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## ARTICLE

# A new green method for the synthesis of silver nanoparticles and their antibacterial activities against gram-positive and gram-negative bacteria

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This study aims to evaluate the capability of *Ageratum conyzoides* and *Mikania micrantha* extracts to synthesize silver nanoparticles (AgNPs) and their antibacterial capability against gram-positive and gram-negative bacteria. Several properties of the synthesized AgNPs, including plasmonic, biomolecule bonding, shape, size, and antibacterial, were investigated. Ultraviolet–visible (UV–vis) spectroscopy was employed for characterizing their plasmonic properties. Functional groups on the produced AgNPs were investigated by Fourier-transform infrared (FT-IR) spectroscopy. The size and shape of the AgNPs were identified using the field-emission scanning electron microscopy (FESEM). Inhibition zone measurement was carried out for evaluating the antibacterial capability. This study showed that the extracts of *A. conyzoides* and *M. micrantha* were able reducing agents as evidenced by the formation of the spherical AgNPs. UV–vis spectroscopy, FT-IR spectroscopy, and FESEM confirmed the physicochemical characteristics of AgNPs. AgNPs that were synthesized using *M. micrantha* were slightly smaller than those produced using *A. conyzoides*. In general, the present work establishes that the synthesized AgNPs have antibacterial capability depending on their size and synthesis procedure.

**KEYWORDS**

*Ageratum conyzoides*, *Mikania micrantha*, silver nanoparticles

## 1 | INTRODUCTION

The use of silver nanoparticles (AgNPs) has been increasing on a global scale particularly in the fields of textiles, technology, health, and food because of their remarkable features.<sup>[1,2]</sup> Among these, the health sector has been predicted

to show a significant consumption of AgNPs. AgNPs were demonstrated to show antibacterial properties against various bacteria and also to be able to show antiviral properties, especially against the HIV-1 virus.<sup>[3]</sup> In addition, AgNPs have anti-inflammatory activity, and have been tested on animals and humans. They can also be used in

cardiovascular implants, catheter devices, bone cement, bio-diagnosis, dentistry, and anticancer treatment.<sup>[4]</sup> Moreover, AgNPs have good electrical and heat conductivity so that they can be applied in electronic devices.<sup>[5]</sup>

In general, AgNPs can be synthesized using several methods, including physical, chemical, and biological.<sup>[6]</sup> It is unfortunate that chemical or physical synthesis has the potential to be dangerous to the environment.<sup>[1]</sup> Thus, new studies have been appearing on the synthesis of AgNPs using biological means (green synthesis) by employing natural ingredients. Basically, the synthesis of AgNPs needs three essential ingredients: a precursor (Ag source, usually silver nitrate [AgNO<sub>3</sub>]), a reducing agent (to reduce Ag<sup>+</sup> ions to Ag<sup>0</sup>), and a stabilizing agent (to avoid agglomeration). For biological synthesis, it is necessary to employ natural materials such as bacteria, fungi, algae, plants, or yeast. This method of synthesis offers many advantages such as low cost since the reducing and stabilizing agents can be obtained freely. Also, biological synthesis can be carried out at room temperature and quickly.<sup>[5]</sup>

A common precursor used to synthesize AgNPs is AgNO<sub>3</sub>. When dissolved in water, AgNO<sub>3</sub> forms ions of Ag<sup>+</sup> and NO<sub>3</sub><sup>-</sup>. Furthermore, the role of a plant extract as a reducing and stabilizing agent begins when the plant extract is mixed with AgNO<sub>3</sub> solution. The reducing agent is responsible for reducing Ag<sup>+</sup> ions from AgNO<sub>3</sub> fractions to Ag<sup>0</sup> particles. In addition, the stabilizing agent is employed to stabilize the produced AgNPs to avoid agglomeration. In general, plants containing flavonoids and terpenoids can be used for this synthesis. For instance, the main terpenoid, eugenol, present in *Cinnamomum zeylanisum* extracts was found to have the capability to act as a bioreduction agent of gold(III) chloride trihydrate (HAuCl<sub>4</sub>) and AgNO<sub>3</sub>, forming nanoparticles.<sup>[7]</sup> The flavonoid transforms from the enol form to the keto form, which can release a reactive hydrogen atom that is able to reduce metal ions to form nanoparticles.<sup>[8]</sup>

Synthesis procedures for producing AgNPs using roots, seeds, fruits, and leaves are well established. However, there is very little information available in the literature regarding the exploration of the capability of weed extracts for synthesizing AgNPs. In addition, the large use of weeds may also support the environment, particularly in agriculture, since they can be linked with the negative impact by monopolizing resources.

Aligning with the aforementioned research necessity, in this study we explored the capability of the weeds *Ageratum conyzoides* and *Mikania micrantha* to synthesize AgNPs. These weeds are commonly found in Indonesia and Malaysia. They basically contain flavonoids and terpenoids.<sup>[9]</sup> In addition, *M. micrantha* contains phenolic compounds.<sup>[10]</sup> Moreover, we investigate the capability of the produced AgNPs to inhibit gram-negative (*Escherichia coli*) and gram-positive (*Bacillus cereus*) bacteria.

## 2 | EXPERIMENTAL

### 2.1 | Materials

*M. micrantha* and *A. conyzoides* were obtained from the area surrounding the Universiti Teknologi Malaysia (UTM), Johor, Malaysia. AgNO<sub>3</sub> and nutrient agar (NA) were supplied by the Sigma-Aldrich. The bacteria *B. cereus* and *E. coli* were provided by the Centre for Environmental Sustainability and Water Security (IPASA), UTM.

### 2.2 | Extraction procedure for *A. conyzoides* and *M. micrantha*

*A. conyzoides* leaves (15 g) were washed in a 500-mL glass beaker using tap water three times and then by the ultrapure water three times. Furthermore, 200 mL of the ultrapure water was poured into the glass beaker containing the washed leaves and then boiled on a hot plate at 250°C for 30 min. It was then cooled to room temperature and filtered using a nylon filter. The obtained leaf extract was stored at 7°C for further use. The same procedure was applied for *M. micrantha*.

### 2.3 | Synthesis of AgNPs

For *A. conyzoides*, 0.5 g of AgNO<sub>3</sub> powder was taken in a 250-mL Erlenmeyer flask and dissolved in 100 mL of ultrapure water. One hundred milliliters of the leaf extract was mixed into the AgNO<sub>3</sub> solution slowly. Next, the solution was stirred using a magnetic stirrer at 50 rpm for 24 hr.

For purification, the AgNP solution was transferred to a 50-mL centrifuge tube and then centrifuged at 6,000 rpm for 20 min. The pellets obtained from the centrifugation process were washed using ultrapure water and re-centrifuged. The obtained pellet was dried at 80°C for further characterization. The same procedure was carried out for *M. micrantha*.

### 2.4 | Plasmonic investigation

Ultraviolet-visible (UV-vis) spectroscopy was used to characterize the plasmonic properties of AgNPs using a UV-vis spectrometer (Perkin-Elmer, No. 101N4110104). For this, 2 mL of the AgNP solution was injected into the UV-vis sample tube. The results of UV-vis data were processed using the Lambda 25 software. This measurement was carried out at a resolution of 1 nm and a scan speed of 960 nm/min and in the wavelength range 300–700 nm.

### 2.5 | FT-IR characterization

For the Fourier-transform infrared spectroscopy (FT-IR) characterization, AgNP<sub>5</sub> were mixed with potassium bromide in the ratio 1:100 to produce AgNP pellets. Next, the pellets were pressed hydraulically using 10 tons pressure. The pressed pellets were then placed on the spectrometer

holder. The functional groups of the synthesized AgNPs were identified from their spectra recorded by a spectrometer (PerkinElmer Frontier-GPOB model 96046). This characterization was carried out over the spectral range  $650\text{--}4,000\text{ cm}^{-1}$  at a resolution of  $4\text{ cm}^{-1}$  and with the accumulation of 10 scans at room temperature.

## 2.6 | Field-emission scanning electron microscopy and energy dispersive X-ray spectroscopy characterization

Morphology of the AgNPs was studied using a field-emission scanning electron microscopy (FESEM, Zeiss Supra 35VP). The equipment was operated at 5 kV acceleration voltage with a magnification of  $\times 50,000$ . The AgNPs were also characterized using SEM with an energy dispersive X-ray spectroscopy attachment (SEM-EDX, Hitachi S-3400N) to determine the elemental composition of the produced nanoparticles. It was operated at a voltage of 15 kV and equipped with the Bruker Quantax software.

## 2.7 | Antibacterial investigation

A filter paper was cut into disks of 1.1 mm diameter. Then, 0.05 g of  $\text{AgNO}_3$  was dissolved in 10 mL of ultrapure water and 10 mL of the leaf extract. Further, the filter paper disks were immersed in the AgNP solution.

In the present study, *B. cereus* and *E. coli* were used for the antibacterial investigation. Into a Petri dish containing NA, 0.1 mL *B. cereus* was poured. Three disks soaked in the AgNP solution were placed on the agar surface. The agar plate containing the bacteria was then stored in an incubator for 24 hr to determine the inhibition zone by measuring the clear area surrounding the filter paper containing the AgNP solution deposited on the plate.

## 3 | RESULTS AND DISCUSSION

### 3.1 | UV-vis characteristics

Figure 1 shows the UV-vis spectra of the synthesized AgNPs using *A. conyzoides* and *M. micrantha* extracts. It can be seen that the AgNPs synthesized using *A. conyzoides* extract has an absorbance peak at  $\sim 540\text{ nm}$ , as shown in Figure 1a. In addition, an absorbance peak at  $\sim 481\text{ nm}$  can be found for the AgNPs synthesized using *M. micrantha* extract (Figure 1b).

It is well known that the UV-vis spectra can be used to estimate the number of AgNPs produced during synthesis. Higher production of AgNPs is generally indicated by an increase in the absorbance. This is because the absorbance is the amount of light absorbed by the particles. Using this logic, the spectrum of AgNPs synthesized using *A. conyzoides* suggests a higher production of AgNPs compared those produced using *M. micrantha*.

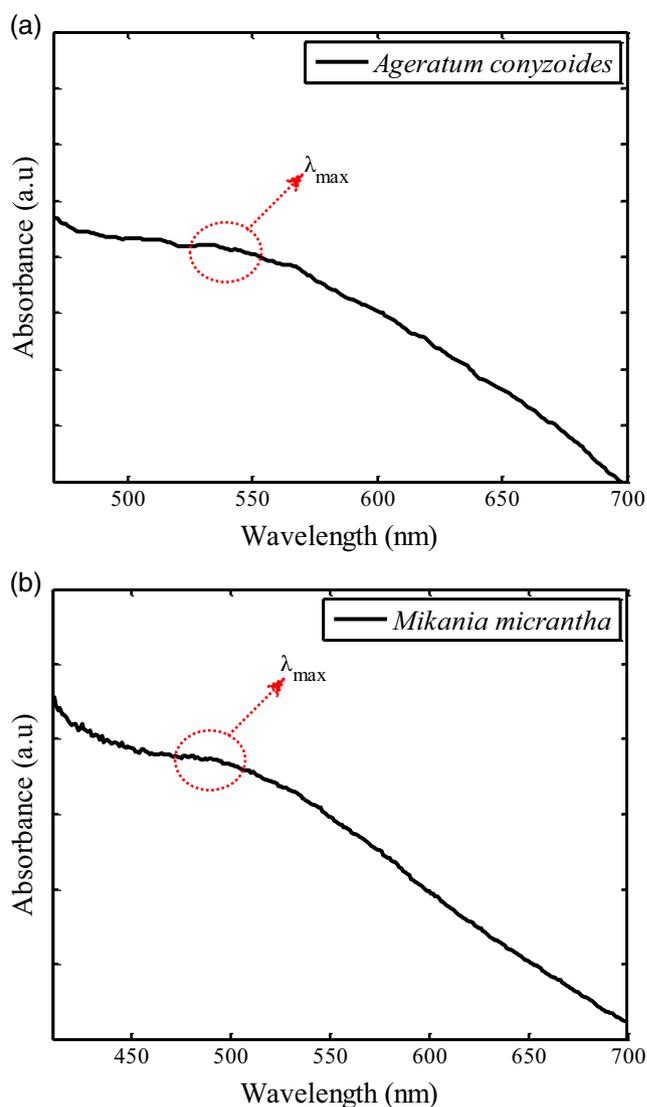


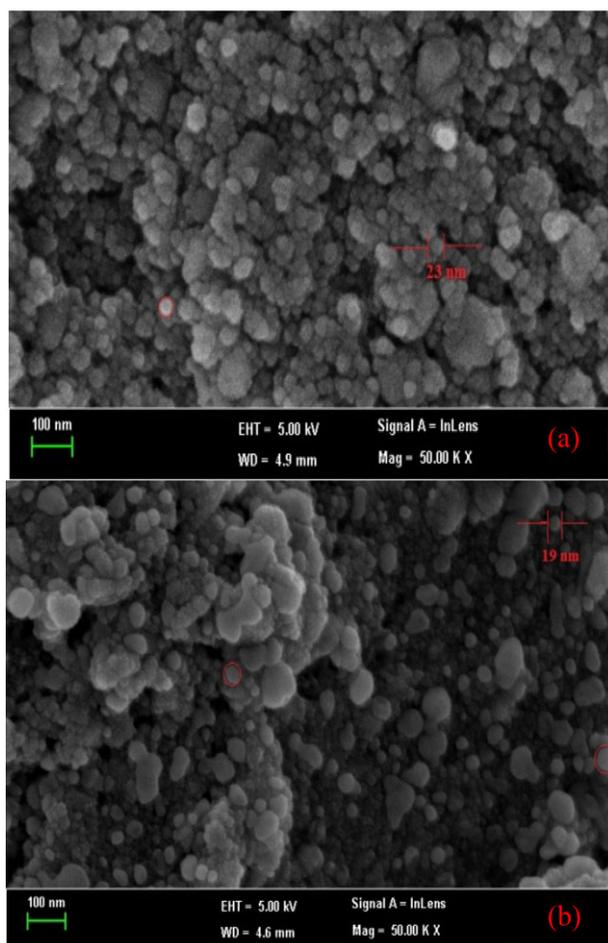
FIGURE 1 UV-vis spectra of the synthesized AgNPs using (a) *Ageratum conyzoides* L. and (b) *Mikania micrantha*

It is well established that the plasmonic properties of AgNPs are strongly affected by their size and the surrounding media.<sup>[11]</sup> In general, the results of this study are similar to those previous studies that also investigated the UV-vis spectra of AgNPs in the range  $450\text{--}500\text{ nm}$ .<sup>[11]</sup> UV-vis spectroscopy is a commonly used method to study the absorption in the ultraviolet and visible spectral regions. It is widely employed to characterize the plasmonic properties of AgNPs. Several studies have confirmed the correlation between the UV-vis spectrum and properties of AgNPs. A single peak in the UV-vis spectrum can be correlated with uniform, spherical AgNPs.<sup>[12]</sup> Studies have also confirmed that AgNPs of irregular shapes show two or more peaks in the UV-vis spectrum depending on their symmetry. In addition, the larger the AgNPs, the higher the wavelength of maximum absorbance.<sup>[13]</sup> Moreover, an increase in the absorbance can be associated with a higher production of the synthesized AgNPs.<sup>[14]</sup>

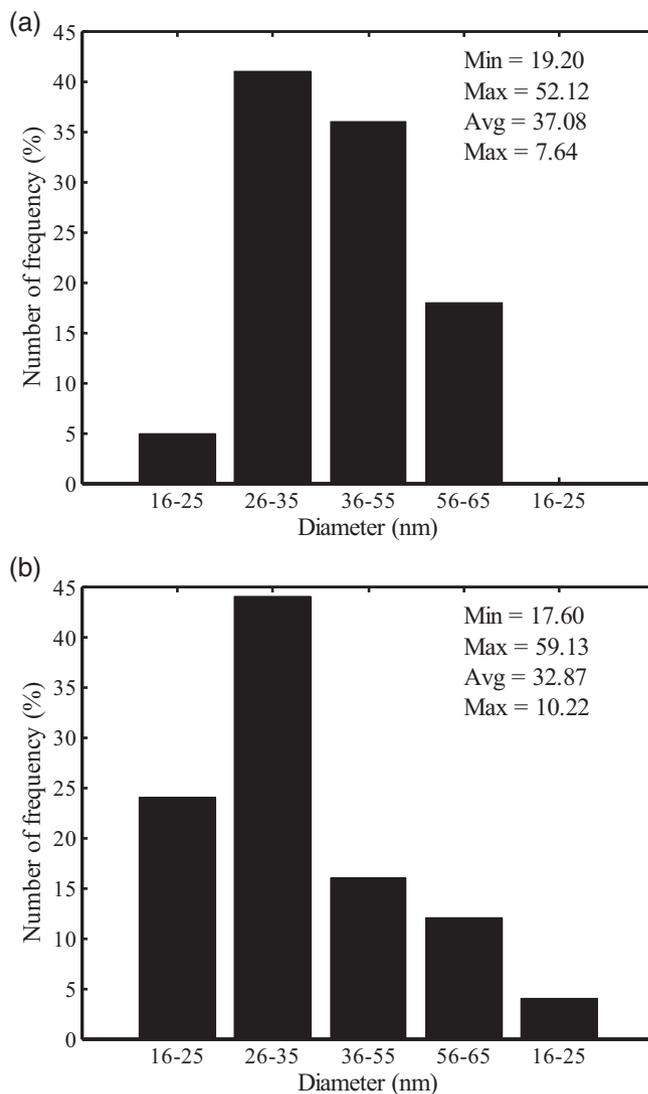
### 3.2 | Morphology of AgNPs

The size of AgNPs was estimated by randomly measuring selected samples from the FESEM data following the procedure established elsewhere.<sup>[5,15,16]</sup> The FESEM images of AgNPs synthesized using *A. conyzoides* and *M. micrantha* are shown in Figure 2. This study shows that the synthesized AgNPs using *M. micrantha* extracts have a wider particle distribution range (17–59 nm) (see Figure 3a) compared to those synthesized using *A. conyzoides* (19–52 nm) (Figure 3b). In general, the AgNPs synthesized using *A. conyzoides* have a larger size of  $37.0 \pm 7.6$  nm compared to those using the *M. micrantha* extract, which is  $33.0 \pm 10.2$  nm.

