulted in the same  $\lambda_{\max}$  but higher absorbance, indicating the formation of more AgNPs with almost the same size distribution. However, further increase of Ag<sup>+</sup> ions to 7.4 and 16.7 mM shifted  $\lambda_{\max}$  to the red region and decreased the maximum absorbance, indicating the formation of larger AgNPs [6]. These different characteristics of AgNPs produced under different concentrations of Ag<sup>+</sup> ions can be explained by the limited amount of reducing and protecting agents in the PPE. When Ag<sup>+</sup> ion concentration was low, the present reducing and capping agents can effectively reduce Ag<sup>+</sup> ions and protect AgNPs from agglomeration. When the concentration of Ag<sup>+</sup> ions was too high, the limited pectin could not protect all the AgNPs produced, which resulted in their agglomeration into larger and less particles [28, 32]. This result implies that a careful choice of Ag<sup>+</sup> concentration is required to synthesize AgNPs with low sizes.

#### 3.4. Influence of PPE Amount on the Formation of AgNPs.

In our study, PPE played the role of a capping agent that protects AgNPs from agglomeration. Therefore, its amount used in the reaction mixture must be an essential factor in controlling the amount and size of the produced AgNPs. Figure 6 shows that PPE volumes lower than 0.25 mL did not change the color of the reaction mixture, associating with no peak around 430 nm. This was possibly due to the low reaction rates at low concentrations of reducing agents from PPE. The inset table in Figure 6 shows that increasing the PPE volumes higher than 0.5 mL resulted in higher maximum absorbance at lower values of  $\lambda_{\max}$ , indicating the formation of more AgNPs with lower sizes.

**3.5. Stability Test of AgNPs.** To evaluate the effectiveness of using PPE pectin as a protecting agent for AgNPs, we compared the stability of AgNPs/PPE with that of AgNPs commonly synthesized using NaBH<sub>4</sub> as the reducing agent and trisodium citrate as the protecting agent (Figure 7).

In this test, 0.1 M NaCl was used as a coagulating agent to accelerate the agglomeration of AgNPs [27]. After 120 min of NaCl addition, AgNPs protected by citrate ions were significantly agglomerated, as expressed by the reduction in the peak absorbance. At the same time, AgNPs/PPE showed a slight reduction in the peak absorbance, indicating a superior protective effect of pectin against agglomeration of nanoparticles. This is due to the electrosterically stabilizing effect of pectin molecules. They surround the surface of AgNPs to form a polymeric capping layer with negative charges of carboxylate groups. This negative surface charge induces repulsions between AgNPs when they approach each other, thus reducing the chance of their collisions (electrostatic stabilization). Moreover, the polymeric surrounding layer further reduces the chance of their agglomeration upon collisions (steric stabilization). In other studies, polymers (polyvinylpyrrolidone and branched polyethyleneimine) also showed a stabilizing effect better than citrate anions for AgNPs in solutions containing monovalent and divalent coagulants [27, 41].

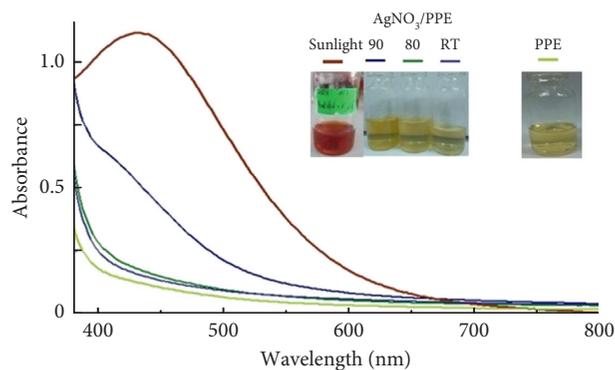


FIGURE 1: UV-vis spectra and appearance of PPE and  $\text{AgNO}_3/\text{PPE}$  after 30 min under different reaction conditions: at room temperature (RT), 80°C, 90°C, and under sunlight.

