

An Investigation of Green Synthesis of Silver Nanoparticles Using Turkish Honey Against Pathogenic Bacterial Strains

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Abstract: The green methodology is one of the most rising production methods since this strategy is simpler than other alternate strategies, eco-friendly, and offers minimal time exhaustion for the production. In this study, silver nanoparticles (AgNPs) were synthesized via the green synthesis method using Sidr honey (SH) and Rhododendron honey (RH), and their characterization was determined. A certain proportion of honey for the silver ion was prepared, and the color alteration which demonstrated the development of AgNPs was observed during the production. The characterization of AgNPs has been identified using UV-vis spectroscopy, FTIR, TEM, SEM, and EDX. As a result, SH-AgNPs and RH-AgNPs were determined with the maximum surface plasmon resonance (SPR) absorption at 443 nm and 456 nm; the average particle size of SH-AgNPs, and RH-AgNPs was 14.3 nm and 14.7 nm, respectively. SH-AgNPs (1:1-5mM) and RH-AgNPs (1:1-5mM) showed more improved antibacterial activity than others against *S. aureus* with an inhibition zone 14 mm. Further, the synthesized nanoparticles can be used to manufacture antibacterial drugs and may open the door to enhance the antibacterial activity of honey.

Keywords: silver nanoparticles; green synthesis; honey; antibacterial activity.

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1. Introduction

Honey is one of the most widely consumed natural products in food and medicine. Honey is very important because of its medicinal properties and unique nutrition value, which contain more groups of useful substances for human health, which are used as anti-inflammatory, antioxidant, antimicrobial, and bacteriostatic properties [1-3], in addition to sunburn healing and wound effects. Usually, honey is a more concentrated solution that contains 16% of water, a large amount of carbohydrates which are around 80-85%, 0.1-0.4% protein, antibiotics, vitamins, antioxidants, enzymes, amino acids, and around 0.2% of ash content [4]. Turkey is one of the biggest honey and bee wax producers globally. It has ranked as the fourth largest honey producer in recent years, only after China, USA, and Argentina [5].

Rhododendron honey (RH), which is also known as “mad honey” in public, is obtained from Rhododendron plants widely grown, especially in the Black Sea Region of Turkey [6,7]. It also contains phenolic compounds, which are important agents of secondary metabolites.

These phenolic compounds are special biological active compounds that act as antioxidants and antimicrobials [8-10]. Sidr honey (SH) has strong antimicrobial activities and contains a high percentage of antioxidants that prevent cell damage inside the body [11].

Metal nanoparticles have important applications in numerous research areas. The importance is due to metal nanoparticle's accurate surface area due to distinctive physicochemical characteristics that promote antimicrobial, anticancer activity, chemical stability, electronic, magnetic, catalytic, and optical properties [12-14].

Silver-containing substances were used mostly for medical and therapeutic benefits in the former times, even before gaining information about bacterial infections [15]. At present, Silver nanoparticles (AgNPs) have gained tremendous recognition in nanotechnology owing to their enormous properties such as chemical stability, good conductivity, catalytic, and particularly antimicrobial as well as anti-inflammatory potency [16]. In addition, AgNPs are used in applications such as antimicrobial applications, cosmetic products, bio-sensor materials, electronic components, catalytic applications, and composite fibers [17-20]. AgNPs can be synthesized using physical methods such as electrochemical [21], photochemical [22], and microwave-assisted synthesis [23]. In addition, it can be synthesized using chemical methods, for example, wet chemical synthesis [24], reduction in solutions [25], and biological methods such as bacterial-mediated synthesis [26]. Among these, the physical method gives low yield, requires high energy, the chemical method absorbs toxic material to the metal surface as a result of using chemicals, is difficult to purify, and contains biological risk [27]. The green synthesis method has been the most preferred and researched alternative method compared to other methods due to its ease of preparation, cost-effectiveness, and environmentally friendly nature [19,28,29].

Recent reports on the synthesis of silver nanoparticles using honey-based systems have also suggested their simplicity and usefulness in antimicrobial applications [30,31]. To the best of our knowledge, the synthesis of silver nanoparticles using Rhododendron honey has not been utilized, and their bacteriostatic capability against pathogenic bacterial strains was investigated. The present work investigated and optimized the green synthesis of AgNPs using SH and RH. Therefore, the purpose of this study was to reveal the physical and chemical characterizations and antibacterial activity of these two types of AgNPs-honey (SH and RH) produced in Turkey. Moreover, the structure, morphology, and biologic activity of AgNPs were studied.

2. Materials and Methods

2.1. Collection and preparation of honey samples.

In this study, natural honey samples were collected from different regions of Turkey. Sidr honey (SH) is obtained from the Mediterranean region, while rhododendron honey (RH) is obtained from the Black Sea region of Turkey. Silver nitrate (AgNO_3) was obtained from Sigma-Aldrich with a $\geq 99.5\%$ purity. In preparing the solutions, deionized water was used for all stages of the experiments.

2.2. Green synthesis of silver nanoparticles.

At first, 2 g of honey samples were dissolved in 10 ml of deionized water and then stirred for 5 min to obtain a dilute and homogeneous solution of 20% (v/v). For green synthesis

of AgNPs, different concentrations of AgNO₃ solutions (1, 3 and 5 mM) were prepared and different volume ratios of 1:1, 1:3 and 1:5 (AgNO₃: honey samples (v/v)) were applied at 1mM AgNO₃ concentration. The effect of changing the reducing agent's different volume ratios and concentrations on AgNPs synthesis at different time intervals was investigated to obtain the optimum condition.

2.3. AgNPs detection and characterization.

To characterize AgNPs, UV-vis absorption spectra of the synthesized AgNPs were recorded on a UV-vis spectrophotometer in the range of 300 to 800 nm. The different volume ratios of 1:1, 1:3, and 1:5 (AgNO₃: honey samples (v/v)), the concentration of AgNO₃ solutions, and the color change of AgNPs synthesis were visually monitored at different time intervals (1-24 h) and measured by UV-Vis spectrometer (Thermo Scientific Evolution™ 201/220). All functional groups likely to be found in NPs obtained from SH and RH were determined by FTIR spectroscopy. Pure SH and RH were used for the background. FTIR spectra were recorded in the range of 500-4000 cm⁻¹ at a resolution of 3-4 cm⁻¹. The morphology of the synthesized AgNPs was performed using Scanning Electron Microscopy (SEM) (JEOL-JSM-7001F), and Energy Dispersive X-ray (EDX) analysis (JEOL-JSM-7001F) was performed to determine the elemental composition of the synthesized AgNPs. The energy dispersive spectrometry (EDS) analysis demonstrates the presence of significant silver content. Transmission electron microscopy (TEM) images were obtained on a JEOL-JSM 7001F using a STEM detector with an accelerating voltage of 30 kV. The particle sizes were calculated using the ImageJ software [32].

2.4. Antibacterial tests.

The antibacterial activity of the synthesized AgNPs was tested against gram-negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive bacteria; *Bacillus cereus*, *Staphylococcus aureus* by the standard agar well diffusion method. A swab of pathogenic bacteria was applied on an agar plate of MHA (Mueller Hinton Agar) medium. Wells were made on agar plates using a good puncher, and the wells were loaded with concentrations of 50 µL of the synthesized AgNPs. The plates were incubated for 18 h at 37 °C, and the antibacterial activity was obtained by measuring the zone of inhibition around the wells. Amoxicillin was used as the standard antibiotic and its dose at 2048 µg/ml.

3. Results and Discussion

3.1. UV-Visible spectral analysis.

The change of color from light-yellowish to reddish-brown was observed due to the excitation of electrons, which indicates the reduction of Ag⁺ into Ag⁰ in the synthesis media [33,34]. The synthesized AgNPs absorption peaks were observed up to 24 h (Fig. 1 and Fig. 2). Fig. 1 shows the UV-vis absorption spectra of synthesized AgNPs with different ratios of SH and 1mM AgNO₃ (1:1, 1:3 and 1:5 (v/v)). After 30 min, the UV-vis absorption spectrum shows a weak and broad absorption peak at 350-600 nm, and the intensity of the peak increases up to 6 h, which is shown in Fig. 1. The increased peak represented the characteristic surface plasmon resonance (SPR) absorption of AgNPs formation [35,36]. This means that the concentration of the AgNPs is shown in a gradual increase of SPR peaks intensity for 6 h, but