These findings are in line with the UV–vis spectra as shown in Figure 1. It is seen from Figure 1 that the AgNPs synthesized using *A. conyzoides* have a maximum in the UV–vis spectra at 540 nm. In addition, the synthesized AgNPs using *M. micrantha* have a maximum at 481 nm. From the UV–vis spectra, it can be concluded that the size of the AgNPs synthesized using the *A. conyzoides* extract was larger than that of NPs made using the *M. micrantha* extract. A previous study found that increasing the size of spherical nanoparticles from 8 to 99 nm increased their peak absorption from 517 to 575 nm.<sup>[13]</sup>



**FIGURE 2** FESEM images for AgNPs synthesized using (a) *Ageratum conyzoides* and (b) *Mikania micrantha*



**FIGURE 3** Size distribution of AgNPs synthesized using (a) *Ageratum conyzoides* and (b) *Mikania micrantha*

Several studies have reported that AgNPs ranging in the range 3–1,000 nm can be fabricated using green synthesis. For instance, AgNPs ranging from 15 to 89 nm could be produced when they were synthesized using *Ficus carica*.<sup>[17]</sup> In addition, AgNPs of ~70 nm could be produced when they were synthesized using *Calliandra haematocephala* extracts.<sup>[18]</sup> Spherical AgNPs ranging from 20 to 30 nm were obtained using *Acalypha indica*.<sup>[19]</sup> An alternative study by employing *Calotropis procera* was able to produce AgNPs of diameter ranging from 150 to 1,000 nm.<sup>[20]</sup> AgNPs in the range 3–6, 3–22, and 3–18 nm could be produced by employing *Allium sativum*, *Zingiber officinale* Rosc., and *Capsicum frutescens*, respectively.<sup>[21]</sup> Table 1 summarizes several plant extracts used for synthesizing AgNPs.

### 3.3 | EDX of the synthesized AgNPs

Figure 4 shows the EDX results of the AgNPs synthesized using *A. conyzoides* and *M. micrantha*. The graphs show the highest energy peak at 3 keV, suggesting the presence of

TABLE 1 Synthesis of AgNPs using several plant extracts

Plant extracts	Size (nm)	Shape	Ref.
<i>Acalypha indica</i>	20–30	Spherical	19
<i>Calotropis procera</i>	150–1,000	—	20
<i>Allium sativum</i>	3–6	Spherical	21
<i>Zingiber officinale</i> Rosc.	3–22	Spherical	
<i>Capsicum frutescens</i>	3–18	Spherical	

silver. This study found the silver atom content of 81.37% and 89.61% for the AgNPs synthesized by *A. conyzoides* and *M. micrantha*, respectively, as listed in Table 2. The table indicates that the samples have higher silver content compared to other detected elements such as oxygen, carbon, and chlorine. Other elements detected in the EDX characterization possibly came from the *A. conyzoides* and *M. micrantha* extracts.

Similar results were obtained in a previous study, with the highest peak observed at 3 keV when AgNPs were synthesized using *Carica papaya*, *Manihot esculenta*, and

*Morinda citrifolia*.<sup>[5]</sup> The presence of an energy peak at 3 keV, which is characteristic of silver atoms, is due to the resonance of silver, which has an energy level equal to 2.984 or 3 keV.<sup>[22]</sup>

### 3.4 | FT-IR characteristics

The FT-IR spectra of the synthesized AgNPs are presented in Table 3. The spectra were used to determine the functional groups of AgNPs. The spectra of AgNPs synthesized by *A. conyzoides* and *M. micrantha* extracts have peaks at 2,960, 2,330, 1,612, and 1,180  $\text{cm}^{-1}$ . The peak at 2,960  $\text{cm}^{-1}$  is possibly due to the C–H stretching, which is a common feature of aliphatic compounds. In addition, the peak at 2,960  $\text{cm}^{-1}$  indicates the presence of functional groups of C=C belonging to the aromatic component. Both aliphatic and aromatic components are present in flavonoid compounds in *A. conyzoides* and *M. micrantha*.

The peak at 1,180  $\text{cm}^{-1}$  can be related to C–O–C or C–OH, which are components of ether or alcohol. The peak at

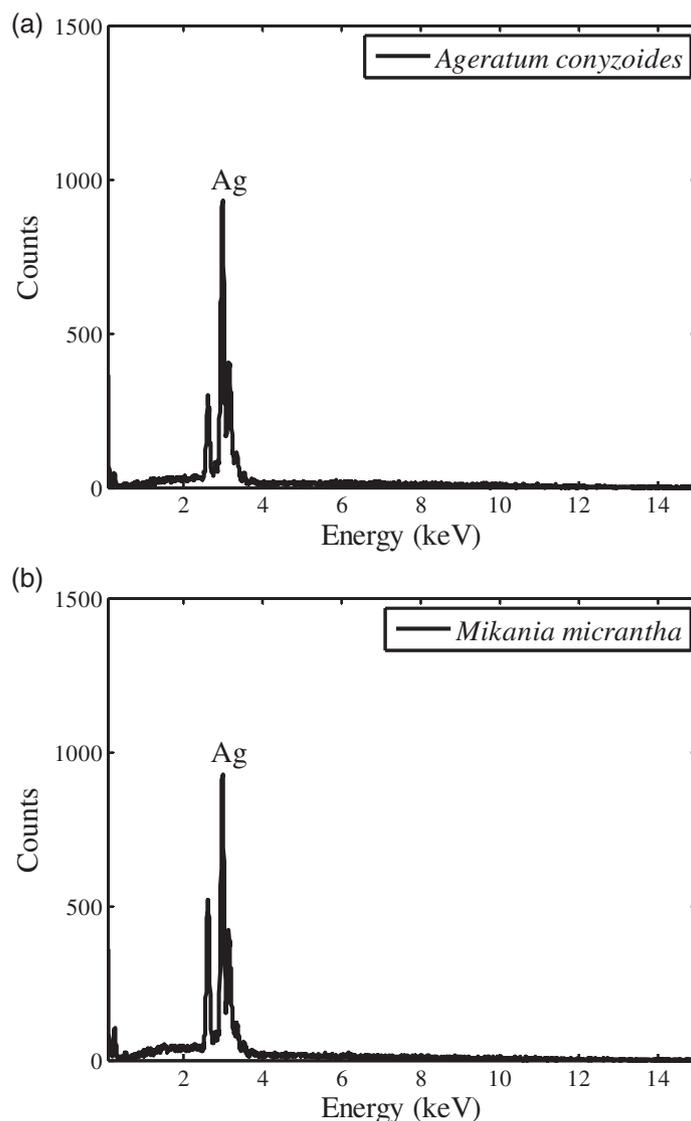


FIGURE 4 EDX spectra of AgNPs synthesized using (a) *Ageratum conyzoides* and (b) *Mikania micrantha*

**TABLE 2** Elemental analysis of AgNPs synthesized using the proposed plant extracts

Plant extract	Element	Mass (%)
<i>Ageratum conyzoides</i>	Silver	81.37
	Chlorine	11.95
	Carbon	5.31
	Oxygen	1.37
<i>Mikania micrantha</i>	Silver	89.61
	Chlorine	6.92
	Carbon	2.69
	Oxygen	0.78

**TABLE 3** FT-IR analysis of AgNPs synthesized using the proposed plant extracts

Plant extract	FT-IR peak (cm <sup>-1</sup> )	Possible assignment
<i>Ageratum conyzoides</i>	1,180	C–O–C
	1,612	C=O
	2,330	–C≡N
	2,960	–CH
<i>Mikania micrantha</i>	1,180	C–O–C
	1,612	C=O
	2,330	–C≡N
	2,960	–CH

1,612 cm<sup>-1</sup> can be assigned to C=O stretching. In addition, the peak at 2,330 cm<sup>-1</sup> can be associated with the nitrile components with the –C≡N functional group. Nitrile is one component of aglikon compound that has antioxidant properties.

Functional groups of AgNPs depend highly on the reducing agents, stabilizing agents, and synthesis procedures. For instance, peaks at 3,548, 3,487, and 3,379 cm<sup>-1</sup> belong to –CH (alkane), that at 1,720 cm<sup>-1</sup> belongs to C=O (ketone), the peak at 825 cm<sup>-1</sup> belongs to C–H, the band at 1,218 to the phenyl ring, and the peak at 1,049 cm<sup>-1</sup> to C–O or C–N (amino), as also reported in previous work.<sup>[18]</sup> The use of *Ficus carica* extract resulted in peaks at 1,625 cm<sup>-1</sup> of N–H and 1,422–384 cm<sup>-1</sup> of C=C.<sup>[17]</sup>