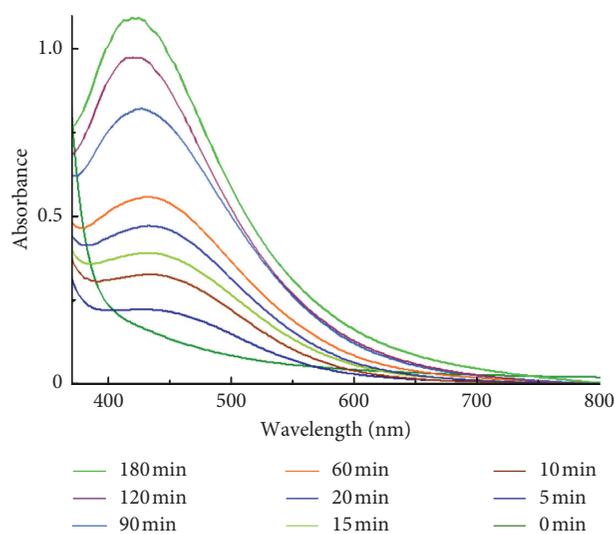


FIGURE 2: Time-dependent UV-vis spectra of  $\text{AgNO}_3/\text{PPE}$  under sunlight irradiation.

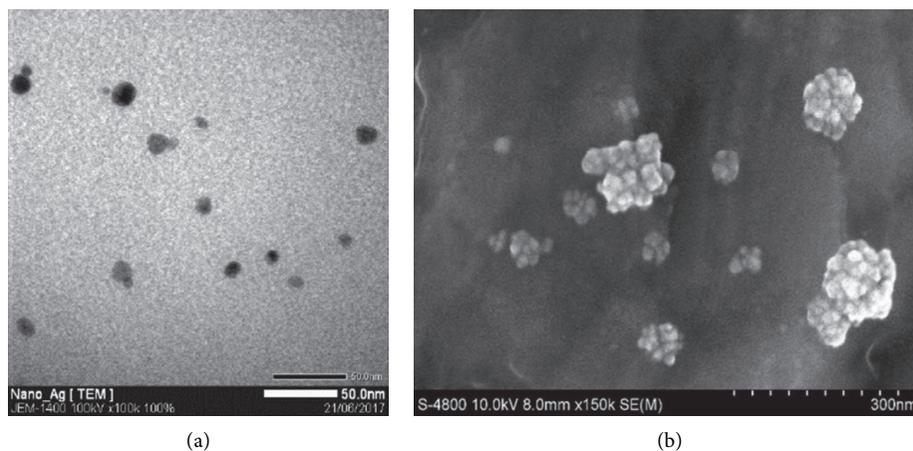


FIGURE 3: (a) TEM image of  $\text{AgNPs}/\text{PPE}$  and (b) SEM image of  $\text{AgNPs}$  clusters after precipitated together with pectin from PPE.

3.6. *Antibacterial Activity of  $\text{AgNPs}/\text{PPE}$ .* Based on the diameters of the inhibition zones in the antibacterial tests (Figure 8 and Table 1), the order of antibacterial strengths

( $p < 0.05$ ) was kanamycin >  $\text{AgNPs} = \text{AgNO}_3 > \text{PPE}$ . PPE itself possessed antibacterial effects, possibly due to the presence of citric acid and/or phytochemicals from pomelo

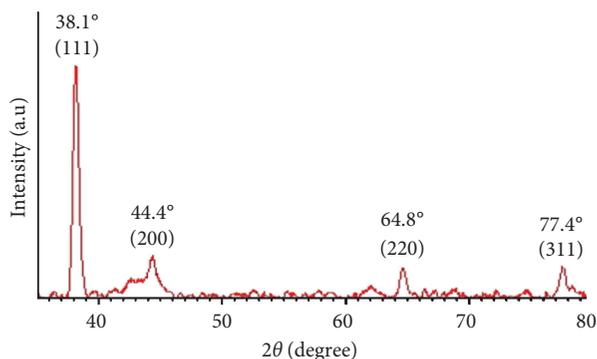
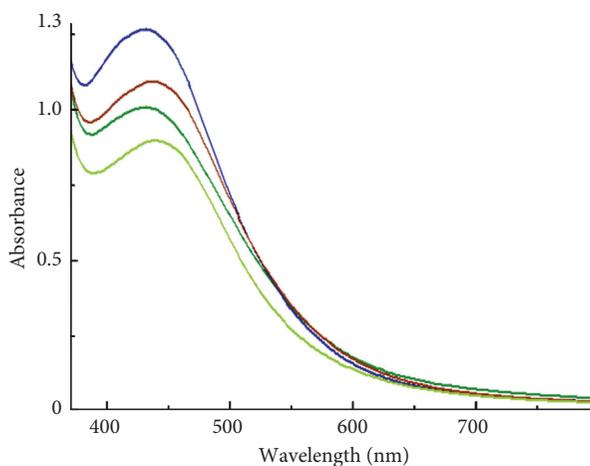


