



Antibacterial activity of silver nanoparticles synthesized from leaf and flower extracts of *Galinsoga formosa*

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ABSTRACT

Silver Nanoparticles (Ag-NPs) are progressively exercised as an antimicrobial agent among myriad applications. The rapid emergence of microbial resistance to conventional antibiotics by multidrug-resistant pathogens has become a threat to the global health community. Traditionally used herbal plants are good sources of bioactive phytochemicals and *Galinsoga formosa* is one of them. Thus, Ag-NPs are biologically synthesized, intending to evaluate the antibacterial activity of *G. formosa*. Disc diffusion assay was used to assess the antibacterial activity of leaf and flower crude extracts distinctly as well as of green synthesized Ag-NPs *in vitro*. The biosynthesis of Ag-NPs was primarily confirmed by brownish color solution and later by UV-visible spectrophotometer. Ag-NPs synthesized from *G. formosa* leaf, and flower extract showed antibacterial activity against gram-positive (*S. aureus*, *S. mutans*, and *S. epidermidis*) and gram-negative (*K. pneumoniae* and *P. aeruginosa*) bacteria, where gram-negative bacteria were more sensitive than gram-positive bacteria. The highest zone of inhibition was observed against *P. aeruginosa* (13.33±0.58 mm) by applying Ag-NPs synthesized from *G. formosa* flower extract. In contrast, the lowest zone of inhibition was observed against *S. epidermidis* (6.33±0.58 mm). Antibacterial activity of Ag-NPs from flower and leaf extracts was considerably higher as compared to their respective crude extract. Further, Ag-NPs from the flower extract was exhibited more growth inhibitory response than the leaf extract. Hence, the findings of this research suggested that the synthesized Ag-NPs from *G. formosa* leaf and flower extract were exhibited antibacterial activity. Such synthesized Ag-NPs might help to develop new drug for combating against various diseases.

INTRODUCTION

The gradual resistance of antimicrobial drugs, especially antibacterial agents, has become a serious issue and a global threat to the public health community [1,2]. In the US, over 2.8 million antibiotic-resistant infections occur annually, and quite 35,000 people are died according to the antibiotic resistance threats reports 2019 [3]. Antimicrobial resistance is one

of the highest ten global public health challenges declared by the World Health Organization (WHO), and in Bangladesh, up to 80% of deaths occur in intensive care units (ICUs) [4]. However, medicinal plants are commonly used in rural areas as a remedy for various diseases and such plants can find a solution for these challenging bacterial infections because they are rich source of biologically active compounds like antimicrobials

[5]. Plants have been using as prophylaxis for a long time since they possess secondary metabolites with several biological activities, such as antimicrobial, antioxidant, antifungal, and anti-inflammatory [6,7]. The disposition to use plants has augmented due to having trepidation from side effects of synthetic chemical drugs as well as the resistance of drugs to a specific treatment [8,9]. *Galinsoga*, a genus of flowering herbs in the family of Asteraceae, has been being used especially flower as folk medicine owing to different medicinal activities [10]. In specific, *G. formosa* is commonly used to alleviate gum pain, mouth infections, and serves as an anti-inflammatory, antimicrobial, or wound healing agent [11].

Nanotechnology to medicine (nanomedicine) has profound application in the pharmaceutical and biotechnology industries [12–15]. In the advent of bacterial multidrug resistance, silver nanoparticles provided a radically new solution to the problem as well as in other fields [16]. The biological synthesis of silver nanoparticles can enhance the antimicrobial activity of plant bioactive compounds in the crude extract as well as the activity of conventional inactive antibiotics [17–19]. In modern nanoscience and nanotechnology, silver nanoparticles play a key role in medicine, and because of their distinctive features like the crossing of brain-blood barrier, it has concentrated on their potential uses in the detection and treatment of cancer [20,21]. Ag-NPs can be synthesized in several ways, where conventional physical and chemical techniques appear to be very costly and challenging. Still, biological approaches are likely to be simple, fast, non-toxic, effective, and environmentally sustainable [22–25]. Because of its broad-spectrum efficacy and low cytotoxicity, it has been shown to be effective as an antiseptic and antimicrobial against both gram-positive and gram-negative bacteria. [26,27]. Therefore, this research aimed to investigate the medicinal value of *G. formosa* leaf and flower extracts in terms of antibacterial activity by the synthesis of Ag-NPs against five pathogenic bacteria.

