

Green synthesis and antibacterial potential of artemisia vulgaris extract in silver nanoparticles against wound bacteria

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Abstract

Background: *Artemisia vulgaris* (*A. vulgaris*), a well-known Chinese traditional herb, is reported to have antibacterial properties, making it a potential agent for wound healing. In our project, we have developed *A. vulgaris* in silver nanoparticles to enhance its effect. This study investigated the antibacterial effects of the synthesised AgNP on common wound bacteria.

Objectives: The objectives of this study were to synthesise *A. vulgaris* in silver nanoparticles and to investigate the anti-bacterial effect on wound bacteria.

Methods: The AgNP was synthesised by the green synthesis method and characterisation tests were carried out to confirm the presence of AgNP in the formulation. The disc diffusion test, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) tests were carried out to investigate the antibacterial effects of AgNP on common wound bacteria. The AgNP was also tested on probiotics using the disc diffusion test to investigate its effect on probiotics.

Results: The characterisation tests have confirmed the presence of AgNP in the formulation. The AgNP containing all plant concentrations were able to inhibit the growth of all bacteria tested but it required a higher concentration to inhibit the gram positive bacteria. The AgNP had less inhibitory effects on probiotics compared to antibiotics and silver nitrate alone. However, statistical analysis showed that the antibacterial effect of the treatment was statistically insignificant.

Conclusion: The AgNP demonstrated anti-bacterial effects on both gram positive and gram negative wound bacteria, but the effect of the treatment was not statistically significant.

Keywords: *Artemisia vulgaris*, silver nanoparticles, antibacterial, wound bacteria

1. Introduction

Wound infection affects cuts, burns, surgical wounds, and diabetic wounds when pathogens invade through the wound opening, resulting in poor wound healing, gangrene, disability, sepsis, and death (Efron & Barbul, 2001). Normal skin flora may become pathogens that invade wound openings when given the right circumstances. Some of these bacteria have

developed resistance towards the standard antibiotic treatment (Stapleton & Taylor 2007). Hence, the development of new antibacterial agents is important to overcome such resistance. *Artemisia vulgaris*, a Chinese traditional herb, is one of the plants which has a potential antibacterial effect. Commonly known as mugwort, it is often used as a traditional remedy or culinary herbs. It is much favoured by the Chinese because it is claimed to have numerous health benefits. It has produced positive results in previous antimicrobial and antioxidant studies (Temraz & El-Tantaway, 2008; Manindra, M. et al, 2016) It is also traditionally used to treat various types of health ailments, such as bleeding, menstrual problems, and skin problems (Fetrow & Avila, 2004).

Herbal agents such as *A. vulgaris* face challenges in terms of drug delivery and bioavailability due to their poor stability and rapid elimination. The extract of *A. vulgaris* can be incorporated into a novel delivery system such as silver nanoparticles to enhance its antibacterial effects while providing a safer alternative for the treatment of wound infection. Silver nanoparticles have been used as a carrier for many herbal agents and shown to have synergistic effects (Aparna, et al, 2015; Orsuwan, et al, 2017). Therefore, in this study, silver nanoparticles containing *A. vulgaris* extracts (AgNP) were tested on common wound bacteria to determine its effectiveness as an antibacterial agent.

2. Methods

2.1. Chemicals and reagents

Silver nitrate was obtained from ACROS Organics™ and used at a concentration of 0.1M to formulate silver nanoparticles. Mueller-Hinton Media was procured from Sigma-Aldrich.

