Scientific Research Journal,
Vol 22, No 1 (2025) 223-241
https://doi.org/10.24191/srj.v22i1.17045

Elucidation Study of Bioavailable Potential Among Plant-based Silver Nanoparticles Fabricated from Several Local Fruit Peels

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Received: 06 January 2025
Accepted: 12 January 2025
Online First: 01 March 2025

ABSTRACT

Plant-based silver nanoparticles (AgNPs) derived from biological waste (fruits) have piqued researchers' interest due to their remarkable antibacterial and antioxidant characteristics, as well as toxicity studies. This study aimed to elucidate the bioavailable potential of AgNPs from silver nitrate fabricated from A. comosus, G. mangostana, and M. indica peel extracts as green reducing agents. UV-Vis and FTIR analysis corroborated the presence of AgNPs. Antibacterial activity against S. aureus and E. coli was evaluated using the disc diffusion and minimum inhibitory concentration (MIC) techniques. Interestingly, all peel extracts successfully produced AgNPs, resulting in reddish-dark brown alterations with a peak absorbance at 432-440 nm and the presence of the most important functional groups, including O-H, C-H, and C=O, which correspond to the most bioactive compounds in peel extracts used as reducing agents detected using FTIR. The antibacterial activity of produced AgNPs against S. aureus and E. coli was observed to vary with nanoparticle concentration. Higher doses of AgNPs resulted in bigger inhibition zones, with AC-AgNPs exhibiting the highest antibacterial activity compared to the other samples. The MIC values for AC-AgNPs antagonistic towards S. aureus and E. coli were 2.5 and 5 mg/mL, respectively, surpassing GM-AgNPs and MI-AgNPs. This

study emphasizes a sustainable approach to nanoparticles synthesis using local fruit peels of pineapple, mangosteen, and mango, as well as the prospect of creating AgNP-based antimicrobial and increase the value of local fruit waste.

Keywords: Plant-based Silver Nanoparticles; Pineapple; Mangosteen; Mango; Biovailable Potential

INTRODUCTION

Nanotechnology, in which synthesised nanomaterials range from 1 to 100 nanometers (nm), has grown in popularity and holds significance in agriculture, chemistry, food, medicine, and pharmaceuticals [1]. Their large surface-area-to-volume ratio gives them distinct physical, chemical, and biological features. Besides, nanoparticles (NPs) vary in size, shape, and structure, which ostentatious their durability, reactivity, and other feature [2]. These distinct properties make nanoparticles (NPs) better suited for usage than bulk materials with consistent physical properties, regardless of size [3]. In biomedicine, noble metal NPs like copper, gold, platinum, silver, titanium, zinc, and magnesium have several uses [4].

According to [5], among several nanoparticles, silver nanoparticles (AgNPs) have received the recognition for their distinctive traits, particularly their exceptional antibacterial properties. These features make AgNPs promising candidates for appliance in medicine, industry, and environmental science [6]. [7] reported that the use of AgNPs has become prevalent, with an annual global production estimated at over 500 tonnes. Several studies have shown that silver nanoparticles (AgNPs) exhibit superior performance in killing bacteria, fungi, viruses, and multidrug-resistant microorganisms [8]. Their remarkable antibacterial properties are attributed to their small dimensions, which allow them to fight several microbial invaders and penetrate bacteria's cell membranes [9]. Moreover, their broad surface area increases pathogen interaction and absorption, making AgNPs suitable for antibacterial use [10].

