

Green synthesis, characterization and optimization of silver nanoparticles using honey and antimicrobial study with food supplements

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Nanotechnology is an emerging field with a vast role in health, cosmetics and industrial applications worldwide. Nanoparticles of silver varying from 1 to 1000 nm are referred to as silver nanoparticles. In the current study, an attempt has been made for the first time to synthesize silver nanoparticles from two varieties of honey available in sultanate of Oman. The existence of silver nanoparticles was identified by UV visible spectroscopy, which showed a characteristic peak at 450 nm. By varying concentration of silver nitrate, honey, pH and temperature, optimization studies for synthesis of nanoparticles were carried out. These parameters play a vital role not only in synthesizing silver particles, but also help to stabilize the nanoparticles. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM), studies were carried out on the nanoparticles to understand their shape, size, structure and optical properties. Antimicrobial studies of silver nanoparticles with honey individually and synergistic effect in combination with food supplements showed significant zone of inhibition against gram-positive and gram-negative bacteria. These findings revealed that honey-based nanoparticles with food supplements could be used effectively to control common diseases.

Keywords: Antimicrobial, Characterization, Food supplements, FTIR, Honey, Nanoparticles, Optimization, SEM, Synergistic effect, XRD.

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Introduction

Nanotechnology has become one of the finest and most sought after area that researchers are applying across a lot of studies. Chemists, physicist, biologists and engineers gave special attention to synthesize nanoparticles for the development of new generation nanodevices¹. Precision engineered nanomaterial's play a significant role in the diagnosis and treatment of diseases effectively. Nanoparticles are very small, ranging from 1 to 100nm in size², although many metals have been used to synthesize nanoparticles, the silver has attracted great attention from scientists due to its vivid chemical and physical properties³. In view of its properties like small particles size, recyclable nature, active antimicrobial role, biosensor, catalyst and bioimaging characteristics currently, they are the choice of many researchers around the globe. Physicochemical methods of synthesis on nanoparticles exhibit a lot of limitations like high cost or high energy consumption and hence are

disadvantageous in synthesizing nanoparticles. In order to overcome these issues, scientists started to use microorganisms such as bacteria, cyanobacteria, algae and fungus for the synthesis of nanoparticles. However, the standardization of microbial cultures, their optimization and maintenance, pose a difficulty in synthesizing and separating nanoparticles⁴. To avoid such unfavourable procedures it was proposed to initiate the green synthesis of nanoparticles, which are environment-friendly and viable. One such method is to use naturally occurring substances for nanoparticle synthesis⁵. Another alternative approach depended on green, eco-friendly materials such as plants, extractions, honey and tea⁶.

Honey is a wonderful gift of nature, produced by *Apis mellifera* from the nectar of plant and is considered as excellent food and traditional medicine source. The major constituents in Honey are fructose and glucose and also contains essential and non-essential amino acids. A roman doctor in his study suggested that honey could be used as a treatment for stomach disease, wound with pus, haemorrhoids, and to stop cough⁷. It is well recognized that certain

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antibiotics when combined with honey, give superior synergistic activity⁸. Taking honey with lemon water stimulates the formation of bile, which further helps the digestive process⁹. Combinations of ginger rhizome powder and honey are also used to treat different forms of respiratory and gastrointestinal infections in traditional medicine¹⁰ in Oman. The combination of honey and ginger displayed high antimicrobial activity against pathogenic gram-positive and negative bacteria¹¹. Therefore in the present study, the combination of silver nanoparticles synthesized from honey was tested with various food supplements which are used traditionally to treat various human ailments. The food supplements used are turmeric, lemon, garlic and Ginger.

Materials and Methods

Chemicals and reagents

AgNO₃ (ACS reagent), Deionized water, Mountain honey, Cultivar honey, