CHARACTERIZATION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED WITH THE GUAVA FRUIT EXTRACT

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ABSTRACT

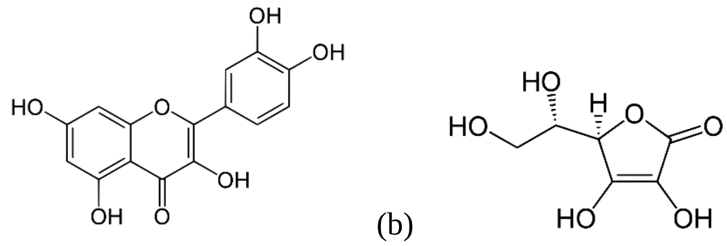
Silver nanoparticles (AgNPs) can be synthesized using biomolecules derived from different plant components and microbial species. In this case, silver nanoparticles that have been produced can be made physiologically active by using plant extracts that contain bioactive cmpounds like polyphenols, flavonoids, or tannins. In this study, biocompatible silver nanoparticles were synthesized from aqueous extract of guava (*Psidium guajava* Linn.) fruit. The formation of nanoparticles (AgNPs-Gv) was confirmed by the color change and by UV-vis spectrometry. The structural and morphological properties of AgNPs-Gv were confirmed by Transmission electron microscope (TEM) and Energy Dispersive X-ray Spectrometer (EDX) analysis and exhibited that particles were spherical in shape with size smaller than 50 nm. The antioxidant activity of AgNPs-Gv was investigated by DPPH method. The antibacterial activity of the synthesized AgNPs-Gv against Gram-negative *E. coli* and Gram-positive *S. aureus* was also examined. The result showed that the synthesized biocompatible silver nanoparticles had high antioxidant and antibacterial properties.

Keywords: Silver nanoparticles, Antioxidant, Antibacterial, Guava

INTRODUCTION

Silver nanoparticles are interest because of their applications in various fields, such as medical, cleaning products, cosmetics and electronic sensor technology, etc. Silver ions have a strong inhibitory effect on a variety of microorganisms (Natkamol Peungsamran and Sirilak Namwong, 2016). Therefore, the use of silver nanoparticles as antimicrobial agent has gained attention. Silver nanoparticles can be synthesized using chemical and physical methods. However, biological synthesis or green synthesis of nanoparticles is an alternative and eco-friendly method for production of nanoparticles. In addition, using plant extracts containing bioactive compounds such as polyphenols, flavonoids or tannins as reducing agents can make the synthesized silver nanoparticles also biologically active.

Guava (*Psidium guajava*) is well known tropical fruit. Its fruit is rich in vitamins A, C, iron, phosphorus and calcium and minerals The guava fruit contains saponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin, quercetin and flavonoids (Naseer *et al*., 2018). The chemical structures of quercetin and ascorbic acid have been shown in Fig.1. In this study, aqueous extract of guava fruit was used for reduction of silver ions and the formation of silver nanoparticles. The green-synthesized nanoparticles were examined by ultraviolet-visible spectroscopy (UV-Vis). Transmission electron microscope (TEM) and Energy Dispersive X-ray Spectrometer (EDX) were used to determine their size. The antioxidant activity and the antibacterial activity of the synthesized silver nanoparticles were also evaluated.



(a) (b)

Fig.1 Chemical structure of (a) quercetin and (b) ascorbic acid

OBJECTIVE

1. To synthesize silver nanoparticles using guava fruit extract as a reducing agent
2. To evaluate the antioxidant activity and antibacterial effect of the synthesized silver nanoparticles

METHODOLOGY

2.1 Preparation of guava extracts

Guava fruit was cut into pieces and wash twice with deionized water. Exactly 100 g of guava fruit was added to 500 ml of deionized water and heated to 80- 90 °C for 1 hour. The extract was separated using cheesecloth twice and stored at 4 °C until used.

2.2 Synthesis of silver nanoparticles

Heat 30 mL aqueous extract of guava to approximately 60-70 °C and slowly drop the 1 mM silver nitrate solution into the extract. Confirmation of the biosynthesis was ensured by observing the color change (clear to brown).