MATERIALS AND METHODS

Sample collection

Fresh *G. formosa* leaves and flowers were collected during the late summer (June 2019) from the Mawlana Bhashani Science and Technology University campus at Tangail, Bangladesh (24°14'09.3"N 89°53'26.2"E)

Extract preparation of *G. formosa* leaf and flower

Freshly collected *G. formosa* leaf and flower were washed with tap water and then rinsed with distilled and deionized water (Total water purification, Dhaka, Bangladesh.) correspondingly to remove dust and other unwanted particles from the surface area. The leaf and flower of the *G. formosa* were ground separately with mortar and pestle. The pulverized leaf and flower were mixed with deionized water and filtered with a cotton cloth and Whatman No.1 filter paper respectively. Then it was centrifuged at 6000 rpm for 10 minutes. Finally, the extracts were stored at 4 °C for further use.

Synthesis of Ag-NPs from *G. formosa*

Different concentrations (10 mM and 5 mM) of AgNO₃ (Merck KGaA, Germany) solutions were mixed with *G. formosa* leaf and flower extract at a ratio of 2:3 to a final volume of 10 mL. The extracts were mixed properly and kept at room temperature for 24h in a dark place to avoid photoactivation of AgNO₃ until the brownish color was formed [28,29]. For the separation of silver nanoparticles, the mixtures were subjected to centrifugation at 9000 rpm for 15 minutes. Removal of excessive silver ions was completed through the rinsing of precipitates of Ag-NPs. Finally, Ag-NPs solutions were obtained by re-dispersed in deionized water, and the sample was then stored at 4°C for further analysis.

UV- visible spectroscopy analysis

The reduction of silver ions in silver nanoparticles was monitored by a UV-visible spectrophotometer (T60 UV-visible Spectrophotometer, PG Instruments, UK) at the wavelength range of 280 - 600 nm. The absorbance of silver nanoparticle aliquots was measured after they were diluted with deionized water.

Disc diffusion method for antibacterial activity

The antimicrobial activities of synthesized Ag-NPs were tested using disc diffusion bioassay described by Bakht *et al.* (2011) with slight modification [30]. Six hours old bacterial cultures on MHA (Mueller Hinton Agar; Becton, Dickinson, and Company) plates were uniformly spread with the glass spreader. Three discs of 5 mm diameter were placed on each MHA plate

which contained 15 µl synthesized Ag-NPs from plant extract as sample, deionized water without sample as negative control, and ampicillin and azithromycin (Himedia, India) as positive control. The test samples were incubated at 37°C for 18 hours, and then the zone of inhibition was measured. Diameters of the inhibition zone formed were measured in millimeters using a measuring scale. To better understand the entire study, a pictorial representation of the procedure is outlined in Figure 1.

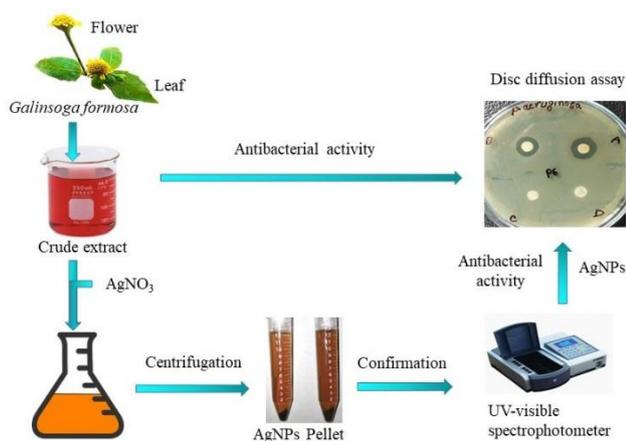


Figure 1. Overview of the antibacterial activity of synthesized Ag-NPs from *G. Formosa*.

Statistical analysis

One way analysis of variance (ANOVA) and independent-sample t-test were performed by Statistical Packages for Social Sciences (SPSS Statistics for Windows, Version 20. Armonk, NY: IBM Corp) software, where $p < 0.05$ was considered as significant and Microsoft Excel 2013 were used for graphical evaluations. All experiments were repeated three times and results were displayed as Mean \pm SD.