The emergence of antibiotic resistance has become a worldwide issue, rendering numerous traditional antibiotics ineffective and presenting a

substantial threat to public health [11]. Overuse and misuse of antibiotics regularly allow bacteria to become more resilient and adaptive, resulting in resistance to today's common antibiotics [12]. Due to that, it has jeopardised thousands of lives and may reach 10 million people by 2050 [13]. After seeking numerous solutions, AgNPs have become a novel antibacterial agent for overcoming this global catastrophe. Correspondingly, global demand for environmentally conscious and sustainable synthesis methods for AgNPs is growing as more industries, including medicine, are employing them. AgNPs conceivably synthesised using biological, chemical, and physical methods [14]. Nevertheless, conventional physical and chemical methods use harmful chemicals and inadvertently aggravate environmental problems. According to [15], chemicals such as sodium borohydride, polyvinyl alcohol, and oleylamine are usable as reducing and capping agents in the fabrication of AgNPs with greater yields and adjustable sizes. Moreover, the chemicals also potentially reduce the biocompatibility of the acquired AgNPs owing to alterations in the surface properties or the presence of residues throughout the synthesis process. Hence, it is vital to seek approaches that can generate AgNPs that are safe for living organisms and the environment.

Biological methods manipulate the extraction of plant in the synthesis of AgNPs because they enriched with numerous phytochemicals that serve as reducing and capping agents. An incorporation of plant extracts as reductants can also address the issue of fruit waste accumulation by converting them into valuable sources. Seeds, peels, flowers, stems, and leaves are plant parts that generate substantial quantities of fruit waste [16]. Some of the fruits that contribute significantly to the accumulation of waste are *Mangifera indica, Garcinia mangostana* and *Ananas comosus* [17]. By utilising these fruity wastes as potential reductants, the green production of AgNPs offers a promising approach to minimise the environmental impact of chemical agents and fulfil sustainability standards. In addition, AgNPs obtained from these natural sources have biocompatible qualities that make them effective antibacterial agents in confronting the increasing threat.

This study specifically focused on elucidation study of the bioavailable potential of AgNPs from silver nitrate ($AgNO_3$) using bioactive compounds from local fruits such as *Ananas comosus, Garcinia mangostana,* and *Mangifera indica* peel wastes as green-reducing agents, characterize using UV-visible spectroscopy (UV-Vis) and Fourier transform infrared

spectroscopy (FTIR). Then, determine and differentiate on antibacterial efficacy of AgNPs from *A. comosus, G. mangostana,* and *M. indica* waste extracts, the disc diffusion and minimum inhibitory concentration (MIC) method is utilised hostile to *Staphylococcus aureus* and *Escherichia coli*.

EXPERIMENTAL METHODOLOGY

Plant Sample

The plant materials that were utilized in this experimental were the peel of MD2 pineapple (*Ananas comosus)*, mangosteen (*Garcinia mangostana*) and mango 'susu' (*Mangifera indica*).

Preparation of Peel Extracts

The peels from *A. comosus, M. indica,* and *G. mangostana* were carefully removed and cut into and subsequently washed twice with distilled water. Then, the samples dried in an oven at 50 °C for 24 hours. After drying, the peels were pulverised using a blender. Three beakers were prepared and labelled for each type of peel extract. Approximately 10 g of the fine powder from each type of peel was weighed using an analytical balance and placed into beakers containing 100 mL of distilled water. The mixtures were stirred well and then heated in a water bath at 60 °C. After allowing them to cool for a few minutes, the extracts were centrifuged at 6,000 rpm. The supernatant was then filtered using Whatman No.1 filter paper, and the collected filtrate was stored at 4 °C for further analysis.

Synthesizing AgNPs using *Ananas comosus, Garcinia mangostana,* and *Mangifera indica* Peel Extracts

Prior to synthesis AgNPs, 0.01 M $AgNO_3$ was prepared by adding 1.6987 g of $AgNO_3$ into 1L of distilled water and mixed well. The $AgNO_3$ utilized as a precursor and peel extracts as reducing agents. As for the concentration of 1000 mg/mL AgNPs, a mixture was prepared by combining 45 mL of the 0.01 M $AgNO_3$ solution with 5 mL of peel extract in a conical flask. The mixture was thoroughly mixed and then heated on a hot plate at 60 °C for 60 minutes. The changes in colour of solution were monitored and

recorded for every 10 minutes to ensure complete reaction. The formation of AgNPs, in each of the sample were measured using UV-Vis and FTIR. After analysis, the synthesised AgNPs was diluted to 10 and 100 mg/mL. Lastly, the colloidal composition was sealed and stored in dark at 4 ^{o}C for further use.