peak intensity decreases until 24 h. The presence of organic ingredients of the honey on the surface of AgNPs affects the nanoparticle size and the surrounding media leading to a decrease in the intensity of the SPR absorption band [33,35,36]. We can see in Fig. 2 the UV-vis absorption spectra of the synthesized AgNPs with different ratios of RH and 1mM AgNO₃ (1:1, 1:3, and 1:5 (v/v)). No big difference was observed between RH and SH due to the AgNPs formation. The intensity of the peaks increased, and the formation of AgNPs has occurred similarly. The maximum intensity of the absorption peak was observed in the 1:1 ratio for 6 h (Fig. 1A, D) and (Fig. 2A, D). Besides, the 1:1 ratio was tested with 1, 3, and 5 mM of AgNO₃ solutions for 6 h (Fig. 3), and these ratios were used for further studies.

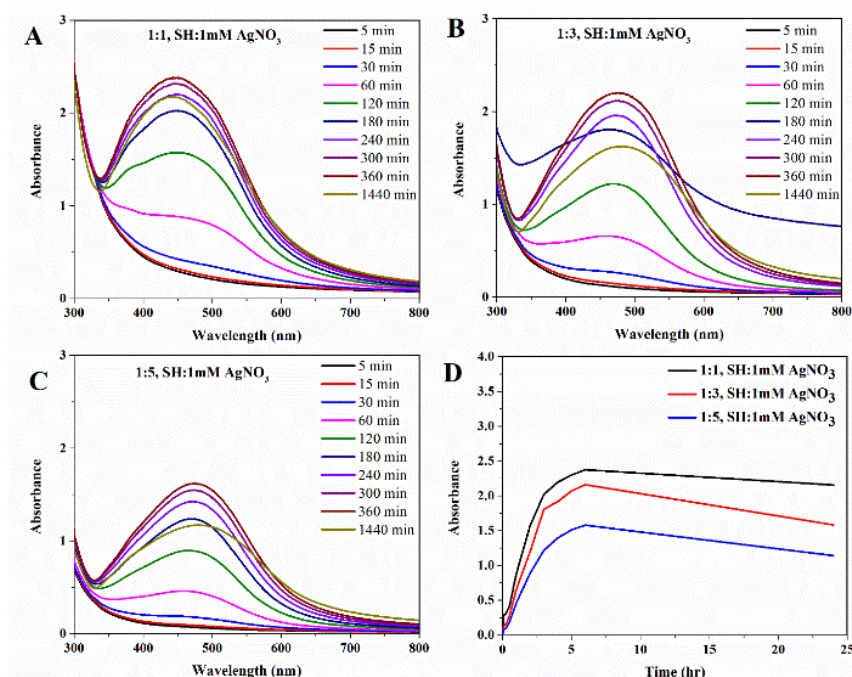


Figure 1. UV-vis absorption spectra different ratios of SH:1mM AgNO₃; (A) 1:1, (B) 1:3, (C) 1:5, and (D) the absorption kinetics of the synthesized AgNPs at the 455 nm with the different time intervals.