RESULTS

Confirmation of the synthesized Ag-NPs by observing visible color change and UV-visible spectral analysis

The *G. formosa* leaf and flower water extracts were used in the synthesis of Ag-NPs. The bio-reductive formation of Ag-NPs was primarily confirmed by visual observation. The addition of aqueous AgNO_3 solution with *G. formosa* leaf and flower extract resulted in the formation of a brownish color solution

after overnight incubation at room temperature in the dark, which indicated the biosynthesis of Ag-NPs. The control AgNO_3 solution without plant extract was not shown any color change under similar conditions. Besides, the formation of Ag-NPs was confirmed by a UV-visible spectrophotometer (Figure 2). The Ag-NPs formed by leaf extract showed a peak near 350 nm to 400 nm wavelength, and a sharp peak was observed around 300 nm for the *G. formosa* flower extract.

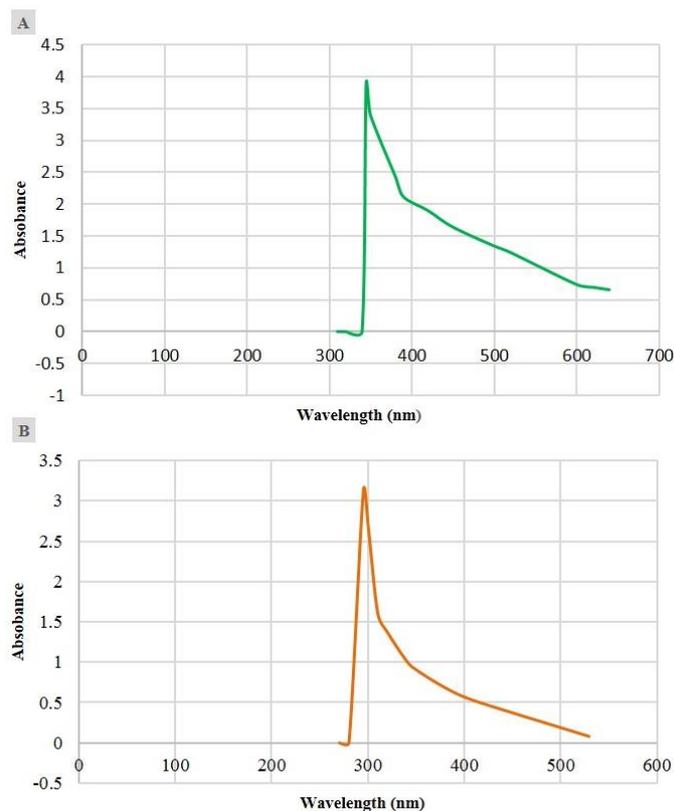


Figure 2. Confirmation of synthesized Ag-NPs of the leaf (A) and flower (B) from *G. formosa* by UV-visible Spectrophotometer.

Antibacterial activity of crude extract of *G. formosa*

The crude extract of *G. formosa* leaves and flowers was applied on 5 distinct bacterial strains to detect their antibacterial activity. Before using, the plant crude extracts were filtered through a millipore filter to remove existing bacteria. Among the five pathogenic strains, three were gram-positive (*S. aureus*; GCA_000013425.1, *S. epidermidis*; GCA_006094375.1, and *S. mutans*; GCA_001558215.1) and two were gram-negative (*K. pneumoniae*; GCA_000240185.2 and *P. aeruginosa*; GCA_000006765.1). However, both of these crude extracts hardly showed considerable antibacterial activity against the bacterial strains.

Higher antibacterial activity of synthesized Ag-NPs than crude extracts

The antibacterial activity of the synthesized Ag-NPs from *G. formosa* was increased despite the limited activity of crude extract. In this experiment, it was observed that silver nanoparticles showed better performance than crude extracts.

Flower extract showed higher antibacterial activity than leaf extract

The Ag-NPs synthesized from *G. formosa* flower extract showed more growth inhibitory response against the tested bacteria. The maximum 13.33 ± 0.58 mm zone of inhibition was recorded for flower extract against *P. aeruginosa*, whereas Ag-NPs from *G. formosa* leaf extract showed an 11.33 ± 0.58 mm zone of inhibition. The synthesized Ag-NPs were more

sensitive against the aforementioned two bacterial strains, where it is clear that Ag-NPs from flower extract were more potent than leaf extract. The detailed result is depicted in Figure 3.