FIGURE 4: XRD pattern of the AgNPs-pectin powder.



AgNO <sub>3</sub> (mM)	$\lambda_{\max}$ (nm)	$A_{\max}$
0.4	432	1.00
0.8	432	1.26
7.4	438	1.09
16.7	440	0.89

FIGURE 5: UV-vis spectra of AgNO<sub>3</sub>/PPE with different Ag<sup>+</sup> concentrations after 30 min of sunlight irradiation. Inset table: maximum absorbance ( $A_{\max}$ ) and  $\lambda_{\max}$  of these mixtures.

peel. The higher antibacterial effect of AgNPs/PPE compared to that of PPE indicates that AgNPs in the PPE possesses intrinsic antibacterial activity.

There have been extensive studies on possible antibacterial mechanisms of AgNPs, which revealed that the main mechanism involves the release of Ag<sup>+</sup> ions from metallic silver [42]. These ions have very high affinities for phosphates, amines, and especially thiols, with which they form a quasi-covalent Ag-S bond (binding energy of approximately 65 kcal/mol) [43]. Moreover, silver ions can bridge several thiol groups, forming an irreversible aggregation of the thiol-bearing biological molecules [44]. Therefore, unlike antibiotics that target specific components of the bacterial life cycle, silver ions attack readily any biological molecules

(DNA, membrane-bound peptides, intracellular peptides, or cofactors) bearing target groups [45].

The results above demonstrate that the antibacterial effects of AgNPs/PPE were not different from those of AgNO<sub>3</sub> at the same 0.8 mM silver concentration. This seems contradictory common sense that silver ions from AgNO<sub>3</sub> should have a higher mobility, and hence a higher antibacterial effect than silver ions released from solid AgNPs. We suggest that the enhanced antibacterial effect of AgNPs/PPE was due to the presence of citric acid in PPE. Citric acid can release (1) H<sup>+</sup> ions, which increase the redox potential of dissolved oxygen E(O<sub>2</sub>, H<sup>+</sup>/H<sub>2</sub>O), and (2) citrate ions, which chelate silver ions and thus reduce the redox potential E(Ag<sup>+</sup>/Ag). These two combined effects of citric acid could

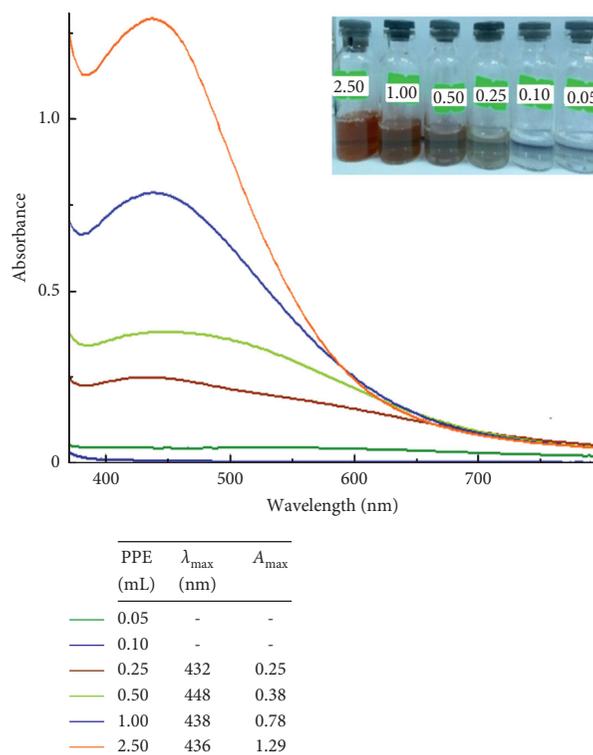


FIGURE 6: Higher amounts of PPE yielded more AgNPs. Inset table: maximum absorbance ( $A_{\max}$ ) and  $\lambda_{\max}$  of the reaction mixtures.

