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Research Article

Najla AlMasoud*, Hajar Alhaik, Malak Almutairi, Asmaa Houjak, Khlood Hazazi, Fatema Alhayek, Sarah Aljanoubi, Ahad Alkhaibari, Asma Alghamdi, Dina A. Soliman, Taghrid S. Alomar*, and Manal A. Awad

Green nanotechnology synthesized silver nanoparticles: Characterization and testing its antibacterial activity

https://doi.org/10.1515/gps-2021-0048 received April 11, 2021; accepted July 14, 2021

Abstract: The green plant-mediated synthesis of silver (GPS-Ag) nanoparticles (NPs) has been increasingly popular due to its eco-friendliness, availability, cost-effectiveness, and the fact that it can be safely handled and possesses a broad variability of metabolites, such as antioxidant and antimicrobial activities. In this current study, the synthesis of AgNPs has been demonstrated using aqueous extracts of fresh leaves of Ficus carica and Salvia rosmarinus (rosemary) that reduced aqueous silver nitrate. This procedure made the synthesis of NPs possible, which was characterized by numerous analytical techniques such as ultraviolet-visible (UV-Vis) spectrophotometry, Fourier transform infrared spectroscopy, transmission electron microscopy (TEM), energy-dispersive X-ray spectroscopy, and dynamic light scattering studies. The visual observation indicated that the colour of aqueous silver nitrate turned brownish vellow after treatment with the fresh leaf extracts and was confirmed by UV-Vis spectra. In addition, the TEM analysis showed that the synthesized NPs well dispersed with average sizes less than 22 nm.

Furthermore, AgNPs and aqueous leaf extracts of *F. carica* and rosemary were examined for their antimicrobial activities against different Gram-positive and Gram-negative bacteria strains. The results indicated that the AgNPs, derived from *F. carica*, have more antibacterial activities than others and inhibited bacterial growth.

Keywords: green synthesis, AgNPs, *Ficus carica*, *Salvia rosmarinus*, antimicrobial activities

1 Introduction

Nanotechnology refers to the science, engineering, and technology carried out at the nanoscale from 1–100 nm. The use of this advanced technology extends to a number of fields such as chemistry, materials science, and other similar disciplines. Moreover, nanotechnology has been used in various applications such as energy, catalysis, food science, biomedicine, wastewater treatment, and electronics [1–3].

Green synthesis methods (green chemistry) are known to be safer in comparison to chemical methods. This is because the former is known to be more sustainable and offer an environmentally safer approach during the synthesis of nanoparticles (NPs) [4]. Some of the novel green chemistry methods such as burgeoning green nanotechnology have proven to be important in newer nanoparticle synthesis techniques. These alternative methods utilize microorganisms and plant-based extracts and have shown to be more effective in the synthesis of NPs [5,6].

Several research studies carried out using silver nanoparticles (AgNPs) reported improved properties, including good electrical and thermal properties, chemical stability as well as catalytic and antimicrobial properties [7–10]. Besides, the physical, mechanical, and structural properties of AgNPs with various sizes and shapes can be modified by the addition of biological agents such as bacteria, fungi, and algae along with their enzymes. Researchers

e-mail: tsalomar@pnu.edu.sa

Hajar Alhaik, Malak Almutairi, Asmaa Houjak, Khlood Hazazi, Fatema Alhayek, Sarah Aljanoubi, Ahad Alkhaibari, Asma Alghamdi: Department of Chemistry, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh 11451, Saudi Arabia

Dina A. Soliman: Department of Botany and Microbiology, Faculty of Science, King Saud University, Riyadh 11459, Saudi Arabia Manal A. Awad: King Abdullah Institute for Nanotechnology, King Saud University, Riyadh 11451, Saudi Arabia, e-mail: mawad@ksu.edu.sa

^{*} Corresponding author: Najla AlMasoud, Department of Chemistry, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh 11451, Saudi Arabia, e-mail: nsalamsoud@pnu.edu.sa