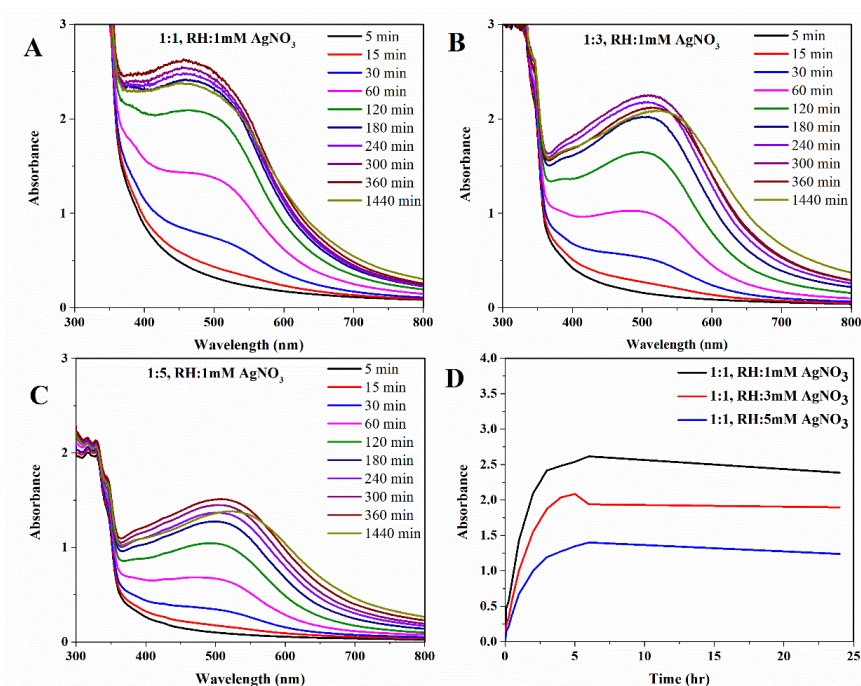


Figure 2. UV-vis absorption spectra different ratios of RH:1mM AgNO₃; (A) 1:1, (B) 1:3, (C) 1:5, and (D) the absorption kinetics of the synthesized AgNPs at the 455 nm with the different time intervals.

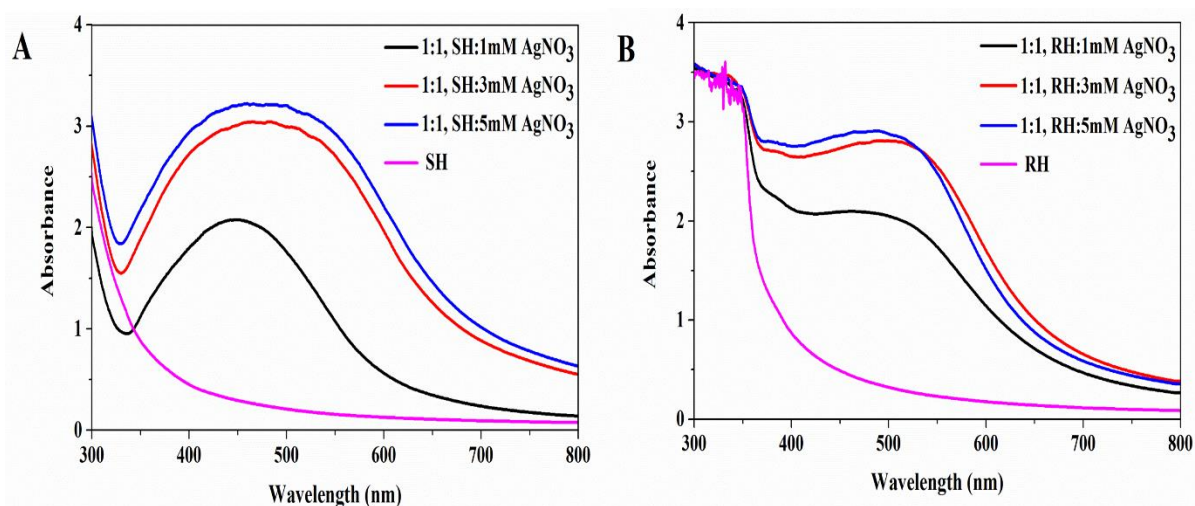


Figure 3. UV-vis absorption spectra at different concentrations of (A) SH-AgNPs and (B) RH-AgNPs.

3.2. Fourier transform infrared spectroscopy (FTIR).

FTIR analysis was performed to detect the presence of various functional groups of biomolecules of honey and honey containing AgNPs. The observed intense bands were compared with standard values to identify functional groups. Representative spectra of both (SH, RH) and SH, RH containing AgNPs manifest absorption peaks in the region 4000–500 cm^{-1} . The FTIR spectrum of both SH and the synthesized AgNPs is shown in Fig. 4A. The FTIR spectra of SH revealed the wide peak at 3272.56 cm^{-1} corresponds to O-H stretching H-bonded alcohols/phenols, and the weak peak at 2930.76 cm^{-1} corresponds to C-H stretching (alkane).