Antimicrobial activity of Ag-NPs was not affected by silver nitrate solutions

Two different concentrations of silver nitrate solutions (10 mM (A) and 5 mM (B)) were used to synthesize Ag-NPs from *G. formosa* plant extract. No remarkable differences were observed in the two different concentrations in terms of their activity (Figure 3). In most of the cases, they pointed very similar results. So, the concentration of silver nitrate solution made no major changes in antibacterial activity by synthesizing particular Ag-NPs.

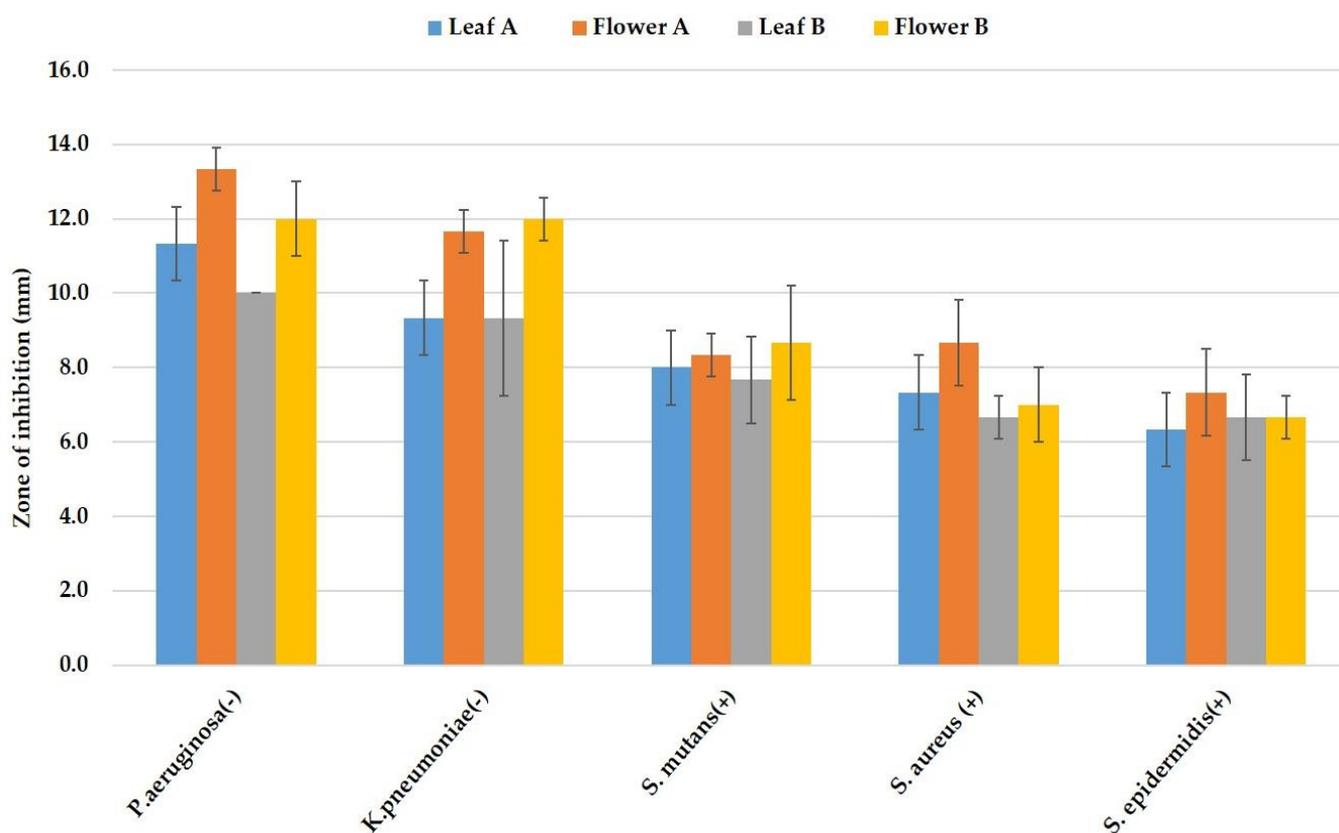


Figure 3. Comparative illustration of the activity of leaf and flower extract at 10 mM AgNO₃ (A) vs 5mM AgNO₃ (B), and gram-negative vs gram-positive bacteria.