2.2. Preparation of plant extracts

The leaves of *A. vulgaris* were collected from a private garden in Kota Kinabalu, Sabah. The herbarium specimen was then sent to the Sabah Forestry Department to be validated by the Forest Research Centre. Around 500g of leaves collected were dried and ground into coarse powder form. The coarse leaf powder was boiled in 100mL of distilled water at 80°C for 3 hours to prepare the aqueous plant extracts in 5%, 10% and 15% w/v concentrations. The extracts were then filtered with common filter paper. (Ahmed, et al, 2016)

2.3. Green synthesis of silver nanoparticles

To synthesise the plant extract into silver nanoparticles, the filtered extracts were mixed with 50mM silver nitrate solution at a ratio of 1:9 v/v (Erjaee, et al, 2017). The 50mM silver nitrate solution was prepared earlier by diluting 0.1N Acros Organics™ Silver nitrate. The mixture of plant extract and silver nitrate was incubated at a room temperature under the stirring condition for 18 hours. The solution was then centrifuged at 13000rpm for 20 minutes

to separate the nanoparticles from the solution. The nanoparticles obtained were washed with distilled water to remove any unwanted materials.

2.3. Characterisation of silver nanoparticles

The UV-visible absorption of the silver nanoparticles was determined in quartz cuvette using the Perkin Elmer spectrometer. The wavelength range was taken from 300 to 800nm. FTIR spectroscopy was obtained using the ATR method and conducted at a room temperature under dry air. The wave range was set to 4000-400 cm^{-1} (Uznanski, et al, 2017). The particle size of silver nanoparticles was analysed using the Malvern Zetasizer Nano Instrument to determine the particle size distribution and surface charge. A high-resolution transmission electron microscope (Hitachi HT 7700) was employed to analyse the surface morphology and size of silver nanoparticles.

2.4. Investigation of antibacterial effects of AgNP on wound bacteria

The antibacterial tests were performed on common wound bacteria, which included *K. pneumonia*, *P. aeruginosa*, *E. coli*, *B. cereus*, *S. aureus*, and two strains of MRSA.

2.4.1. Disc diffusion test

Muller Hinton Agar (MHA) medium was prepared and the bacterial culture of 0.5 McFarland standard was spread thoroughly on the agar plates. The silver nanoparticles containing 5%, 10% and 15% w/v of plant extract were made into a solution and added into the wells made on the agar plates. Antibiotic discs were used as the positive control whereas 50mM silver nitrate was used as the negative control. The plates were incubated overnight at 37°C. The diameter of inhibition zone was indicative of the inhibitory effect of silver nanoparticles on the growth of the bacteria.

2.4.2. Minimum Inhibitory Concentration (MIC) test

The culture medium, bacterial suspension, and formulation samples with plant extract concentration ranging from 0.125mg/mL to 4mg/mL were added into a 96-well plate and then incubated overnight at 37°C. Dyes were added into each well to analyse the results. The MIC is the lowest concentration where bacterial growth is inhibited by 50%.

2.4.3. Minimum Bactericidal Concentration (MBC) test

Using the samples from MIC test, 5 μl of sample was taken from each well and added onto the agar plate. The plates were then incubated at 37°C overnight. The lowest concentration with no bacterial growth observed on the plates was considered the MBC.

2.5. Investigation of antibacterial effects of AgNP on probiotics

The disc diffusion test was carried out on probiotics, namely *L. casei*, *L. rhamnosus*, *L. arabinosus*, and *L. acidophilus*. The procedure was the same as that carried out on wound bacteria.

2.6. Statistical analysis

The Optima Data Analysis software version 2 was used to analyse the data obtained from the antibacterial tests of AgNP. ANOVA test was used to compare variables between groups. Statistical significance was set to <0.05 for all tests.

3. Results and Discussion

3.1. Characterization of silver nanoparticles

In accordance to previous studies, a reduction reaction of silver nitrate to silver takes place when plant extracts are added (Sathishkumar, et al, 2009; Rasheed, et al, 2017). The reaction can also be confirmed by the UV spectrum, where a broad absorption peak can be seen with λ_{max} at 427nm, indicating the presence of silver nanoparticles (Figure 1). The plant extract itself is capable of reducing silver nitrate without the use of synthetic chemical reagents.

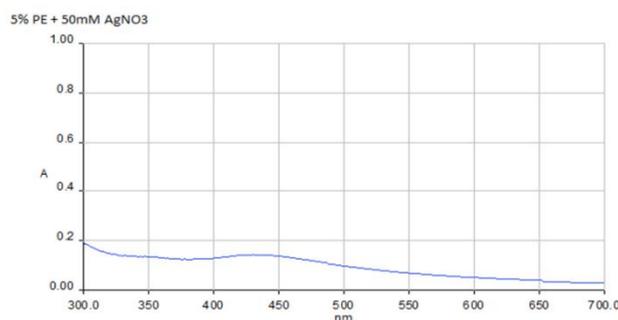


Figure 1: The UV spectra obtained which indicate the presence of silver nanoparticles

From the FTIR spectrum, significant absorption peaks of around 1100cm and 1300cm can be observed (Figure 2) The bands at 1100cm and 1300cm indicate the stretching of alkyl amine and alkyl ketone respectively. These functional groups present in *A. vulgaris* extracts were responsible for such reactions.

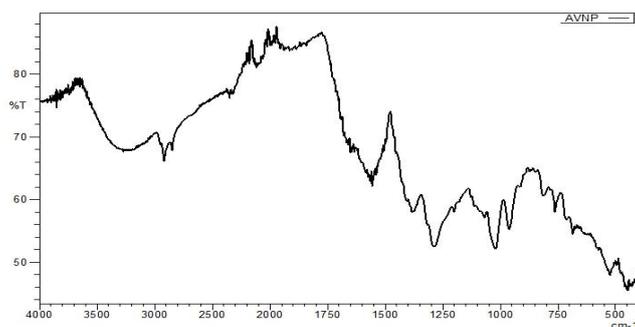


Figure 2: The FTIR spectra showing the functional groups responsible for the reduction reaction to produce silver nanoparticles

The data obtained using zetasizer show that the z-average for the nanoparticles containing 5%, 10%, and 15% plant extracts were 123nm, 240nm, and 237nm, respectively. The particle size estimated by zetasizer seemed to be larger than the usual nanoparticle size. Although the particle size was estimated to be around 200nm, the morphological analysis using TEM showed that most particle sizes fell within 50nm. The zeta potential ranged +20-30mV for all concentrations, indicating that the formulation was stable (Table 1).

Table 1: Particle size and zeta potential of silver nanoparticles containing plant extract

Concentration of plant extract in AgNP	Mean particle size (nm)	Zeta Potential (mV)
50 mg/mL	152.3	+ 24.6
100 mg/mL	224.5	+ 29.0
150 mg/mL	264.4	+ 30.1

The morphological analysis using TEM shows that the nanoparticles displayed a globular shape with a size ranging from 20 to 50nm (Figure 3). The aggregation of silver nanoparticles can be observed, which explains the larger size estimation by zetasizer. It is common that silver nanoparticles aggregate to be in a stable form (Prathna, 2011).

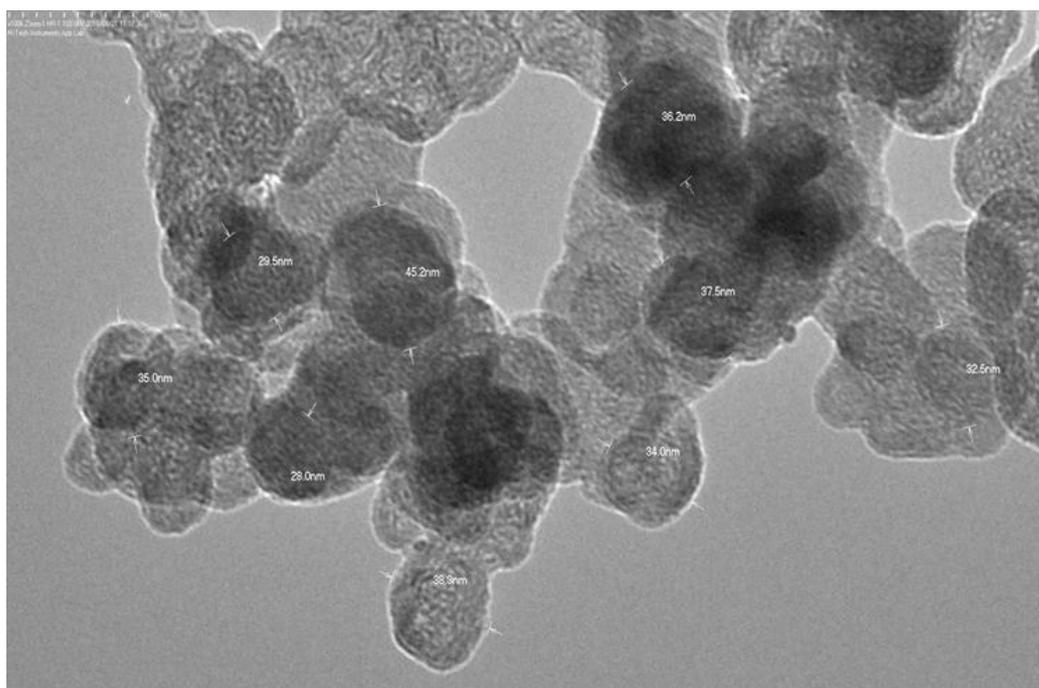


Figure 3: TEM image at 50nm magnification showing the silver nanoparticles with the size ranging between 20 and 50nm

3.2. Investigation of antibacterial effects of AgNP on wound bacteria

3.2.1. Disc diffusion plate test

AgNP with all concentrations of plant extract was able to inhibit the growth of gram positive and gram negative bacteria, although the antibacterial effect was not significant.

(Figure 4). The combination was better than both 50mM silver nitrate alone and plant extract alone. However, the AgNP was still not as effective as the positive control, the antibiotic discs. The bacterial growth inhibition was not affected by the concentration of plant extracts.

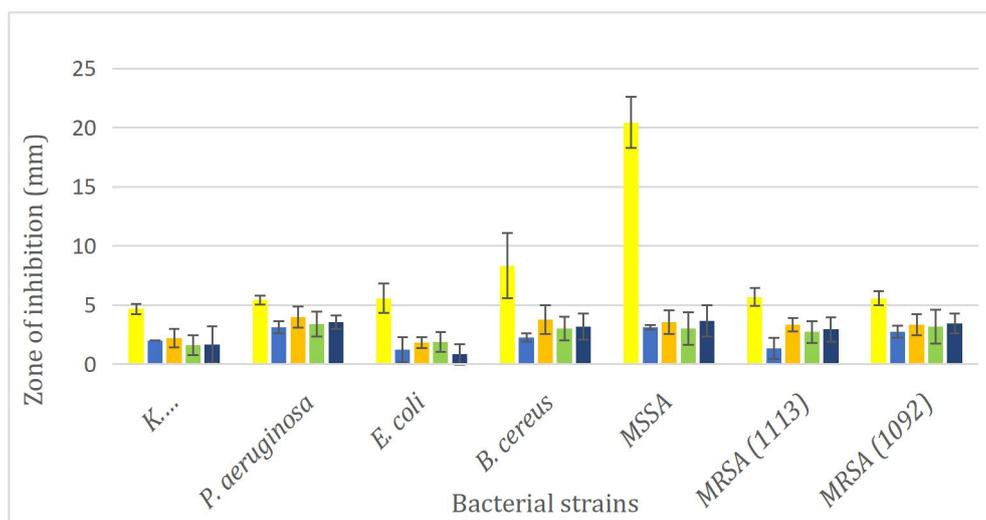


Figure 4: The inhibition of bacterial growth by disc diffusion plate test

The results indicated that the silver nanoparticles and *A. vulgaris* extracts can enhance each other's effect to inhibit the growth of bacteria. According to previous studies, the antibacterial effect of silver nanoparticles is mainly attributed to its small size and electrostatic attraction. (Rasheed, et al, 2017; Prathna, 2011; Nam, et al, 2015). The small size of silver nanoparticles allows it to easily penetrate the bacterial membrane. The small size of nanoparticles provides a high-surface-to-volume ratio, allowing them to have an increased contact area on the bacterial surface so that a greater amount of silver ions can exert the bactericidal effects towards the bacteria (Nam, et al, 2015; Bondarenko, et al, 2013).

The positively-charged nanoparticles and negatively-charged cell surface of gram negative bacteria cause an electrostatic attraction, which eases the diffusion of nanoparticles into bacterial cells (Pazos-Ortiz, E., et al, 2017). The permeation of nanoparticles into bacteria may result in the disruption of protein synthesis, alteration of bacterial structure, and cell death. The plant extract of *A. vulgaris* has a minimal bacterial effect compared to silver nitrate alone. The methanolic extract of *A. vulgaris* is slightly better than the aqueous extract. Terpene compounds found in *A. vulgaris* may contribute to its antibacterial effect (Zengin & Baysal, 2014)

3.2.2. Minimum Inhibitory Concentration (MIC)

From the MIC values, the silver nanoparticles containing *A. vulgaris* extract were effective in inhibiting both the gram positive and gram negative bacteria (Figure 5). The gram negative bacteria, *E. coli* and *K. pneumonia*, had the lowest MIC value, 0.25 mg/mL. Both methicillin susceptible and resistant strains of *S. aureus* had the highest MIC value of 1.00 mg/mL.

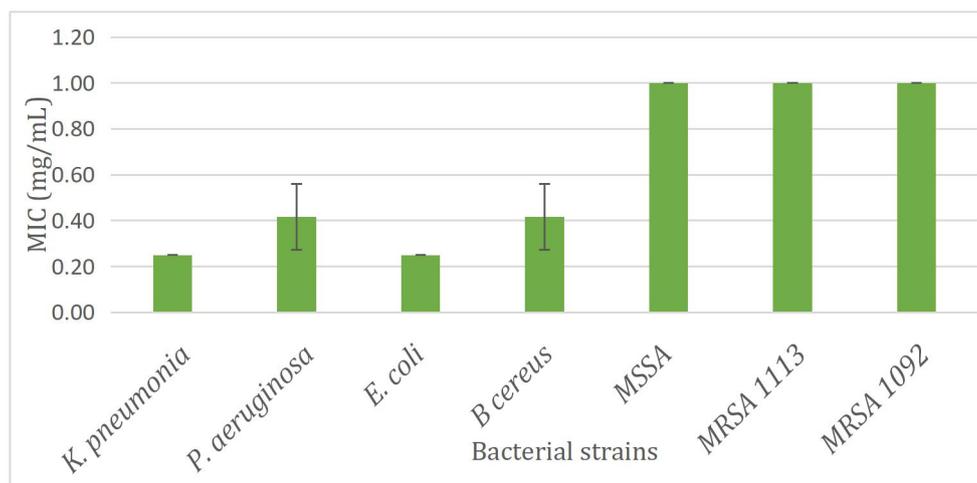


Figure 5: The minimum concentration required to inhibit bacterial growth by 50%

Although silver nanoparticles containing *A. vulgaris* extract were effective in inhibiting the growth of gram positive and gram negative bacteria, the results suggested that the inhibition towards gram negative bacteria was more prominent than that on gram positive ones. From the MIC values, it can be observed that a higher concentration of the formulation was required to inhibit the growth of gram positive bacteria compared to gram negative bacteria. The thick cell wall of gram positive bacteria contains a higher amount of peptidoglycan which causes the silver ions to adhere on the cell wall, resulting in a poorer antibacterial effect (Dakal, et al, 2016). The cell membrane of gram negative bacteria possesses lipopolysaccharides which are negatively charged. This promotes the adhesion of silver nanoparticles, causing the bacteria to be more susceptible to the antibacterial effect (Dakal, et al, 2016). The mechanism of *A. vulgaris* extract between gram negative and gram positive bacteria, however, is still not fully understood.

3.2.3. Minimum Bactericidal Concentration (MBC)

From the MBC values, it is shown that 4mg/mL of silver nanoparticles containing *A. vulgaris* extract, which was the highest concentration, was unable to kill the bacterial population of all the strains tested.

Table 2: Results of the MBC test

Bacteria	Minimum Bactericidal Concentration (mg/mL)
<i>K. pneumonia</i>	~4
<i>P. aeruginosa</i>	>4
<i>E. coli</i>	>4
<i>B. cereus</i>	>4
MSSA	>4

3.3. Investigation of antibacterial effects of AgNP on probiotics

AgNP demonstrated lower antibacterial effects towards probiotics when compared to standard antibiotics (Figure 6). It is also noted that plant extract alone had less inhibition towards the growth of probiotics compared to AgNP. This suggests that the plant extracts exhibit a protective effect towards good bacteria so that the cells can be protected from the damaging effects of silver nanoparticles.

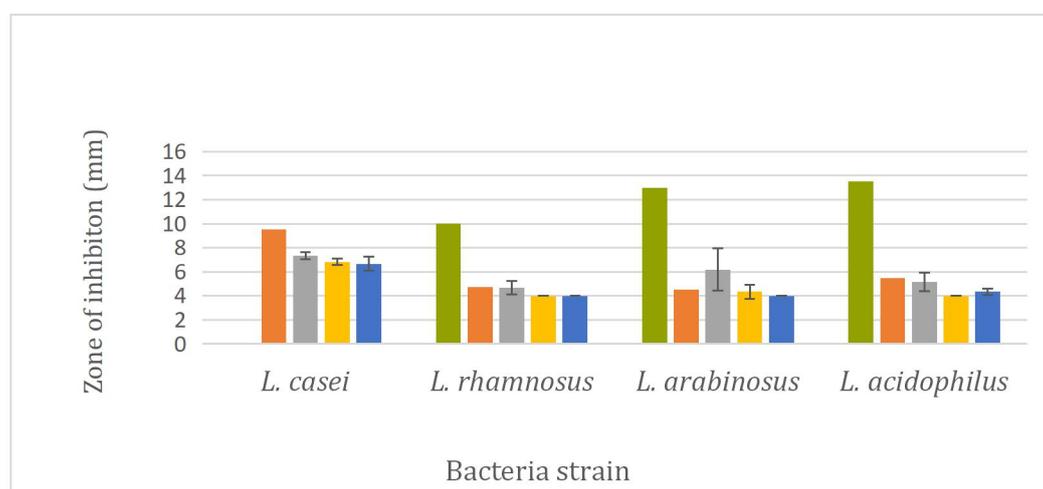


Figure 6: The inhibition of probiotics growth by disc diffusion plate test

A study has shown that a diet containing *A. vulgaris* was linked to an increase in intestinal bifidobacteria (Lee, et al, 1995). In a case report, *A. vulgaris* has been shown to speed up the wound healing process of an anaconda snake, indicating skin protective effects. In previous studies, *A. vulgaris* has shown cell protective effects, including hepatoprotective effects and less cytotoxic effects towards normal cells when compared to cancer cells (Gilani, et al, 2005; Saleh, et al, 2014)

4. Conclusion

A. vulgaris extracts and silver nanoparticles enhance each other's effect to inhibit the growth of bacteria, although not significantly. The plant extracts also exhibit a protective effect, protecting the probiotics from the damaging effects of treatment. Hence, silver nanoparticles containing *A. vulgaris* extract are a potentially safer alternative to the standard antibacterial treatment.

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