^{*} Corresponding author: Taghrid S. Alomar, Department of Chemistry, College of Science, Princess Nourah Bint Abdulrahman University, Riyadh 11451, Saudi Arabia,

used an isolated bacterium to synthesize AgNPs (30-60 nm) biologically [11–14], with improved catalytic activity and a stable hydrazine oxidation reaction. Another study carried out by Abdel-Raouf involved a rapid biogenic process using Laurencia catarinensis, a marine red alga [15,16]. More recent studies have employed several plant-based extracts for the synthesis of AgNPs, including extracts of blackberry fruit [17], Sacha inchi shell biomass and leaf [18,19], natural rubber latex, aloe vera [20], rambutan peel [21], clove [22], coffee, green tea [23], and leaves of Coccina grandis [24]. Some of the more recent uses of natural extracts for nanoparticle synthesis include rosemary extracts: the use of this plant and others resulted in the approval of the use of R. officinalis by the European Union as an efficient natural food preservative [25]. R. officinalis has proven to be beneficial for a number of medical conditions, for example useful as an anti-inflammatory and hepatoprotective [26]. Rosemary was used to acquire aqueous extracts as reductants for the synthesis of AgNPs [27] and the MgO nanoflowers (MgONFs) [28]. The successful green synthesis of AgNPs through the dried fruit extract of Ficus carica was described previously [29] and it was shown that the synthesis was reasonably nontoxic, and thus, it can be employed as a capable anticancer agent. Ficus carica is known to be used as a healing agent in many medical applications as a cardiovascular, respiratory, and as an anti-inflammatory agent [30].

The chemical profile of Salvia rosmarinus was carried out by Leporini et al. [31]. The chemical composition of the essential oil from Salvia rosmarinus was studied by modern analytical techniques such as gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). 1,8-Cineole, α-pinene, camphor, and trans-caryophyllene were the most present compounds. In addition, phytochemical studies on F. carica revealed the presence of numerous bioactive compounds such as phenolic compounds, phytosterols, organic acids, anthocyanin, triterpenoids, coumarins, and volatile compounds such as hydrocarbons, aliphatic alcohols, and a few other classes of secondary metabolites from different parts of F. carica. Most species of F. carica contain phenolic compounds, organic acids, and volatile compounds. Phenolic acids such as 3-0- and 5-0-caffeoylquinic acids, ferulic acid, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, psoralen, bergapten, and organic acids (oxalic, citric, malic, quinic, shikimic, and fumaric acids) have been isolated from the water extract of the leaves of *F. carica* L. [32,33].

This study summarizes and investigates the use of Ficus carica and rosemary-based extracts to reduce Ag+ ions to synthesize AgNPs. The synthesized AgNPs were analyzed to confirm and study their characteristics using Fourier transform infrared spectroscopy (FTIR), scanning

electron microscopy, energy-dispersive X-ray spectroscopy (EDX), ultraviolet-visible absorption spectroscopy (UV-Vis), and transmission electron microscopy (TEM). The antibacterial potentials of the green synthesized AgNPs have been evaluated against various strains of bacteria.

2 Materials and methods

2.1 Preparation of NPs using Ficus carica and Salvia rosmarinus leaves extracts

The leaves of Ficus carica and Salvia rosmarinus (rosemary) were collected from well-grown trees in the farms in Saudi Arabia, Riyadh. The collected leaves were washed thoroughly with tap water, until no residual impurities were left; then they were washed with deionized water and airdried. About 10 g from each dried Ficus carica and rosemary leaves were macerated separately in 100 mL of boiling distilled water. The mixtures were kept overnight, and the extracts were filtered using filter papers.

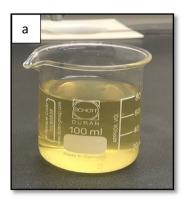
Roughly 5 mL from the aqueous extracts were mixed with 50 mL of AgNO₃ (1.0 mM) separately; both mixtures were then placed on an electric heater and magnetic stirrer at 60°C until brownish silver colloid was observed, indicating the formation of AgNPs (Figures 1 and 2), which were stable for several weeks in glass conical flasks covered with foil at room temperature.