Gram-negative bacteria are more sensitive than gram-positive bacteria to synthesized nanoparticles

The synthesized Ag-NPs were investigated for their antibacterial activity assay against both gram-positive (*S. aureus*, *S. epidermidis*, and *S. mutans*) and gram-negative (*K. pneumoniae* and *P. aeruginosa*) bacteria by disc diffusion method. In this study, a maximal 13.33 ± 0.58 mm zone of inhibition was recorded for *G. formosa* flower extract and 11.33 ± 0.58 mm for *G. formosa* leaf extract against *P. aeruginosa* (Figure 4a; P-5 and Figure 4b: P-6; Table 1). On the contrary, the lowest zone of inhibition was observed against *S. epidermidis* both for *G. formosa* leaf and flower extract (Figure 4a; P-4 and Figure 4b: P-9; Table 1). Among the five tested pathogenic bacteria, the synthesized Ag-NPs from *G. formosa* was more effective towards gram-

negative bacteria as compared to gram-positive bacteria (Figure 4a and 4b; Table 1). The comparison of such differences between gram-negative and gram-positive bacteria was disclosed in Figure 3.

The synthesized nanoparticles from *G. formosa* leaf and flower extract exhibited minimal growth inhibitory effects against *S. epidermidis* (Figure 4a: P-4 and Figure 4b: P-9) and *S. aureus* (Figure 4a: P-1 and Figure 4b: P-7) as well as *S. mutans* (Figure 4a: P-3 and Figure 4b: P-8) but showed maximal growth inhibitory effects against *K. pneumoniae* (Figure 4a; P-2 and Figure 4b: P-10) and *P. aeruginosa* (Figure 4a: P-5 and Figure 4b: P-6). Surprisingly, no zone of inhibition was found for ampicillin (25 $\mu\text{g}/\text{mL}$) against those strains, but azithromycin (30 $\mu\text{g}/\text{mL}$) showed a broad-spectrum antibacterial activity (Figure 4a and 4b).