Characterisation

UV-Visible Spectrophotometer

Initial characterization of AgNPs was performed using UV-Visible spectroscopy. The samples were diluted three times before fill in quartz cuvette and analysed. The UV-Vis spectra of the AgNPs solution were recorded using a Shimadzu UV-1601 spectrophotometer (300 to 800 nm). Distilled water was used as blank to adjust the baseline.

Fourier-transform Infrared (FTIR) Spectroscopy

The functional groups responsible in reducing silver ions to silver nanoparticles was identified using FTIR spectra. An aliquot of sample was taken and analysed using PerkinElmer FT-IR Spectrometer Frontier with a wave region of 400-4000 cm^{-1}. The FTIR spectra produced were evaluated and recorded to determine the functional groups contained in each peel extract and AgNPs derived from each extract.

Disc Diffusion Method

Mueller-Hinton agar (MHA) plates were divided into three quadrants by marking the outer, and bottom of the plate and labelled each quadrant with the positive control, negative control, and AgNPs. In the laminar flow hood, 100 µL of fresh overnight cultures of bacteria were aseptically collected from the subculture broth and evenly spread onto the MHA plates using a sterile hockey stick. All plates containing pathogenic bacteria were then allowed to air dry. Subsequently, sterile blank discs were impregnated using sterile forceps with samples of silver nanoparticles derived from peel extracts of varying concentrations (10, 100, and 1000 mg/mL), as well as distilled water (serving as the negative control). The impregnated discs were then placed onto the agar plates, each corresponding to its labelled quadrant.

Standard antibiotic ampicillin was utilized as the positive control. Then, the plates were inverted and incubated for 18 hours. Following the incubation period, the diameter of the clear area surrounding each disc, known as the zone of inhibition, was measured using a ruler and recorded.

Minimum Inhibitory Concentration Method

Minimum inhibitory concentration (MIC) assays were conducted against S. aureus and E. coli to assess the antimicrobial activity of the synthesised AgNPs. The sterile test tubes were prepared and labelled with AgNPs concentrations, a positive control, and a negative control. Different concentrations of AgNPs derived from *A. comosus, G. mangostana,* and *M. indica* peel extracts (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 0.125 mg/mL, and 0.0625 mg/mL) were made through the two-fold serial dilution method. A stock solution of AgNPs at 20 mg/mL was prepared.

Firstly, 5 ml of nutrient broth was added to each test tube, including the positive and negative controls. Then, to make 10 mg/mL in Tube 1, 5 ml of 20 mg/mL AgNPs stock solution was added to the test tube containing 5 ml of nutrient broth and mixed well. Next, transfer 5 ml from Tube 1 to Tube 2 and mix well to make 5 mg/mL in the second test tube. This process was repeated, transferring 5 mL from the second test tube to the third test tube with 5 mL of nutrient broth, to achieve a concentration of 2.5 mg/mL. The serial dilutions continued similarly to prepare test tubes with final concentrations of 1.25 mg/mL, and 0.625 mg/mL. In test tube 5, 5 mL of the mixture was discarded to make 5 mL as the final volume. Then, each test tube was inoculated with 1 mL of an overnight bacterial culture and incubated at 37 °C for 24 hours. The positive control contained only nutrient broth and bacteria without AgNPs, while the negative control contained nutrient broth and AgNPs without bacteria.

After incubation, each test tube was examined for bacterial growth. The presence of turbidity indicated bacterial growth, while clear solutions indicated inhibition of bacterial growth. For more precise quantification, optical density readings at 600 nm (OD600) were taken using a spectrophotometer. The MIC was corroborated by identifying the lowest concentration of AgNPs which resulted in an OD600 reading comparable to the negative control.

RESULTS AND DISCUSSION

Characterisation of Silver Nanoparticles using UV-Visible Spectroscopy

UV-visible spectroscopy is a prevalent tool for characterising nanoparticles (NPs) by encountering their surface plasmon resonance (SPR) absorption peaks, thus illustrating their formation [18]. Since the valence band and conduction band in the silver atoms absorb light energy, the electrons resonate between these two bands and give rise to the SPR band [15]. Figure 1 displays the absorbance peak of the synthesised AgNPs from three different peel extracts, measured using UV-vis spectrophotometer (Shimadzu UV-1601) within the wavelength range of 300–800 nm. The peak is measured and compared with the plasmon resonance of Ag which ranges from 391 to 460 nm [19].

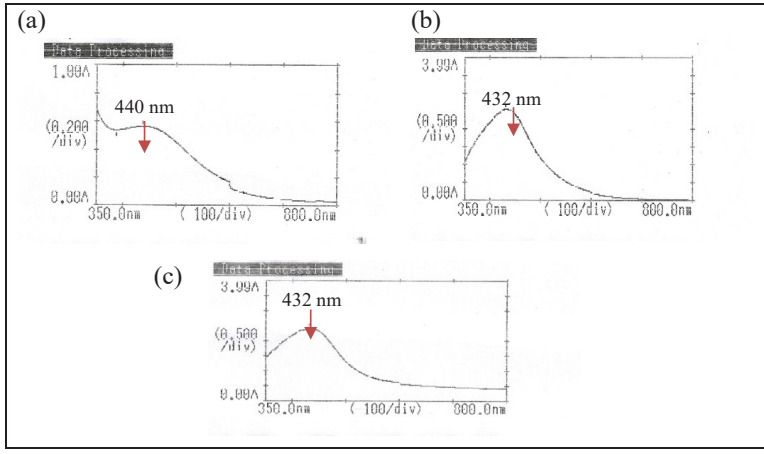

Figure 1: Absorbance peak of synthesized AgNPs derived from (a) *A. comosus*, (b) *G. mangostana* and (c) *M. indica* peel extracts.

As illustrated in Figure 1, the absorption peaks for AC-AgNPs are found at 440 nm while for GM-AgNPs, and MI-AgNPs are at 432 nm, respectively. Besides, the results also show broad peaks for all the samples. The results obtained are consistent with previous research, which found that the maximum wavelength of AC-AgNPs is 445 nm, GM-AgNPs is 430 nm and MI-AgNPs is 412 to 434 nm [20]. Furthermore, the spectrum of all

AgNPs synthesised are broad, indicating polydispersion [21]. Polydispersity could be caused by a wide range of secondary metabolites with possibly varied reduction characteristics, influencing AgNP nucleation and development [22]. As a result, the presence of absorption peaks between 432 and 440 nm indicates the synthesis of silver nanoparticles from pineapple, mangosteen, and mango peel extracts.

Characterisation of Silver Nanoparticles Using Fourier Transform Infrared (FTIR) Spectroscopy

Fourier transform infrared (FTIR) spectroscopy is utilized to analyse the biological components and their functional groups that are involved in the reduction, capping, and stabilisation of silver nanoparticles. Both the peel extract and the nanoparticles are subjected to infrared (IR) radiation through the PerkinElmer FT-IR Spectrometer Frontier within the range of 400 to 4000 cm^{-1}. Figures 2, 3 and 4 illustrate the infrared spectra of the peel aqueous extract and synthesised AgNPs. The distinctive peaks obtained are determined by comparing them with FT-IR findings from previous research that utilise green synthesis techniques for AgNPs.