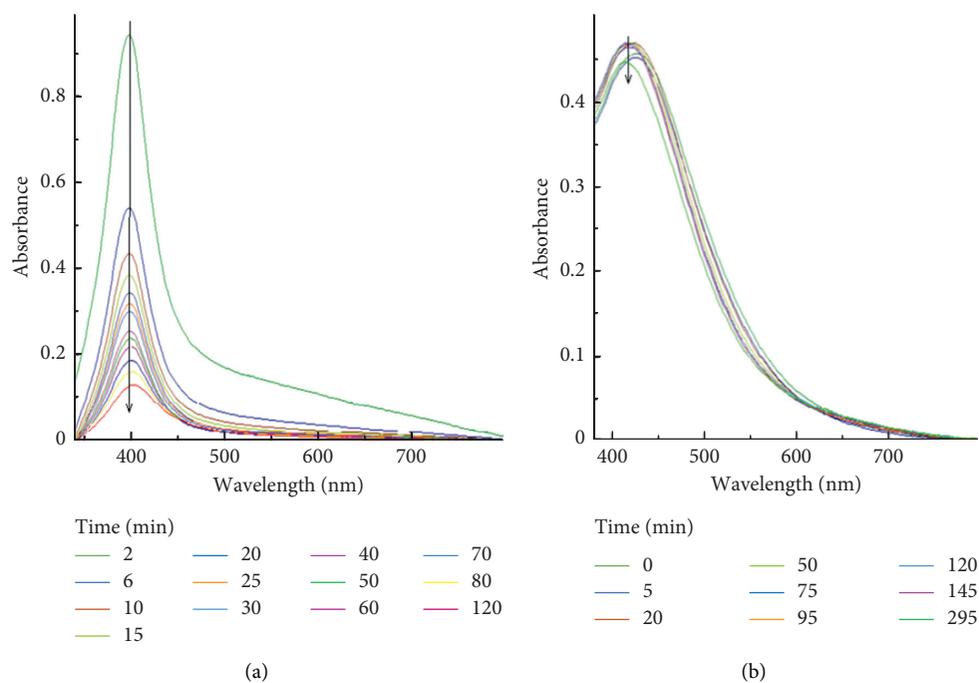


FIGURE 7: Time-dependent UV-vis spectra of AgNPs solution in the presence of 0.1 M NaCl. (a) AgNPs prepared using  $\text{NaBH}_4$  and trisodium citrate; (b) AgNPs/PPE.