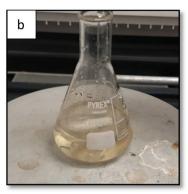
The precise mechanism involved in the reduction and stability of silver ions has not been clearly understood until now because biomolecules vary from plant to plant [34]. As a result, more research is needed into the synthesis of AgNPs with plant extracts in order to identify the right biomolecules that serve as the capping and stabilizing agents. Nonetheless, the trapping of AgNPs ions on the protein surface due to electrostatic interactions between silver ions and proteins in plant material extract has been proposed as a probable mechanism implicated in the bioreduction of Ag. Proteins decrease AgNPs ions, causing them to change the secondary structure and generate silver nuclei. Silver nuclei are formed by further reducing Ag ions and their build-up at the nucleus, resulting in the production of AgNPs [35].

2.2 Characterization of the formation of nanoparticles

A variety of analytical techniques were used to examine the formation of NPs. UV 2450 Spectrophotometer (Shimadzu

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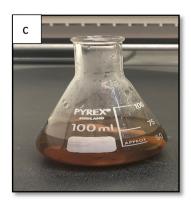


Figure 1: Visual observation of (a) the *Ficus carica* extract, (b) after 5 min of mixing the *Ficus carica* extract with silver nitrate solution, and (c) the synthesis of AgNPs (after 15 min the colour changed to brown).

Corporation, Kyoto, Japan) was employed to carry out UV-Vis spectral analysis in a range of 100 to 800 nm. Furthermore, the particle size distribution of biosynthesized AgNPs was obtained using the dynamic light scattering (DLS) method using a Zetasizer (Nano series, HT Laser, ZEN3600 Malvern Instruments, Malvern, UK). The synthesized AgNPs and functional groups of the extracts were also studied using FTIR. The aim of the study was to analyse and evaluate the interaction between the extracts and AgNPs in the wavenumber range of 400–4000 cm⁻¹. Moreover, the TEM (Zeiss, Germany, EM 10C-200 kV) was utilized to observe the shape, size, and morphology of the synthesized NPs. Elemental analysis was performed using EDX coupled with a JEM-2100F transmission electron microscope; this was carried out to confirm the presence of silver in the suspension.

2.3 Antimicrobial activity

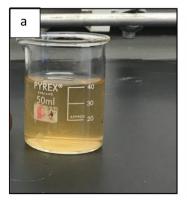
Three different types of bacteria were used in this study, including *Escherichia coli* ATCC35218, *Staphylococcus aureus*

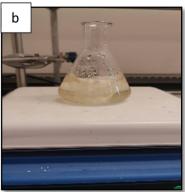
ATCC 43300, and *Bacillus cereus* ATCC 11778 (clinical isolate), which were obtained from King Khalid University Hospital, Riyadh, Saudi Arabia. In this work, the antimicrobial activity of the produced AgNPs was tested using a well-cut diffusion method. About 14 g of nutrient agar was dissolved in distilled water (500 mL) and autoclaved at 121°C and 15 psi for 45 min. The solid medium was punched with a sterile cork borer to make a well (4 wells in each plate). The leaf extract was used as a control, and three different concentrations of AgNPs were used (2, 4, and 6 μ L), followed by a gradual addition until the hole was filled and incubated at 37°C for 24 h. Then, the zone was measured after the incubation and expressed in millimeters of the diameter.

3 Results and discussion

3.1 UV analysis

Initial stages of the reduction illustrated a change in the colour of the mixture from almost colourless to brown





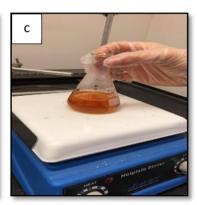


Figure 2: Visual observation of (a) the rosemary extract, (b) after 5 min of mixing the rosemary extract with silver, and (c) the synthesis of AgNPs (15 min after mixing) the colour changed to brown.

when the silver nitrate solution was mixed with the agueous extract of the leaf, which indicated the formation of AgNPs due to the surface plasmon resonance (SPR) of AgNPs [36,37], which might be the primary signature of the nanoparticle. In this work, different analytical methods were used to study the characterization of AgNPs. One of the main methods to detect and evaluate the formation of NPs in an aqueous solution is UV-Vis spectroscopy. The confirmation and stability of the synthesized AgNPs was by UV-Vis spectroscopy since the plasmon band of Ag is sensitive to the size and shape of the formed NPs [38]. The silver ions are reduced to silver atoms due to the components in the plant extract [39]. Figure 3 shows the UV-Vis spectra of the silver nanoparticle formation for both F. carica and rosemary extracts and were measured from 200 to 800 nm. Generally, broad peaks at higher wavelengths indicate particle size growth, whereas a narrow peak at a shorter wavelength confirms the formation of smaller-sized AgNPs. Figure 2a and b shows sharp and intense surface plasmon resonance (SPR) bands at 450 nm, corresponding to the characteristic SPR of synthesized AgNPs using both plants extracts and this result is in agreement with the previously reported results that AgNPs having wavelength (λ_{max}) values in the range between 400 and 500 nm [40] indicate the formation of good quality AgNPs [41].

3.2 DLS analysis

The size distribution and the average size of the synthesized AgNPs were carried out using the DLS technique as shown in Figure 4. From Figure 4, it can be noted that the average size and polydispersity index (PdI) of the synthesized AgNPs using *F. carica* and rosemary leaves extracts

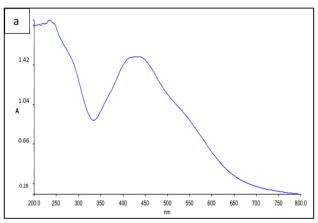
were found to be 295 and 0.31 nm, and 61.44 and 0.42 nm, respectively. The measured z-average and PdI of the synthesized NPs illustrate that these NPs, are monodisperse, verifying the data presented by the producer and UV-Vis spectroscopy [42].

3.3 Fourier infrared spectroscopy analysis

FTIR techniques were performed to demonstrate the functional collections of the green synthesized NPs [43] and the leaves extracts of *Ficus carica* and rosemary. Figures 5 and 6 illustrate the FTIR spectra of the synthesized NPs by using an aqueous extract of *Ficus carica* leaves and the *Ficus carica* leaf extract, respectively.

FTIR was employed to investigate the synthesis of AgNPs using rosemary leaves and to detect the possible biomolecules accountable for the reduction of Ag⁺ ions and capping the bio-reduced AgNPs synthesized by the plant extract. The absorption bands in the FTIR spectrum (Figures 5 and 6) indicate the presence of active functional groups in the synthesized AgNPs. To obtain a good signal/noise ratio, the FTIR transmission spectra were recorded in the region 400–4,000 cm⁻¹.

FTIR analysis of the synthesized AgNPs (Figure 5a) shows major peaks at 1,057, 1,386, 1,620, and 3,417 cm⁻¹. The peak at 1,075 cm⁻¹ is attributed to primary alcohols, 1,386 cm⁻¹ arises due to NO₃ and may be due to C–N stretching vibrations of aliphatic and aromatic amines. In addition, the peak at 1,620 cm⁻¹ is due to the nitro compounds, whereas the peak at 3,417 cm⁻¹ is attributed to OH stretching in alcohol and phenolic compounds. The FTIR spectrum shows the presence of biomolecules of *Ficus carica* leaves in the solution of AgNPs (Figure 5b).



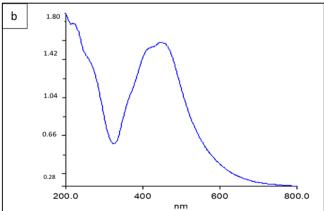


Figure 3: UV-Vis absorption spectra of AgNPs synthesized by an aqueous (a) F. carica and (b) rosemary leaves extracts.

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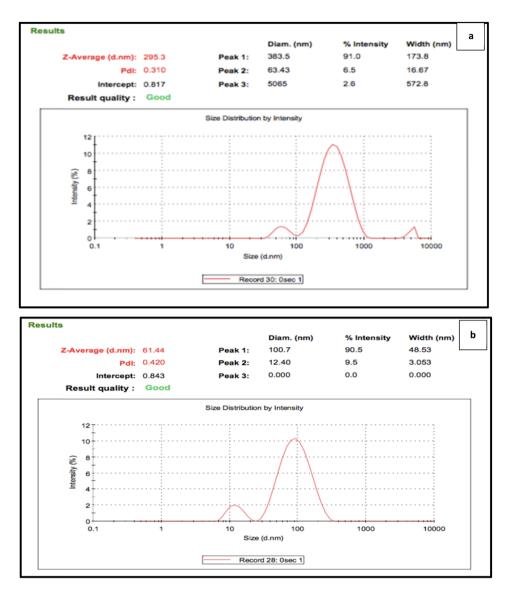


Figure 4: DLS analysis of the synthesized AgNPs using (a) Ficus carica and (b) rosemary extracts.

On the other hand, in the spectrum of rosemary aqueous extract (Figure 6b), peaks at 1,610, 1,384, and 1,057 cm⁻¹ have been attributed to enzymes, amides, and proteins, which appear to be responsible for the decrease of metal ions when using vegetable materials for the synthesis of metal NPs [38]. Some IR bands common to the rosemary aqueous extract appeared in the synthesized AgNP sample (Figure 6a), but the transmittance level of the plant extract bands was weakened after interaction with AgNPs and shifted to 3,431 cm⁻¹ (O–H stretching), 2,366 cm⁻¹ (alkyls C–H stretching), 1,630 cm⁻¹ (assigned to amide I, arising due to carbonyl stretch in proteins) and 1,058 cm⁻¹ corresponding to C–O, C–N stretching vibrations of the aliphatic amines or alcohols/phenols, representing the presence of polyphenols in the rosemary extract [44,45].

3.4 TEM and EDX analysis

The shape and size of the synthesized AgNPs extracted from *F. carica* and rosemary fresh leaves were carried out using TEM analysis (Figure 7a and b). From Figure 6, it can be noted that the shape of the NPs is spherical with a few agglomerations. The obtained results are in line with previous methods (UV and DLS). The UV results display a wide SPR band due to the adsorption of compounds in the extract of leaves on the surface of NPs. In addition, the DLS results illustrate the monodispersity index of the synthesized AgNPs using *Ficus carica* and rosemary; this finding shows that the synthesized particles vary in size and display no agglomeration [46,47]. Furthermore, the EDX spectrometry was carried out and provided both

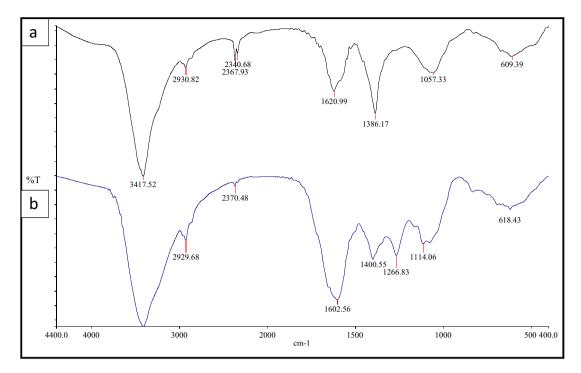


Figure 5: FTIR spectra of (a) the synthesized NPs by using an aqueous extract of Ficus carica leaves and (b) the Ficus carica leaf extract.

quantitative and qualitative details about the elements that were used in the formation of the NPs. Metallic silver nanocrystals generally display peaks around 3 keV because of their surface plasmon resonance [48], and the synthesized NPs using *F. carica* and rosemary displayed greater counts at

3 keV because of the presence of Ag (Figure 7c and d) respectively. On the other hand, the signals of oxygen, carbon, potassium, and chlorine atoms were detected in both synthesized AgNPs. These elements could have acted as capping organic agents attached to the surface of AgNPs [49].