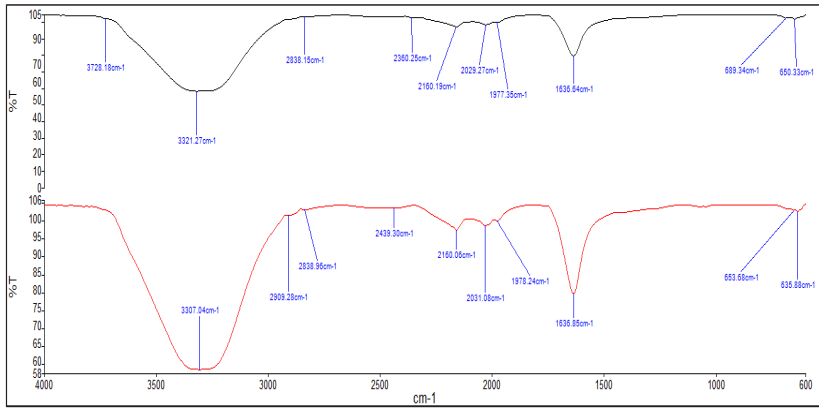

Figure 2: FTIR analysis spectra of *A. comosus* extract (red line) and green synthesized AgNPs derived from *A. comosus* peel extract (black line).

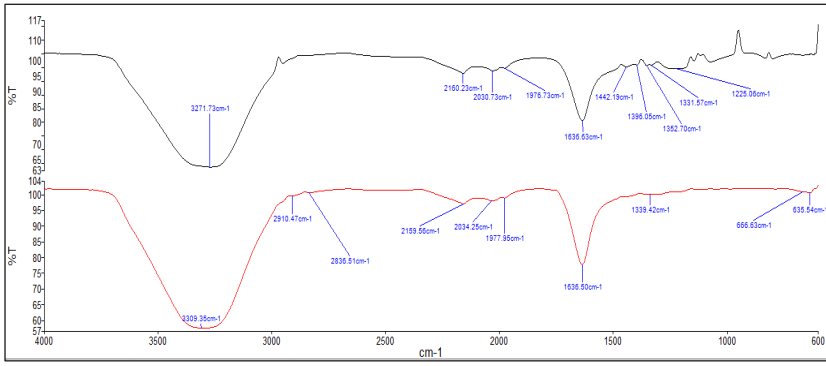

Figure 3: FTIR analysis spectra of *G.mangostana* extract (red line) and green synthesized AgNPs derived from *G. mangostana* peel extract (black line).

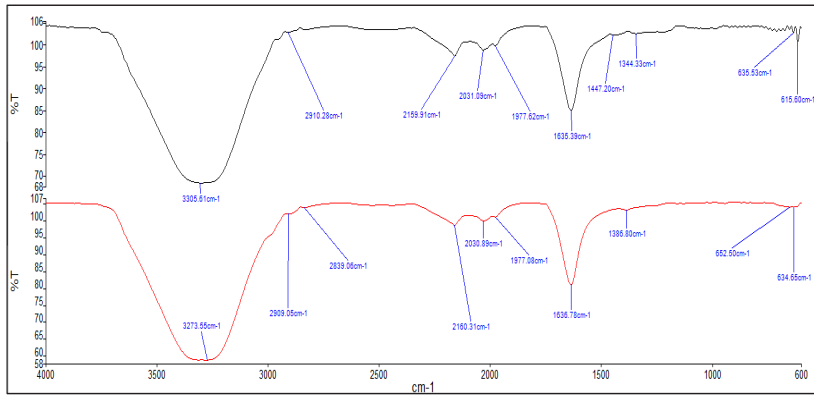

Figure 4: FTIR analysis spectra of *M. indica* extract (black line) and green synthesized AgNPs derived from *M. indica* peel extract (red line).