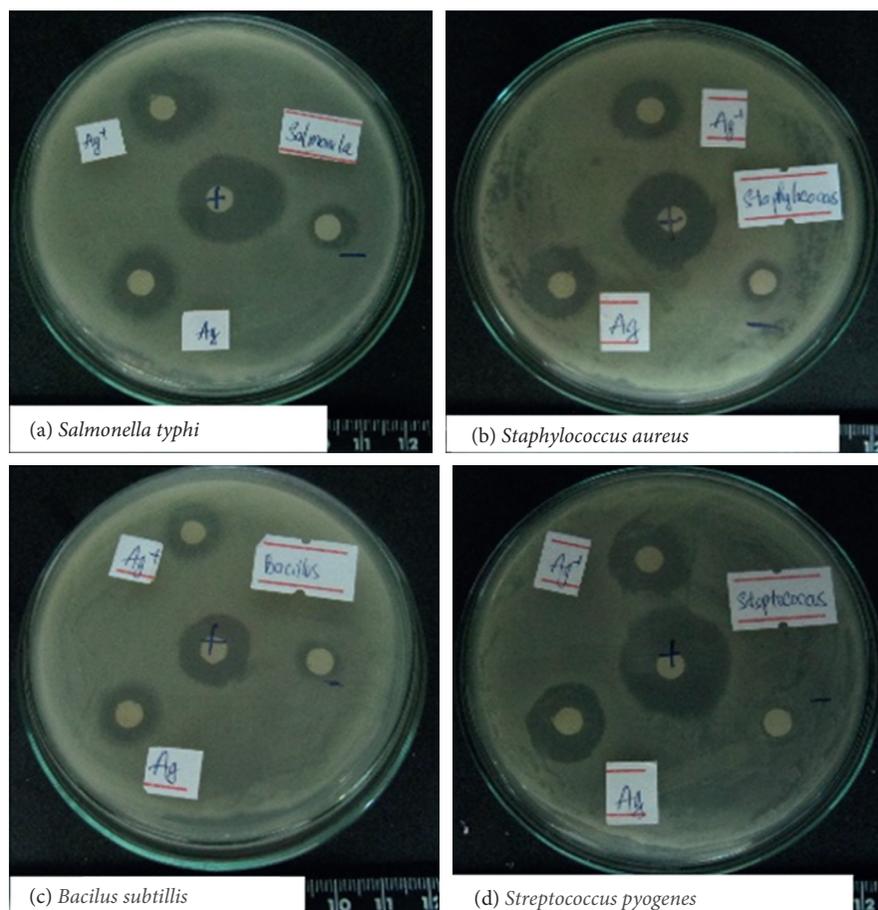


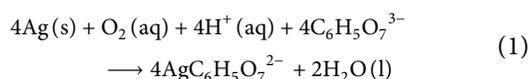
FIGURE 8: Antibacterial activity of AgNPs in comparison with  $\text{AgNO}_3$ , kanamycin (+), and PPE (-).

TABLE 1: Diameters of inhibition zones (mm) in the antibacterial tests of AgNPs/PPE, PPE, kanamycin, and  $\text{AgNO}_3$  against four bacterial strains.

Bacteria	Kanamycin (+)	PPE (-)	$\text{AgNO}_3$	AgNPs/PPE
<i>Salmonella typhi</i>	$17.5 \pm 1.5^a$	$10.4 \pm 0.2^c$	$13.0 \pm 1.9^b$	$13.9 \pm 0.2^b$
<i>Staphylococcus aureus</i>	$17.4 \pm 1.2^a$	$8.9 \pm 0.1^c$	$12.4 \pm 1.9^b$	$11.6 \pm 1.1^b$
<i>Bacillus subtilis</i>	$13.8 \pm 1.5^a$	$8.5 \pm 0.2^c$	$11.5 \pm 0.7^b$	$11.5 \pm 0.5^b$
<i>Streptococcus pyogenes</i>	$17.7 \pm 4.2^a$	$8.3 \pm 0.1^c$	$10.8 \pm 0.8^b$	$11.2 \pm 1.5^b$

The results are expressed as mean  $\pm$  SD ( $n=3$ ). The means in a row with different letters <sup>a</sup>, <sup>b</sup> or <sup>c</sup> are significantly different ( $p < 0.05$ ).

enhance the oxidative dissolution of AgNPs into Ag (I) according to the following overall reaction:



For AgNPs and  $\text{AgNO}_3$ , the inhibition zone against the Gram-negative *S. typhi* was significantly larger than the other three Gram-positive strains ( $p < 0.05$ ). This higher resistance of Gram-positive strains is consistent with other studies and can be explained by the thick peptidoglycan cell walls that resist the penetration of silver ions into the bacterial cell [15, 46].

## 4. Conclusions

In this study, we proved that direct sunlight facilitates the formation of AgNPs in acidic pomelo peel extract (PPE). The flavonoids in PPE are supposed to be the reducing agents for silver ions and pectin as the capping agent for AgNPs. The AgNPs synthesized in PPE demonstrated enhanced stability against agglomeration due to the protective effect of pectin. AgNPs/PPE showed antibacterial effects comparable to those of  $\text{AgNO}_3$ , possibly due to the presence of citric acid and other components in PPE. The obtained PPE containing AgNPs, pectin, and phytochemicals from pomelo peels can be utilized further to produce antibacterial and antioxidant films in food packaging and medical applications.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

The author declares that there are no conflicts of interest regarding the publication of this paper.

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