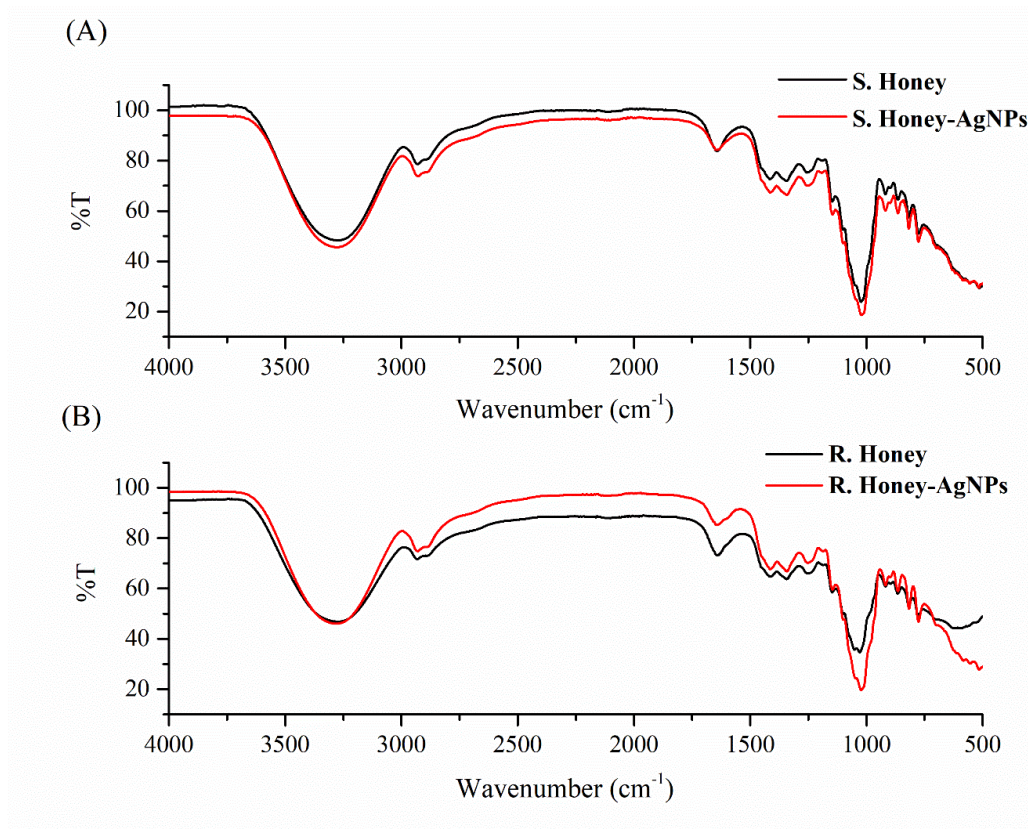


Figure 4. FTIR spectra of (A) SH, SH-AgNPs and (B) RH, RH-AgNPs.

Weak bands at 1643.95, 1416.41, and 1255.99 cm^{-1} correspond to C=O stretching (protein amide I group), deformation vibrations of (O–H) in the C–OH group, and stretching vibrations of (C–O) in carbohydrates, respectively [2]. The strong peak at 1023.04 cm^{-1} corresponds to C–O stretching vibrations indicating the presence of vinyl ether. When AgNPs were synthesized, the bands shifted to 3276.76, 2929.47, 1643.88, 1415.85, 1254.77, and 1020.75 cm^{-1} , respectively. The band at 1643.88 cm^{-1} was detected for natural protein, showing that binding with AgNPs didn't change the honey protein nature [17,37]. The changes seen in the FTIR spectra of the SH-AgNPs are due to the exploitation of these compounds in the reduction and capping of silver nanoparticles. The functional groups involved in RH and the formation of AgNPs using FTIR spectroscopy are shown in Fig. 4B. The broad peak at 3271.98 cm^{-1} in the spectra indicates the existence of O–H group of alcohol [38]. Weak bands at 2931.85, 1642.97, 1415.91, 1252.63, and 1028 cm^{-1} are associated with stretch vibration of C–H aromatic compound stretching, C=O stretching (mainly from carbohydrates), C=C stretching, C–O stretching, and C–O vinyl ether, respectively [39]. After AgNPs synthesis, the bands shifted to 3281.28, 2930.30, 1643.36, 1415.45, 1253.87, and 1023.98 cm^{-1} bands with lower intensity due to the reduction and capping of AgNPs.

3.3. SEM/EDX and TEM analyses.

The SEM and TEM images are presented in Fig. 6 and Fig. 6. It is seen from the figure that most of the silver nanoparticles were well dispersed without any aggregation, and few aggregated particles were also observed. The images reveal spherical shapes and uniform distributions of the nanoparticles. The average particle size of SH-AgNPs and RH-AgNPs are 14.3 nm and 14.7 nm, respectively (Fig. 6a, b).

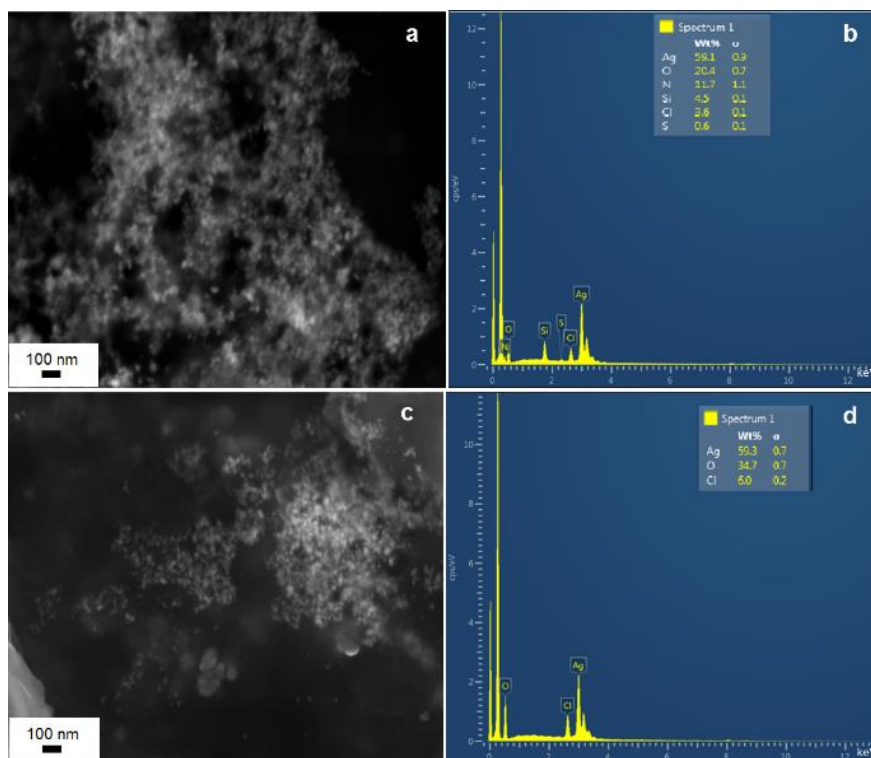


Figure 5. SEM and EDX analysis of the SH-AgNPs (a,b) and RH-AgNPs (c,d).

The EDX analysis of SH-AgNPs and RH-AgNPs showed relative percentage compositions of Ag, O, N, Cl, S, and Si elements. EDX spectrometers have confirmed the presence Ag signal of the AgNPs in the synthesized AgNPs. The percentage of Ag metal found

in occurrence with other chemical elements was found to be substantial, about 59.3%. The other elements originated from honey ingredients such as glucose, fructose, organic acids, vitamins, and minerals and served as capping organic agents bound to the surface of the AgNPs [40].

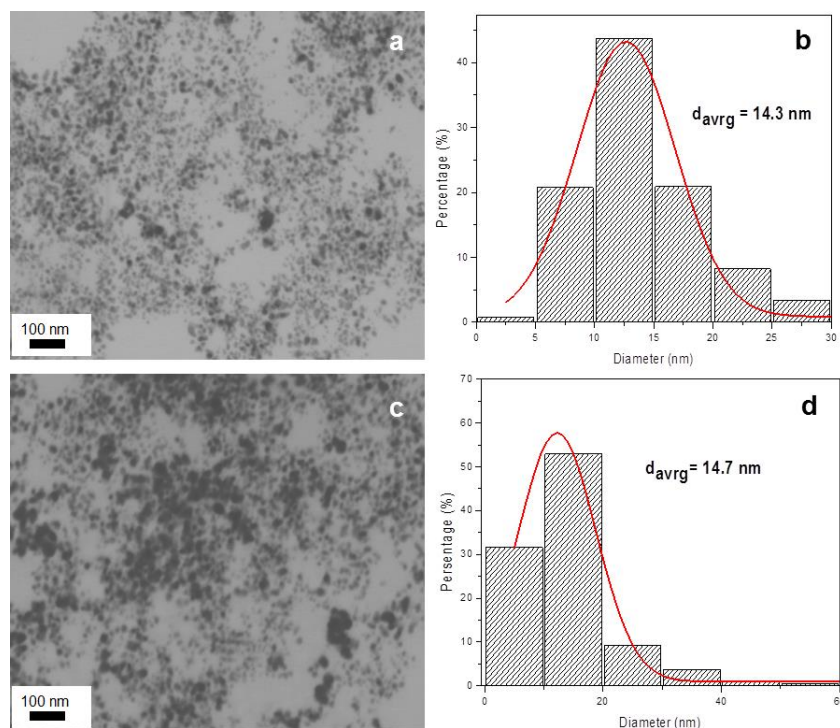


Figure 6. TEM images and particle size distributions of the SH-AgNPs (a,b) and RH-AgNPs (c,d).

3.4. Antimicrobial activity.

Agar well diffusion method was applied to evaluate the antimicrobial performances of the synthesized AgNPs as described by previous studies [41,42]. Gram-negative bacteria; *Escherichia coli*, *Pseudomonas aeruginosa*, and gram-positive bacteria; *Bacillus cereus*, and *Staphylococcus aureus* were chosen for antibacterial tests. The bacterial growth inhibition was studied in plates loaded with 50 μ L of synthesized AgNPs after 18 h of incubation. The zone of inhibition observed at the various concentrations of synthesized AgNPs in the different bacterial strains, *E. coli*, *P.aeruginosa*, *B.cereus*, and *S. aureus*, are shown in Table 1. The inhibition zone of synthesized AgNPs was measured and found between the ranges of 7 mm to 14 mm (Fig. 7). SH-AgNPs (1:1-5mM) and RH-AgNPs (1:1-5mM) showed more improved antibacterial activity compared to others. Silver nanoparticle free-honey samples showed no significant bacteriostatic properties.

Table 1. Results of the antibacterial activity of SH-AgNPs and RH-AgNPs.

Compounds	Gram-negative		Gram-positive	
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. cereus</i>
SH (1:1-1mM)	7.5 $\pm$ 0.4	ND*	ND*	ND*
SH (1:1-3mM)	9.6 $\pm$ 1.3	10.2 $\pm$ 0.5	9.8 $\pm$ 0.4	10.0 $\pm$ 1.4
SH (1:1-5mM)	10.2 $\pm$ 1.2	12.0 $\pm$ 0.4	14.0 $\pm$ 0.6	11.2 $\pm$ 1.2
RH (1:1-1mM)	ND*	ND*	ND*	ND*
RH (1:1-3mM)	8.7 $\pm$ 0.5	9.8 $\pm$ 0.5	10.5 $\pm$ 0.7	9.0 $\pm$ 1.0
RH (1:1-5mM)	10.1 $\pm$ 0.8	11.2 $\pm$ 0.7	14.0 $\pm$ 0.4	10.0 $\pm$ 0.4
Amoxicillin	28 $\pm$ 0.6	ND*	8 $\pm$ 0	40 $\pm$ 1.8

Values were given as mean $\pm$ standard deviation.

*ND not detected. Antibacterial studies inhibition zone diameter (mm).