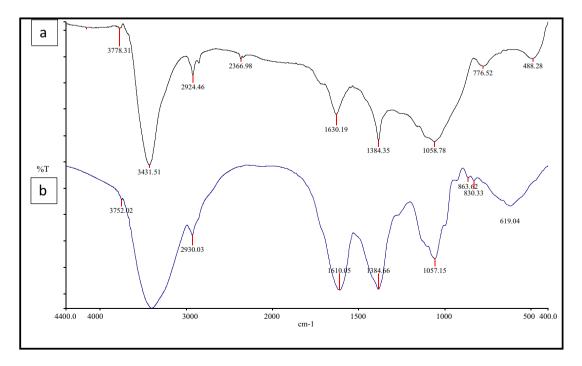


Figure 6: FTIR spectra of (a) the synthesized NPs by using an aqueous extract of rosemary leaves and (b) the rosemary leaf extract.

3.5 Antimicrobial efficacy analysis

The antimicrobial effects of the synthesized NPs and the extracts of *Ficus carica* and rosemary were studied against four different types of pathogenic bacteria including Grampositive *Staphylococcus aureus* (ATCC 43300), *Bacillus cereus* (ATCC 11778), and Gram-negative bacteria *Escherichia coli* (ATCC35218).

As presented in Figures 7 and 8, the synthesized AgNPs from the *Ficus carica* extract show greater antibacterial activity than the synthesized AgNPs using rosemary extract, and poor activity of both extracts against the tested bacterial strains. These results corroborate those obtained by Acay et al. [50] and Logaranjan et al. [51].

Significant activity was seen at three concentrations $(A-2\mu L, B-4\mu L)$, and $D-6\mu L$ of the synthesized AgNPs using the *Ficus carica* extract against *E. coli*, *B. cereus*, and *S. aureus* with zones of inhibition of 10, 16, and 22 mm, 12, 22, and 30 mm, and 35, 40 and 48 mm, respectively

(Figure 8a and b). Similarly, three concentrations $(A-2\,\mu L, B-4\,\mu L)$, and $D-6\,\mu L$) of the synthesized AgNPs using the rosemary extract also exhibited the potent antibacterial activity against *E. coli*, *B. cereus*, and *S. aureus* with zones of inhibition of 10, 16, and 22 mm, 12, 22, and 30 mm, and 35, 40, and 48 mm, respectively (Figure 9a and b).

The *Ficus carica* extract displays activity against *E. coli* and *S. aureus* with zones of inhibition of 10 and 12 mm and for *B. cereus* there was no zone of inhibition; while for rosemary extract, it shows activity against *E. coli*, *B. cereus*, and *S. aureus* with the diameter of zones of inhibition being 6, 3, and 15 mm as shown in Figures 8 and 9.

AgNPs from extracts exhibit remarkable antibacterial activities and could be clarified by the fact that very explicit phytoactive compounds covered the AgNPs from the plant extract. These phytochemicals that might perform as reducing and/or capping agents would be favourably involved in the reduction of AgNPs ions into silver

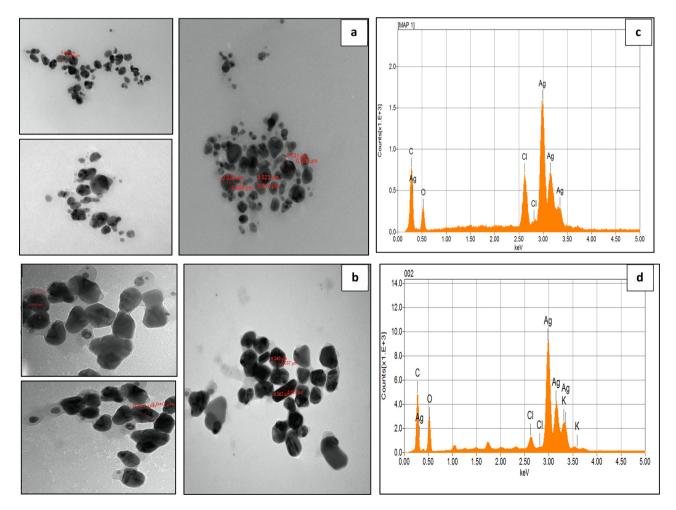


Figure 7: TEM micrographs and EDX spectrum analysis of AgNPs synthesized using F. carica (a and c) and rosemary (b and d) extracts.