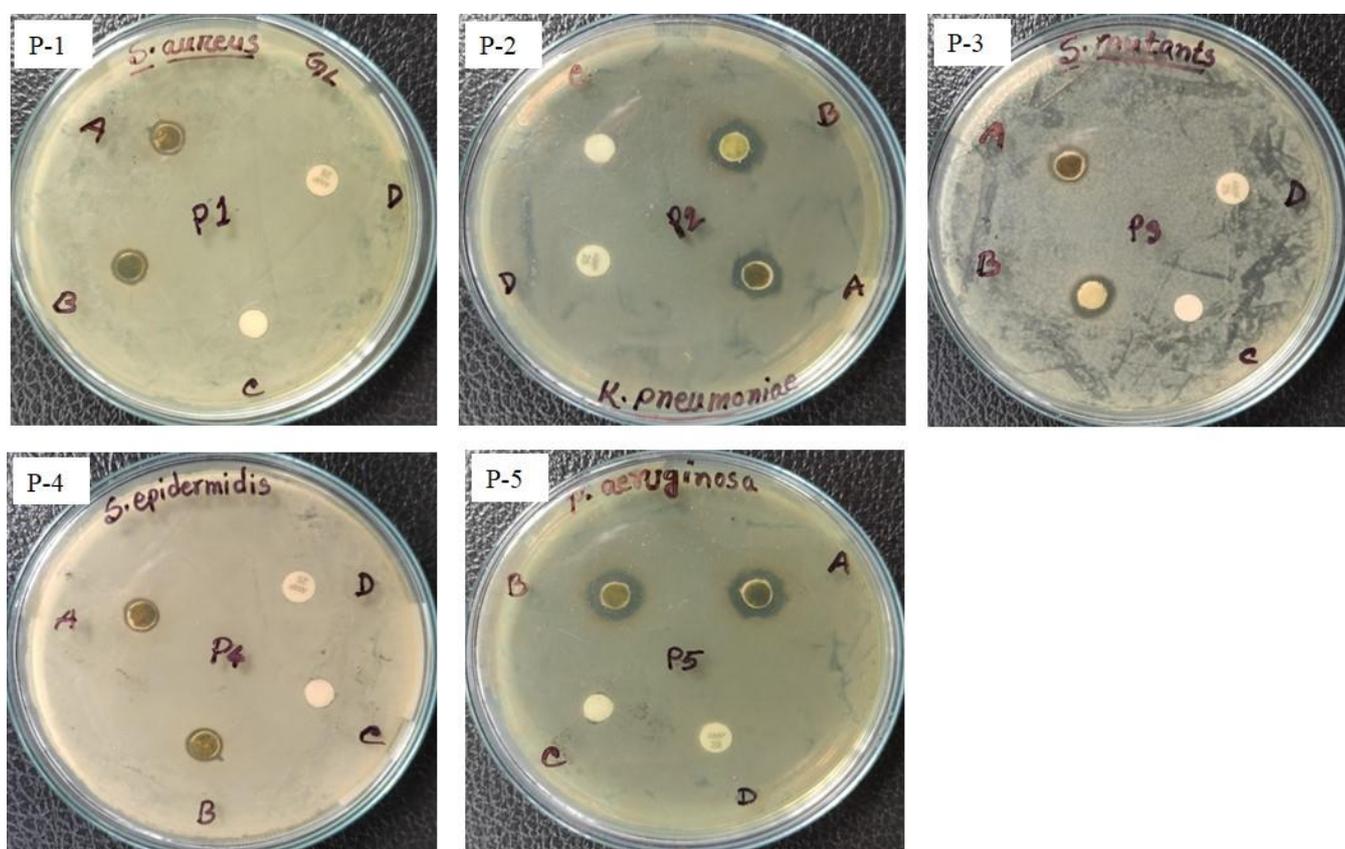


Figure 4a. Antibacterial activity of Ag-NPs synthesized from *G. formosa* leaf extract against *S. aureus* (P-1), *K. pneumoniae* (P-2), *S. mutans* (P-3), *S. epidermidis* (P-4), and *P. aeruginosa* (P-5). Ag-NPs using 10 mM (A) and 5 mM (B) AgNO₃ respectively, a disc with deionized water as a negative control (C), and ampicillin disc (D).