### 3.5 | Antibacterial investigation

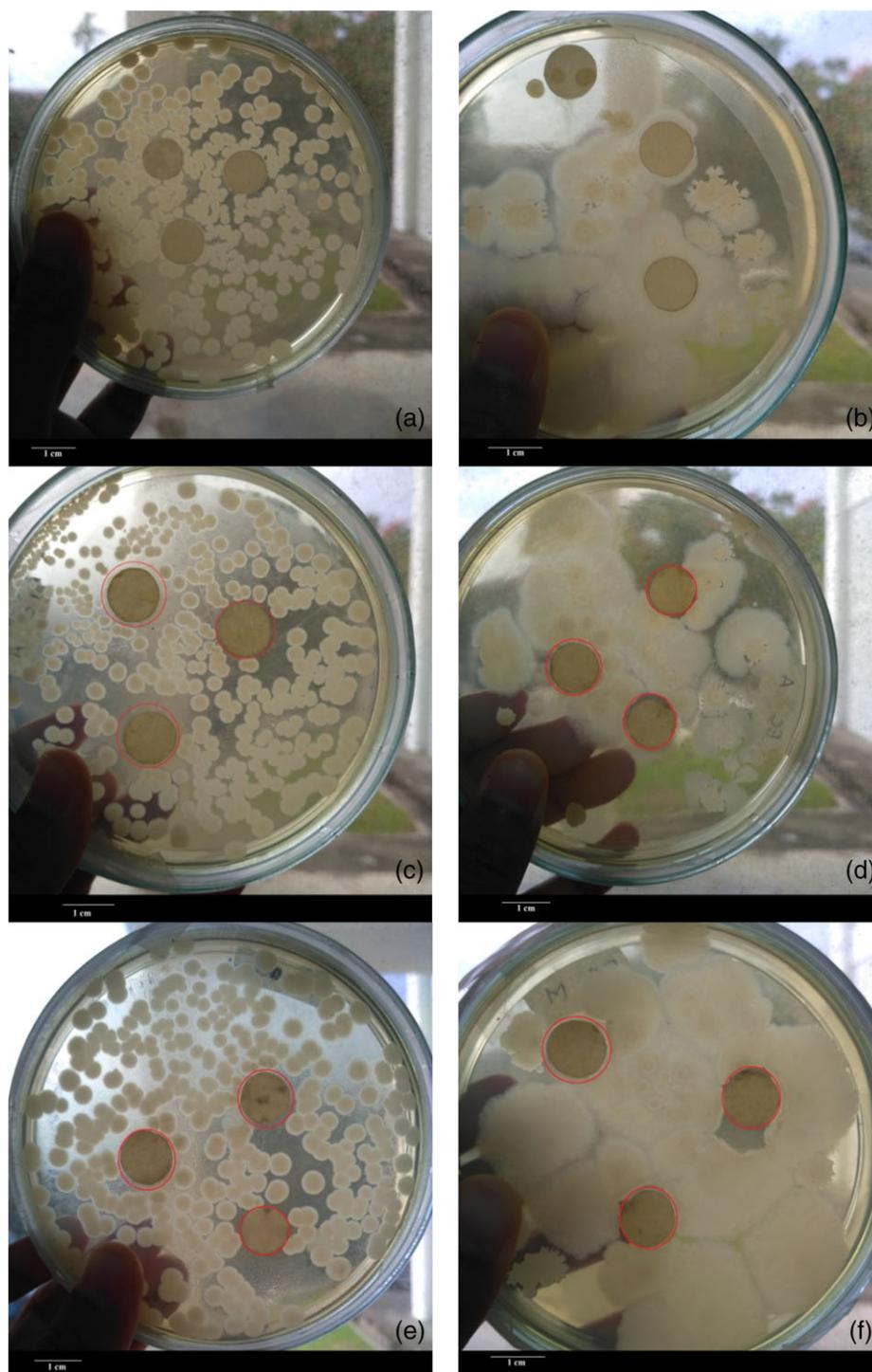
The synthesized AgNPs were tested against *B. cereus* and *E. coli*, and the results are presented in Figure 5. It was observed that AgNPs have antibacterial ability with the formation of the inhibition zone as indicated by the red circle in Figure 5. AgNPs synthesized using *A. conyzoides* have an average zone of inhibition of 1.33 ± 0.06 mm against *B. cereus* and 1.27 ± 0.06 mm against *E. coli*. In addition, the AgNPs synthesized using *M. micrantha* show an average inhibition zone of 1.2 ± 0.00 mm for *B. cereus* and 1.3 ± 0.5 mm for *E. coli*.

In general, the synthesized AgNPs demonstrated antibacterial ability for both gram-positive and gram-negative bacteria. Findings of this study are in line with those reported in the literature. For instance, AgNPs synthesized using *Talinum triangulare* exhibited inhibition zones of 2.35 ± 0.35, 2.55 ± 0.64, and 1.65 ± 0.07 mm against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*, respectively.<sup>[23]</sup> Also, inhibition zones of 6.50 ± 0.40 and 8.90 ± 0.60 were observed when AgNPs were synthesized using *Zingiber officinale*,<sup>[24]</sup> and those synthesized using *Melastoma malabathricum* inhibited the growth of all employed bacteria ranging from 1.17 ± 0.50 to 1.60 ± 0.10 mm and from 1.03 ± 0.06 to 1.60 ± 0.10 mm when they were synthesized using *Clidemia hirta*.<sup>[16]</sup> Table 4 lists a comparison of the zones of inhibition of AgNPs synthesized by green procedures against several bacteria. It can be observed from the table that the zone of inhibition of AgNPs obtained from this study has slightly lower values than those of AgNPs synthesized using *T. triangulare* or *Z. officinale*. However, a similar inhibition zone obtained in this work was found when AgNPs were synthesized using *M. malabathricum* or *C. hirta*. It is well established that the antibacterial capability of AgNPs depends highly on the size, species, shape, and the synthetic procedure as well as the bacteria types. For instance, the reduction of AgNP size from 100 to 5 nm can enhance their antibacterial properties.<sup>[25]</sup>