Figure 2 illustrates prominent frequency shifts between A. comosus aqueous extract and AC-AgNPs which 3307.04 to 3321.27 cm^{-1} for O-H stretching vibrations, 2838.96 to 2838.15 cm^{-1} for C-H stretching vibrations, and 1636.85 to 1636.64 cm^{-1} for C=O stretching vibrations. Additionally, Figure 3 displays intense peaks in *G. mangostana* extract at 3309.35 and 1636.50 cm^{-1}, corresponding to O-H and C=O stretching vibrations, shifted to 3271.73 and 1636.63 cm^{-1}, respectively, for GM-AgNPs. Meanwhile, strong peaks in *M. indica* extract are determined in Figure 4 at 3305.61, 2159.91, and 1635.39 cm^{-1}, which shifted to 3273.55, 2160.31, and 1636.78

cm^{-1}, respectively, for MI-AgNPs. The shifts from 3305.61 to 3273.55 cm^{-1} indicate the presence of O-H stretching vibrations. Peaks between 2159.91 to 2160.31 cm^{-1} and 1635.39 to 1636.78 cm^{-1} signify the presence of C≡N triple bonds and C=O stretching vibrations. Upon comparing the FT-IR spectra of AgNPs and peel aqueous extract, it is observed that certain peaks exhibited shifts, alterations in peak intensity, and the emergence of new peaks.

A prior study [5] found that alcohols and phenols have significant broad vibrations of the O-H stretch and hydrogen-bonded groups. Furthermore, C=O stretching vibrations are related to carbonyl groups, possibly from ketones or aldehydes, whereas C-H stretching vibrations are characteristic of aliphatic hydrocarbons such as terpenoids [23]. C≡C stretching vibrations indicate the existence of alkynes. The minor shift in these peaks indicates that these functional groups are involved and interact in the production of AgNPs.

According to [5], the aqueous extract of *A. comosus* contains flavonoids, phenolic acids, and various functional groups, including carboxyl, ketones, aldehydes, and carboxyl groups. These functional groups act a part in reducing silver ions to silver during the manufacture of AC-AgNPs. In addition, the G. mangostana extract contains flavonoids, xanthones, and tannins, while the M. indica extract contains flavonoids, polyphenolic compounds, and ascorbic acid. These chemicals may aid in the reduction of silver ions [17]. As an example, flavonoids consist of hydroxyl (-OH) and interact with $AgNO_3$, resulting in the dissociation of Ag^+ and NO_3^- ions in water. The acidic OH groups in phytochemicals donate H^+ ions, resulting in a negative charge. Negatively charged functional groups, such as O^- in phenols, interact electrostatically with Ag^+, reducing Ag^+ ions. NO_3^- ions accept H^+ from phenolic OH groups, resulting in HNO_3. Silver retains its metallic state (Ag^0), resulting in AgNPs [18]. Hence, it is clear that the biological components found in the peel extracts of *A. comosus, G. mangostana*, and *M. indica* serve as efficient reducing agents and capping agents, ensuring the stability of the synthesised AgNPs.

Antibacterial Activity

Disc Diffusion Method

From the results in Table 1, all 1000 mg/mL AgNPs of the sample show the largest inhibition zone against *S. aureus* and *E. coli* which is (13.1 ± 1.1 and 10.7 ± 0.6 mm) for AC-AgNPs, (9.3 ± 1.1 and 7.9 ± 0.1 mm) for GM-AgNPs, and (15.9 ± 1.7 and 9.3 ± 0.4 mm) for MI-AgNPs. On the other hand, the smaller inhibition zone is at 100 mg/ml and the smallest inhibition zone is detected at 10 mg/ml for all AgNPs. The inhibition zones of 10 mg/mL of AC-AgNPs, GM-AgNPs, and MI-AgNPs against *S. aureus* and *E. coli* are (8.0 ± 0.1 and 6.7 ± 1.5 mm), (8.0 ± 1.0 and 5.9 ± 0.8 mm), and (6.4 ± 0.7 and 4.0 ± 1.0 mm), respectively. From the results acquired, it can be suggested that the higher the AgNPs' concentration, the larger the diameter of the zone of inhibition where most of 1000 mg/mL AgNPs shows strong antibacterial activity. According to [24], antimicrobial classified as strong if inhibition zone (>10-20 mm), moderate (5-10 mm) and weak (<5 mm). Besides, from Table 2, there is a statistically significant effect of concentration and sample on the zone of inhibition (p-value <0.05).