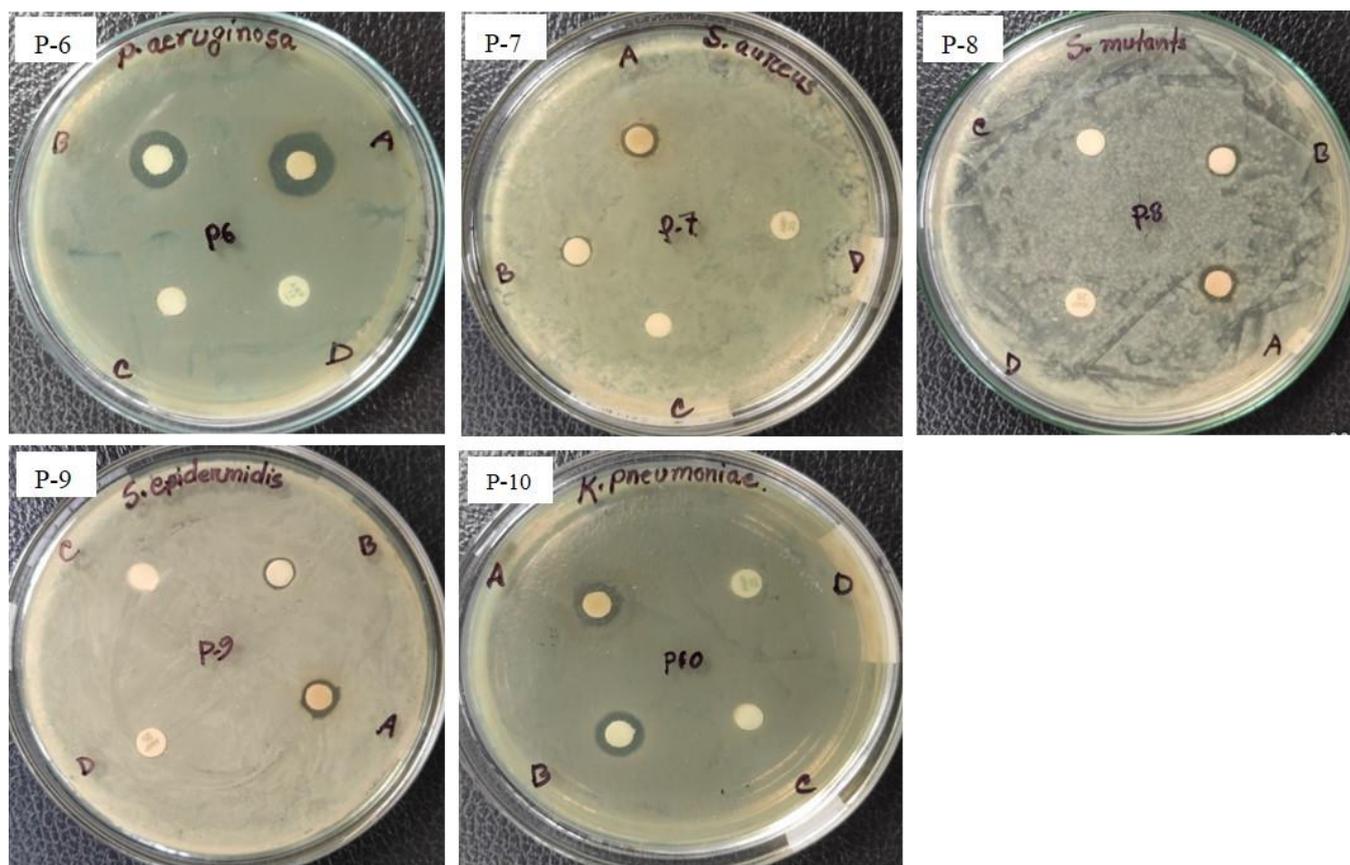


Figure 4b. Antibacterial activity of Ag-NPs synthesized from *G. formosa* flower extract against *P. aeruginosa* (P-6), *S. aureus* (P-7), *S. mutans* (P-8), *S. epidermidis* (P-9), and *K. pneumoniae* (P-10). Ag-NPs using 10 mM (A) and 5 mM (B) AgNO₃ respectively, a disc with deionized water as a negative control (C), and ampicillin disc (D).

Table 1. Antibacterial activity of synthesized Ag-NPs.

| Name of the sample | Plate no. | Bacteria against | Zone of inhibition (A) | Zone of inhibition (B) | Positive control | |
|-------------------------------------|-----------|-----------------------|--------------------------------|--------------------------------|--------------------------|------------------------|
| | | | (Diameter in mm) 90 µg/disc | (Diameter in mm) 90 µg/disc | Azithromycin 30 µg/ml | Ampicillin 25 µg/ml |
| <i>G. formosa</i> leaf extract | P-1 | <i>S. aureus</i> | 7.33±1.53 | 6.67±0.58 | 24.67±0.58 | Resistant |
| | P-2 | <i>K. pneumoniae</i> | 9.33±1.53 | 9.33±2.08 | 25.00±1.00 | Resistant |
| | P-3 | <i>S. mutans</i> | 8.00±1.73 | 7.67±1.16 | 27.33±1.53 | Resistant |
| | P-4 | <i>S. epidermidis</i> | 6.33±0.58 | 6.67±1.16 | 27.67±1.53 | Resistant |
| | P-5 | <i>P. aeruginosa</i> | 11.33±0.58 | 10.00±0.00 | 25.00±1.00 | Resistant |
| | | | (P = 0.007, F = 6.75) | (P = 0.020, F = 4.82) | | |
| <i>G. formosa</i> flower extract | P-6 | <i>P. aeruginosa</i> | 13.33±0.58 | 12.00±1.00 | 25.00±1.00 | Resistant |
| | P-7 | <i>S. aureus</i> | 8.67±1.16 | 7.00±1.00 | 24.67±0.58 | Resistant |
| | P-8 | <i>S. mutans</i> | 8.33±0.58 | 8.67±1.53 | 27.33±1.53 | Resistant |
| | P-9 | <i>S. epidermidis</i> | 7.33±1.16 | 6.67±0.58 | 27.67±1.53 | Resistant |
| | P-10 | <i>K. pneumoniae</i> | 11.67±0.58 | 12.00±0.58 | 25.00±1.00 | Resistant |
| | | | (P = 0.00, F = 26.05) | (P = 0.00, F = 18) | | |