2.3 Characterization of nanoparticles

The reduction of Ag^+ to Ag° in the solution was recorded with an Ultraviolet-visible (UV–vis) spectroscopy at the wavelength range of 200 − 800 nm. Transmission electron microscope (TEM, Hitachi HT7700) was used to determine morphology and particle size. Composition analysis was performed by using Energy Dispersive X-Ray Spectroscopy (*EDS, EDAX RTem*). The synthesized AgNPs-Gv were drop on a carbon coated copper grid and observed at 80 kV.

2.4 Antioxidant test

Weigh 3 mM DPPH, dissolve with 45 mL of ethanol and 5 mL of DI water into a 50 mL volumetric flask and wrap in foil. Then weigh 0.001 g of gallic acid, dissolve it with 1000 µL of 10% ethanol and prepare 1000 µL of AgNPs-Gv sample. Then add the prepared substance to well plate96, keep it in the dark for 4 hours and then measure by Microplate Reader

Prepare 3 mM DPPH solution with ethanol and water in a ratio of 9:1. Aqueous extract of guava fruit and synthesized AgNPs-Gv sample were allowed to react with DPPH solution for 4 h in the dark. Then the absorbance was measured at 517 nm and the radical scavenging activity (RSA) was calculated using the following equation (İlhami and Alwasel, 2023):

$$\text{RSA (\%)} = \frac{(A_C - A_S)}{A_c} \times 100$$

where A_c is the absorbance at 517 nm of the control sample, and A_s is the absorbance at 517 nm that contains the test sample, including guava extracts and AgNPs-Gv.

2.5 Antibacterial test

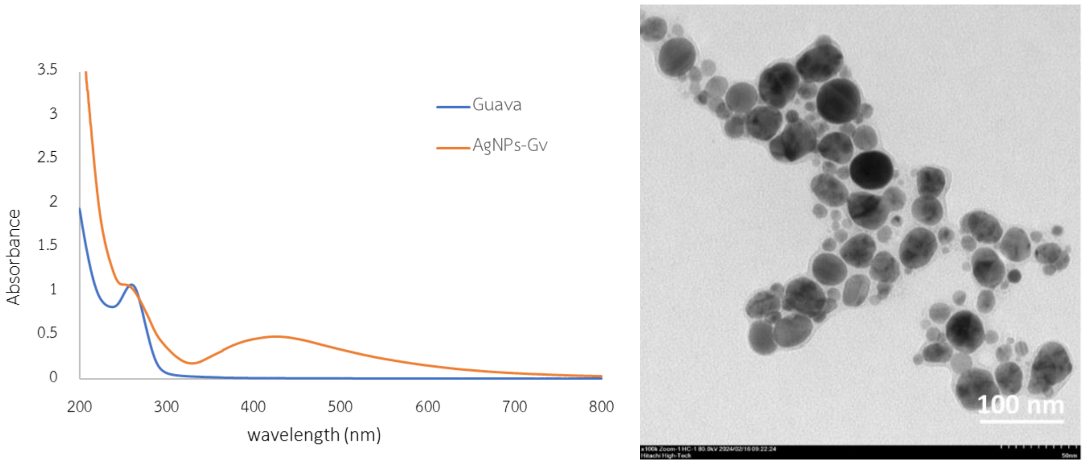
Antibacterial activity of guava extracts and AgNPs-Gv was done against *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S. aureus*) by disc diffusion method. Ampicillin Chloramphenicol and Bacitracin were used as positive controls and deionized water was used as a negative control.

RESULTS

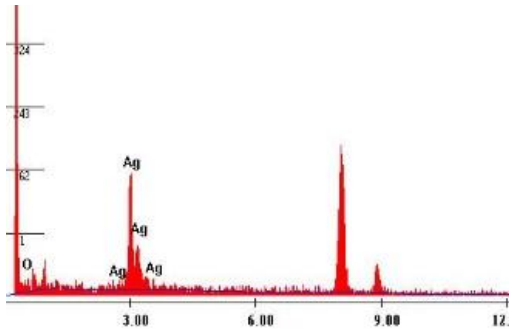
3.1 Characterization of silver nanoparticles