### 3.6 | Antibacterial mechanism

Findings from this work show that the produced AgNPs have antibacterial properties against both the tested bacteria. In this context, there are four well-defined mechanisms related to the antibacterial properties of AgNPs: (a) AgNP adhesion onto bacterial cell wall and membrane surface; (b) penetration of AgNPs into the bacterial cell and the resulting damage to intracellular structures and biomolecules, (c) generation of reactive oxygen species (ROS) and free radicals, and (d) modulation of signal transduction pathways.<sup>[26]</sup>

In the first mechanism, the charge of AgNPs and bacterial cell membrane is crucial for the process. For instance, the positive charge of AgNPs interacts with the negative charge of the cell membrane by electrostatic force, resulting in AgNPs' attachment to cell membranes and finally leading to rupture of the cell wall.<sup>[27]</sup> In the second mechanism, AgNPs are expected to penetrate into the bacterial cells and possibly interact with cellular structures and biomolecules such as proteins, lipids, and DNA, thereby facilitating their deactivation.<sup>[28]</sup> In addition, AgNPs are well known to have the ability to produce ROS and free-radical species such as hydrogen peroxide, superoxide anion, hydroxyl radical, hypochlorous acid, and singlet oxygen, enhancing the oxidative stress in cells.<sup>[29]</sup> In the fourth mechanism, AgNPs can modulate cellular signaling by dephosphorylating tyrosine



**FIGURE 5** Zone of inhibition of (a) without AgNPs against *Bacillus cereus*, (b) without AgNPs against *Escherichia coli*, (c) AgNPs synthesized using *Ageratum conyzoides* against *Bacillus cereus*, (d) AgNPs synthesized using *Ageratum conyzoides* against *Escherichia coli*, (e) AgNPs synthesized using *Mikania micrantha* against *Bacillus cereus*, and (f) AgNPs synthesized using *Mikania micrantha* against *Escherichia coli*. The zone of inhibition is indicated by the red circle

residues on key bacterial peptide substrates, resulting in the inhibition of bacterial growth.<sup>[30]</sup>

Since bacteria such as *B. cereus* and *E. coli* depend highly on an enzyme for metabolizing oxygen to maintain their activity, the presence of AgNPs can interfere with the effectiveness of the enzyme and disable the uptake of oxygen, resulting in the death of the bacteria. Moreover, it is also

found that *A. conyzoides* and *M. micrantha* have antioxidant features. Antioxidants are known to have antimicrobial properties.<sup>[31]</sup> Following this logic, since the AgNPs were synthesized using *A. conyzoides* and *M. micrantha*, it is possible that their surface properties also affect the bacterial growth, as reported in the previous work which also found that antioxidant compounds inhibited the growth of *S. aureus*.<sup>[31]</sup>

TABLE 4 Inhibition zone of AgNPs synthesized using green approaches

Plants extract	Bacteria	Inhibition zone (mm)	Ref.
<i>Talinum triangulare</i>	<i>Staphylococcus aureus</i>	2.35 ± 0.35	23
	<i>Escherichia coli</i>	2.55 ± 0.64	
	<i>Candida albicans</i>	1.65 ± 0.07	
<i>Zingiber officinale</i>	<i>Staphylococcus</i> spp.	6.5 ± 0.4	24
	<i>Listeria</i> spp.	8.9 ± 0.6	
	<i>Bacillus</i> spp.	None	
<i>Ageratum conyzoides</i>	<i>Bacillus cereus</i>	1.33 ± 0.06	This study
	<i>Escherichia coli</i>	1.27 ± 0.06	
<i>Mikania micrantha</i>	<i>Bacillus cereus</i>	1.2 ± 0.00	
	<i>Escherichia coli</i>	1.3 ± 0.50	

#### 4 | CONCLUSIONS

The aim of this work was to evaluate the capability of *A. conyzoides* and *M. micrantha* extracts to synthesize AgNPs and their antibacterial capability against gram-positive and gram-negative bacteria. It was shown that the extracts were good natural reducing and stabilizing agents, as evidenced by the formation of the spherical AgNPs. The size of AgNPs synthesized using *A. conyzoides* extracts was  $37.0 \pm 7.6$  nm, whereas those synthesized using *M. micrantha* extracts had an average size of  $33.0 \pm 10.2$  nm, suggesting that smaller size can be produced by employing these extracts. Moreover, AgNPs synthesized using *A. conyzoides* and *M. micrantha* extracts act as a good antibacterial agents against the gram-positive and gram-negative bacteria models, as evidenced by the inhibition zone observation.

This study enhances the knowledge on AgNP biosynthesis without the use of any chemicals still achieving good result in terms of the NP sizes, which are less than 50 nm. For further improvements, an evaluation of the produced AgNPs on their effects against other organisms is needed, as findings of this study are essential for medical applications. In addition, it is also useful to validate the effects of physico-chemical parameters, including the AgNP concentration effects, on their antibacterial properties.

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