It is consistent with previous study where [25] stated that the zone of inhibition increases linearly with amount of AgNPs. Furthermore, statistically, it shows that antibacterial activities depend on type of AgNPs and its concentration by p-value at 0.023. 1000 mg/mL MI-AgNPs exhibit better antimicrobial performance against both bacteria with a larger inhibition zone than the positive control. These findings also correspond to the quick colour changes where a greater amount of AgNPs formed leads to a larger number of NPs available to interact with and disrupt the cells. Hence, the efficacy of AgNPs derived from pineapple, mangosteen and mango peel, as antimicrobial agent increased with higher concentrations of AgNPs and 1000 mg/mL AgNPs is effective sample in inhibiting bacterial growth.

Table 1: Diameter of zone of inhibition (mm) in mean ± SE for anti-bacterial activity of silver nanoparticles

Bacteria	Sample	Zone of inhibition (mm)				
		Silver nanoparticles concentration (mg/mL)			Positive control	Negative control
		10	100	1000		
S.aureus	AC-AgNPs	8.0 ± 0.0	10 ± 0.6	13.1 ± 1.1	16.3	0.0
	GM-AgNPs	8.0 ± 0.6	9.0 ± 0.6	9.3 ± 0.6	11.0	0.0
	MI-AgNPs	6.4 ± 0.4	10.5 ± 0.3	15.9 ± 2.1	15.5	0.0
E. coli	AC-AgNPs	6.7 ± 0.9	7.0 ± 0.0	10.7 ± 0.3	7.8	0.0
	GM-AgNPs	5.9 ± 0.5	7.3± 0.3	7.9 ± 0.1	7.6	0.0
	MI-AgNPs	4.0± 1.1	7.1 ± 0.6	9.3 ± 0.3	8.1	0.0

Table 2: Two-way ANOVA test for the interaction of zone on inhibition with type of AgNPs and its concentration.

Dependent variable: Zone of inhibition

Source	Sum of square	df	Mean square	F	P-value
Concentration	61.974	2	30.987	6.442	0.018
Sample	16.855	2	8.427	1.963	0.015
Concentration*Sample	54.201	4	13.550	3.156	0.023

Besides, all the synthesised AgNPs display greater antibacterial properties against gram-positive bacteria (*S. aureus*) compared to gram-negative bacteria (*E. coli*). This is because gram-negative bacteria possess an outer membrane that serves as an additional barrier that contributes to their resistance [17]. Even though it has a thin peptidoglycan, the outer membrane consists of lipopolysaccharides, retarding the penetration of antibiotics or AgNPs into the peptidoglycan and cell. In contrast, Gram-positive bacteria lack an outer membrane and cell wall structure, which makes them extra vulnerable to certain antibacterial agents [23]. A similar observation was reported by [26] with green synthesised AgNPs

from pineapple peel extracts. Therefore, AgNPs derived from pineapple, mangosteen, and mango peel extracts, can serve as an effective antibacterial agent against both gram-positive and gram-negative bacteria.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is a crucial quantitative analysis for assessing the effectiveness of new antimicrobial compounds, including nanoparticles, against pathogenic bacteria. MIC testing involves preparing serial dilutions of the antimicrobial agent and exposing the target microorganism to these varying concentrations. This study determined the MIC of silver nanoparticles (AgNPs) produced with three different peel extracts (pineapple, mangosteen, and mango) against *S. aureus* and *E. coli.* They are tested in five different concentrations, which are 10, 5, 0.25, 0.125, and 0.0625 mg/mL and the results are compared with a negative control containing the nutrient broth and AgNPs (1 mg/mL) without bacteria. Figure 5 displays the MIC value of synthesised AgNPs against *S. aureus* and *E. coli.*