The Formation of AgNPs-Gv was monitored by UV–vis spectrum. UV–vis is a simple and widely used technique for monitoring the occurrence of AgNPs. Upon interaction with an electromagnetic field, conductive electrons in the outermost orbit of metal NPs oscillate in resonance with certain wavelengths, this is called Surface Plasmon Resonance. With The excitation of SPR, a color change occurs in the colloidal solutions of AgNPs. The absorbance at around 450 nm is usually taken to approve the reduction of Ag^+ in to Ag°. After addition of guava aqueous extract to silver nitrate solution confirmed by the color change from colorless to brown. (Fig. 1)

The Transmission electron microscope (TEM) shown in Fig. 1(b) shows that AgNPs-Gv is mostly spherical shape and depicted that size of the particles are below 50 nm. The elemental composition was investigated and confirmed by Energy Dispersive X-Ray Spectroscopy (EDX) spectroscopy (Fig.1(c)). Elemental profile of the silver nanoparticles with spectrum indicates that AgNPs-Gv contain primarily Ag, C and O. As given in Fig. 1(c), C and O atoms were noted, which confirmed that the organic contents present in the Guava aqueous extract were responsible for the reduction and stabilization of AgNPs



(a) **(b)**



Element	*Wt %*	*At %*
C	86.57	93.93
O	06.41	05.22
Ag	07.03	00.85

(c)

Fig.1 (a) UV–vis spectra of extract guava and synthesized silver nanoparticles (AgNPs-Gv) (b) TEM images of AgNPs-Gv and (c) EDX analysis of AgNPs-Gv

3.2 Antioxidant properties

The synthesized AgNPs-Gv from Guava Extract were applied for antioxidant tests by DPPH scavenging method. All measurements were performed in triplicate. The results indicate that guava fruit extract showed the significant antioxidant activity equal to gallic acid. The AgNPs-Gv has lower % radical scavenging but still high antioxidant (Fig.2).

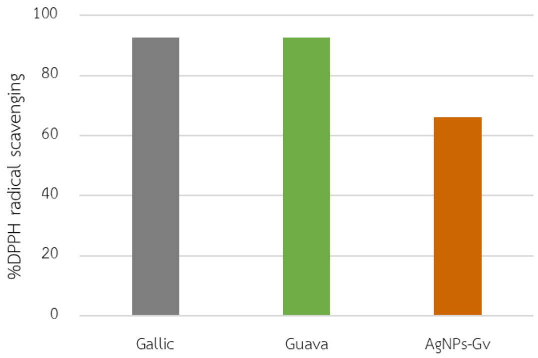


Fig.2 Antioxidant activity of of gallic acid, guava fruit extract and AgNPs-Gv

3.3 Antibacterial property

The results confirmed that the antibacterial activity was shown in Fig. 3. The zone of inhibition against *E.coli* were 1 9 .4 (±0.2) mm, 26.2 (±0.2) mm, and 10 (±0.2) mm for Ampicillin, Chloramphenicol, and AgNPs-Gv, respectively. Bacitracin did not show antibacterial property against *E.coli*. The zone of inhibition against *S.aureus* were 30.5 (±0.3) mm, 22.5 (±0.1) mm, 13.4 (±0.2) mm and 15.5 (±0.2) mm for Ampicillin, Chloramphenicol, Bacitracin and AgNPs-Gv, respectively. No zone of inhibition appeared for deionized water; Gv, guava fruit extract.

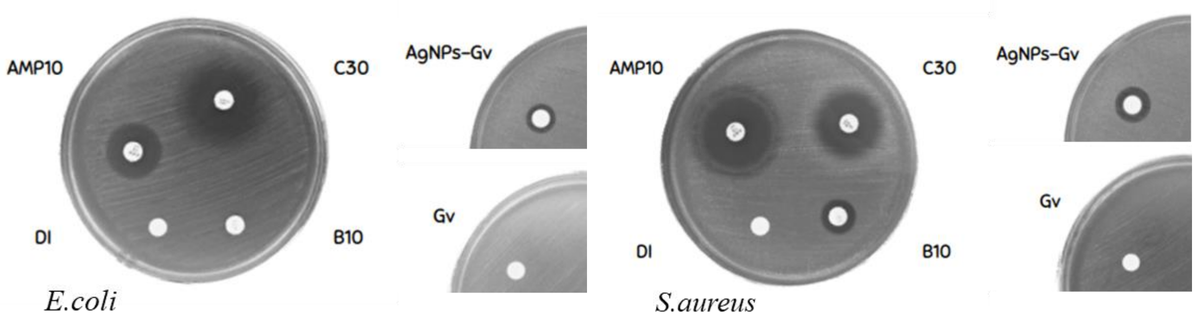


Fig.3 Antibacterial activity of AgNPs-Gv (AMP10, Ampicillin; C30, Chloramphenicol; B10, Bacitracin; DI, deionized water; Gv, guava fruit extract)