DISCUSSION

Bacterial resistance is a burning issue in the medical sector now-a-days, where Ag-NPs might be a solution due to their bactericidal properties. The scientists focus on a variation in the synthesis of nanoparticles for the development of antibiotics against microorganisms as Ag-NPs can improve the bioactivity of natural compounds, but the activity depends on the size of the nanoparticles [31,32]. The biologically synthesized Ag-NPs are eco-friendly, safe, and easy to use [33]. To synthesize Ag-NPs, *G. formosa* leaf and flower crude extract were treating with AgNO₃ solution and initially confirmed by a visual color change to brown [34,35]. This is the primary indication for the formation of Ag-NPs, which is further confirmed by a UV-visible spectrophotometer. After confirmation, the synthesized nanoparticles from *G. formosa* leaf and flower extract were applied on five bacterial strains and exhibited growth-inhibitory response against all the strains, but showed prominent growth inhibitory effects against two strains namely, *K. pneumonia* and *P. aeruginosa*. According to the results, it was observed that the gram-negative bacteria showed more sensitivity to synthesized Ag-NPs than gram-positive bacteria, where flower extract was more potent than leaf extract. Alfuraydi *et al.* (2019) reported that synthesized Ag-NPs using sesame oil cake inhibited the growth of gram-negative bacteria such as *P. aeruginosa*, *K. pneumonia*, and *E. coli*, while *Bacillus subtilis* and *S. aureus* were not sensitive [36]. The outcomes of this study are also in agreement with the previous findings reported on the *Datura stramonium* leaf extract-assisted Ag-NPs and assessment of their antibacterial activity [37]. Senthil *et al.* (2017) observed that Ag-NPs enhanced protein leakage from bacterial cells, where they were more susceptible to gram-negative bacteria than gram-positive bacteria [38]. Such findings indicated that the nanoparticles might inhibit the growth of gram-negative bacteria more specifically than gram-positive bacteria.

However, the exact mechanism remains unclear; the adherence of Ag-NPs to bacterial cells, cell disruption, generation of reactive oxygen species, and free radical production, as well as the regulation of bacterial signal transduction pathways, have been recognized as the most important modes of antimicrobial action [39,40]. The discriminate bactericidal performance of Ag-NPs might depend on distinct interaction patterns between the bacterial cell wall and Ag-NPs. On the contrary, the charge of Ag-NPs is correlated with bacterial cell

damage as gram-negative bacteria are more susceptible to Ag⁺ ions invasion than gram-positive bacteria [41,42]. Due to the difference in peptidoglycan structure, and denser outer membrane, some researchers described that Ag-NPs are more effective in gram-negative bacteria [34,42,43]. The gram-positive bacteria are resistant to the action of the nanoparticles because of their variations in the cell wall structures, and the activity of the nanoparticles towards the pathogens might be interrelated with the diverse alterations in the morphological features between gram-negative and gram-positive bacteria [36]. But several studies showed that Ag-NPs are effective in both gram-positive and gram-negative bacteria [44–47]. Although the antibacterial activity of Ag-NPs depends on particle charge, size, shape, and interaction of phytochemicals, the bacterial cell wall structures might be another important factor. Limitations of this study were smaller sample size, and unavailability of advanced characterization of the synthesized Ag-NPs. While the fresh *G. formosa* is commonly used as an antimicrobial as well as an anti-inflammatory agent, this experiment could not find such strong behavior of fresh aqueous crude extract. It is assumed that there are active ingredients in the plant that need to be identified in further study.

CONCLUSION

The antibacterial activity of bioderived silver nanoparticles from *G. formosa* flower and leaf extract was assessed against five pathogenic bacteria. The findings of this research summarized that the synthesized Ag-NPs inhibited the growth of gram-negative bacteria more specifically as compared to gram-positive bacteria. The synthesized Ag-NPs from flower extract has more antibacterial activity than leaf extract. This might be due to the existence of unique natural compounds or different concentrations of the same compounds in the different parts of the plants. The plant extract might contain novel ingredients that should be identified in further research. Such bioactive natural compounds could be served as supporting materials for the formulation of new medication against various bacterial infections.

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AUTHORS CONTRIBUTION

MRM, AJ, and MAS planned and designed the research. MAS supervised the whole research. MRM, AJ, CB, SAS, TS, and TA conducted the entire laboratory works. MAS, MAZ, and MK arranged the entire facilities for the research. MRM and RM interpreted the results and performed the statistical analysis. MRM drafted the manuscript. MAS, MZA, and MAI have thoroughly edited and revised the manuscript. All authors read and approved the manuscript.

CONFLICTS OF INTEREST

There is no conflict of interest among the authors.

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