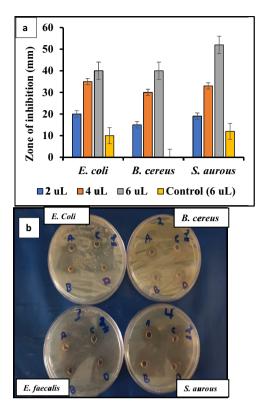


Figure 8: (a) The graphical of the values of inhibition zones (mm); (b) the antibacterial activity of produced AgNPs at A (2 μ L), B (4 μ L), D (6 µL), and C (Ficus carica extract as a control, 6 µL) concentrations against E. coli, B. cereus, and S. aureus.

metal and the phytochemical-assisted synthesis of AgNPs from the plant extract for nucleation, stabilization, and capping [52].

Consequently, the synthesized AgNPs improved the antimicrobial activity owing to the particle size and the aggregation of NPs [50]. In addition, previous studies show that the antibacterial activity depends on the dose of the synthesized AgNPs. In addition, previous studies show that the antibacterial activity depends on the dose of the synthesized AgNPs [53-55]. From Figures 7 and 8, it can be noted that the high concentration of AgNPs causes more antibacterial activity and bacterial cell death, as a result of the interaction between nanoparticles and the protein of bacteria [56,57].

Overall, the interaction between the synthesized NPs and bacteria can be explained as follows:

- (1) the interface among positive charges on the bacteria surface and negative charges in NPs,
- (2) the expulsion of intracellular material that instigates cell death due to physicochemical modifications in the bacterial cell wall, resulting in the NPs stopping DNA replication and respiration of bacteria [58,59].

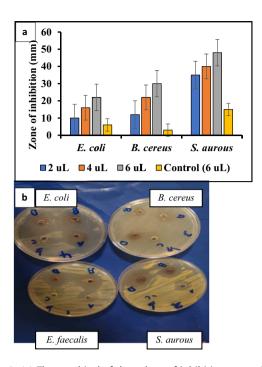


Figure 9: (a) The graphical of the values of inhibition zones (mm); (b) the antibacterial activity of produced AgNPs at A (2 μ L), B (4 μ L), D (6 μ L), and C (rosemary extract as a control, 6 μ L) concentrations against E. coli, B. cereus, and S. aureus.

4 Conclusion

Biological silver nanoparticle synthesis (AgNPs), which is an environmentally friendly method, could be carried out in a variety of applications involving medical treatments. The current study established the non-hazardous materials, eco-friendly, and facile production of AgNPs from plant extracts. The colour shift of the aqueous silver nitrate from colourless to brown with the addition of plant extracts provided the first confirmation of the synthesis of AgNPs. Other analytical methods were employed to study the characterization of NPs, including UV-Vis spectroscopy, FTIR, TEM, DLS, and EDX analysis. These confirmed the successful synthesis of spherical AgNPs and verified the role of extracts' phytochemicals as reducing, stabilizing, and capping agents in the green synthesis of AgNPs.

Furthermore, the biological evaluation of AgNPs obtained through green synthesis exposed good bactericidal properties against E. coli, B. cereus, and S. aureus, all of which are pathogens generally involved in infectious skin diseases. Thus, the wide variety of green synthesized AgNPs as bioactive compounds makes them an ideal agent for controlling infectious agents and useful in other pharmaceutical areas.

Funding information: This research was funded by the Deanship of Scientific Research at Princess Nourah bint

Abdulrahman University through the Fast-Track Research Funding Program.

Author contributions: Najla AlMasoud, Taghrid S. Alomar, and Manal A. Awad: writing – original draft, writing – review and editing, conception, and design of the study. Hajar Alhaik, Malak Almutairi, Asmaa Houjak, Asmaa Houjak, Khlood Hazazi, Fatema Alhayek, Sarah Aljanoubi, Ahad Alkhaibari, and Asma Alghamdi: writing – original draft, methodology, analysis, and interpretation of data; Dina A. Soliman: writing – original draft, and methodology.

Conflict of interest: The authors state no conflict of interest.

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