CONCLUSION

The AgNPs-Gv were successfully synthesized. The absorption SPR peaks appeared around 450 nm. TEM-EDX analysis exhibited that particles were spherical in shape with size smaller than 50 nm. The synthesized AgNPs-Gv showed antioxidant and antibacterial properties. The results suggested that AgNPs prepared by green synthesis can be considered as an alternative antibacterial agent.

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REFERENCES

[1] Al-Otibi, F., Al-Ahaidib, R. A., Alharbi, R. I., Al-Otaibi, R. M., & Albasher, G. (2020). Antimicrobial potential of biosynthesized silver nanoparticles by *Aaronsohnia factorovskyi* Extract. *Molecules*, 26(1).https://doi.org/10.3390/molecules26010130

[2] Ali, K. A., Yao, R., Wu, W., Masum, M. M. I., Luo, J., Wang, Y., Zhang, Y., An, Q., Sun, G., & Li, B. (2020). Biosynthesis of silver nanoparticle from pomelo (*Citrus Maxima*) and their antibacterial activity against *acidovorax oryzae* RS-2. *Materials Research Express*, 7(1).

[3] Agustina, T. E., Handayani, W., & Imawan, C. (2020). The UV-VIS Spectrum Analysis from Silver Nanoparticles Synthesized Using Diospyros maritima Blume. Leaves Extract. Advances in Biological Sciences Research, 14. 411 – 419.

[4] Asif, M., Yasmin, R., Asif, R., Ambreen, A., Mustafa, M., Umbreen, S. (2022). Green Synthesis of Silver Nanoparticles (AgNPs), Structural Characterization, and their Antibacterial Potential. Dose Response. 20(1):15593258221088709. doi: 10.1177/15593258221088709.

[5] Dhand, V., Soumya, L., Bharadwaj, S., Chakra, S., Bhatt, D. & Sreedhar, B. (2016). Green synthesis of silver nanoparticles using Coffea arabica seed extract and its antibacterial activity. *Materials Science and Engineering*, 58, 36–43.

[6] İlhami,G., & Alwasel, S. (2023). "DPPH Radical Scavenging Assay" Processes 11, no. 8: 2248. doi: 10.3390/pr11082248

[7] Kandylis, P., Kokkinomagoulos, E. (2020). Food Applications and Potential Health Benefits of Pomegranate and its Derivatives. *Foods*, 9, 122. https://doi.org/10.3390/foods9020122

[8] Keshari A. K., Srivastava R., Singh P., Yadav V.B., & Nath G. (2020). Antioxidant and antibacterial activity of silver nanoparticles synthesized by *Cestrum nocturnum*. *J Ayurveda Integr Med*. 11(1). 37-44. https://doi.org/10.1016/j.jaim.2017.11.003.

[9] Limei, Y., Mouming, Z., Bao, Y., Qiangzhong, Z., & Yueming, J. (2007). Phenolics from hull of Garcinia mangostana fruit and their antioxidant activities. *Food Chemistry*, 104, 176-181.

[10] Naseer, S., Hussain, S., Naeem, N., Pervaiz, M., Rahman, M. (2018). The phytochemistry and medicinal value of Psidium guajava (guava). *Clin Phytosci* 4, 32 doi: 10.1186/s40816-018-0093-8.

[11] Peungsamran, N. and Namwong, S. (2016). The Coated Paper with Silver Nanoparticles and its Antibacterial Activity. *Key Engineering Materials*, 675, 265-268.

[12] Wanna, C., Boonman, N., & Phakpaknam, S. (2023). Antioxidant and antidiabetic properties of *Garcinia cowa* Roxb. extracts from leaves, fruit rind, and stem bark in different solvents. *Plant Science Today*, 10, 3 https://doi.org/10.14719/pst.2455.