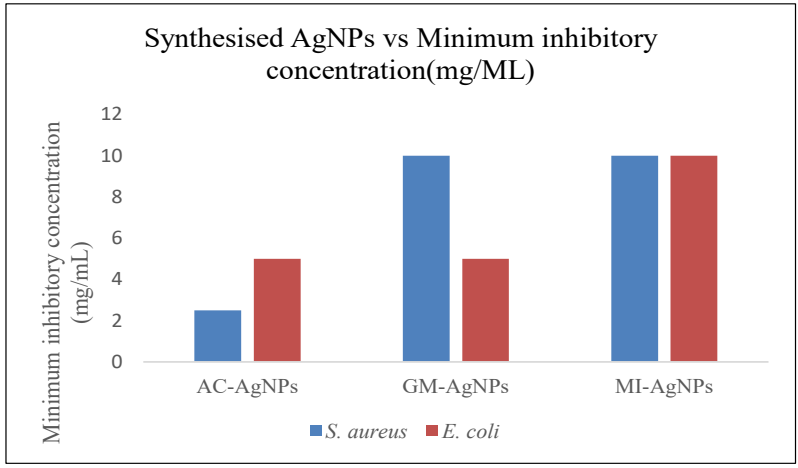

Figure 5: Minimum inhibitory concentration of three different synthesised AgNPs against *S. aureus* and *E. coli.*

As shown in Figure 5, the MIC value against *S. aureus* is detected at 2.5 mg/mL for AC-AgNPs and 10 mg/mL for both GM-AgNPs and MI-AgNPs, while turbidity is observed in the lower concentrations. Besides, the MIC value against *E. coli* is observed at 5 mg/mL for both AC-AgNPs and

GM-AgNPs. No visible bacterial growth was detected at this concentration and higher, while turbidity was observed in the lower concentrations (0.25, 0.125, and 0.0625 mg/mL). For MI-AgNPs against *E. coli*, the MIC value is determined at 10 mg/mL where clear solution observed is similar to negative control. Thus, the MIC results indicate that AC-AgNPs exhibit the highest antibacterial activity, with the lowest MIC values against both *S. aureus* and *E. coli* compared to GM-AgNPs and MI-AgNPs.

The ability to inhibit bacterial at the lowest concentration is contributed by the small size of AgNPs that increase its surface area, leading to better interactions with bacterial cells [27]. The positive-charged of AgNPs potentially bind to negatively charged bacteria's cell wall, causing them to break down. The silver ions released binds with biological components such as oxygen, sulphur, and nitrogen, modifying their structural and functional characteristics and leading to cell death [19]. Besides, the penetration of silver ions into cell membrane and attachment to it causes destabilize the membrane integrity, leading to leakage of cellular contents and eventual cell death [28]. Additionally, they induce oxidative stress by producing reactive oxygen species (ROS), which oxidise and deteriorate the lipids, DNA, and proteins, ultimately killing the bacteria [18]. Thus, AgNPs are the potent antimicrobial agents against pathogenic bacteria, specifically, *S. aureus* and *E. coli*.

CONCLUSION

In conclusion, this study advances an understanding of the green synthesis of AgNPs by utilizing the previously undiscovered bioavailable potential of waste extracts from *A. comosus, G. mangostana,* and *M. indica* as eco-friendly reducing and antibacterial agents. These findings not only demonstrate the significance of local fruit waste as a sustainable resource in nanotechnology, but also advocate for ecologically responsible nanoparticles synthesis procedures. As an additional recommendation, this application could be further emphasized in the production of biohydrogen. By harnessing sustainable techniques using plant-based silver nanoparticles, biohydrogen can contribute to a more sustainable energy future, supporting industries ranging from transportation to energy generation while minimizing carbon footprints.

ACKNOWLEDGEMENT

The authors thank Universiti Teknologi MARA, Arau Campus for providing instrumentation and laboratory to carry out the research.

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