It was also noted that our findings are consistent with what was previously reported in the literature [17,18]. The antimicrobial activities of NPs differ according to the size of the NPs, the synthesis condition (concentration of the reduced metal and reducing agent), and the strains type [43].

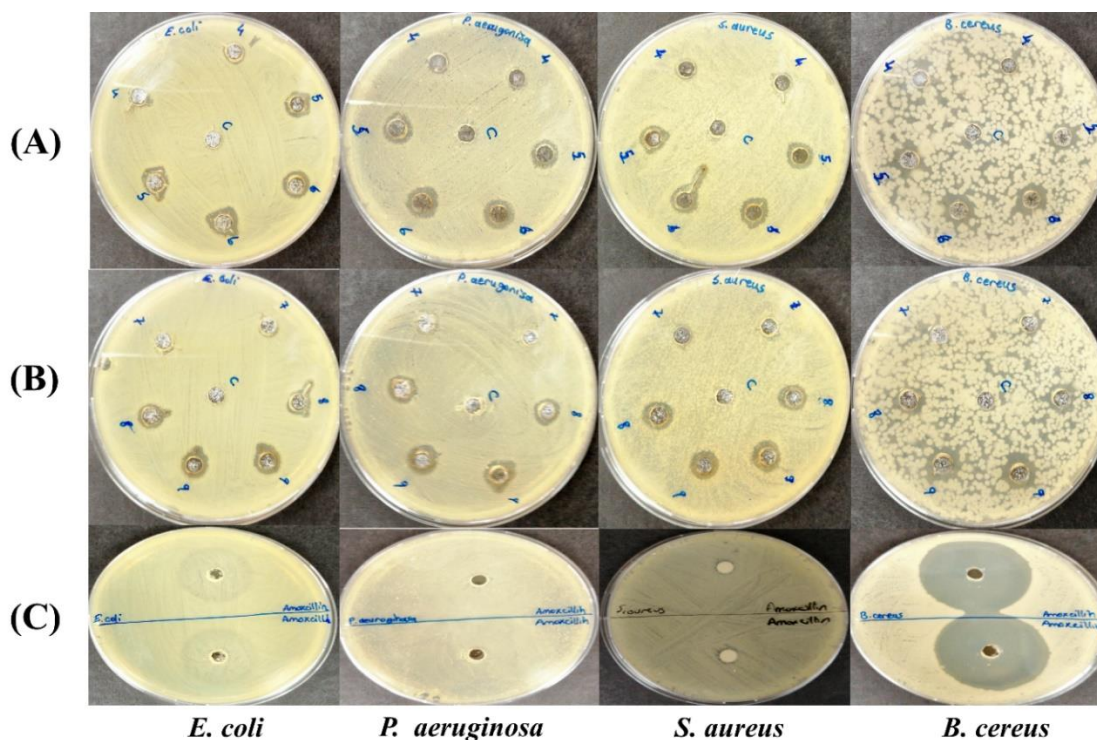


Figure 7. Antibacterial activities of (a) SH-AgNPs, (b) RH-AgNPs and (c) Amoxicillin against *E. coli*, *P. aeruginosa*, *S. aureus*, and *B. cereus*, respectively.

4. Conclusions

A fast, eco-friendly, and convenient green method was used to synthesize SH-AgNPs and RH-AgNPs from AgNO_3 solution using SH and RH honey. SH and RH were demonstrated to be a good source of reducing and capping agents to produce stable and well dispersed AgNPs. AgNPs formation was investigated by UV-vis, FTIR, SEM, TEM, and EDX. The UV-vis spectra of AgNPs exhibit the characteristic peak of 455 nm. FTIR spectra show various functional groups in the formation of synthesized NPs that may result from reducing Ag ions to AgNPs. TEM and SEM analysis demonstrated that the synthesized AgNPs were in spherical shapes, and the average sizes of SH-AgNPs and RH-AgNPs are 14.3 nm and 14.7 nm, respectively. SH-AgNPs and RH-AgNPs showed good antibacterial activity against both Gram-negative and Gram-positive bacteria. Therefore, they can be explored for potential biomedical uses and pharmaceuticals.

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Conflicts of Interest

The authors declare no conflict of interest.

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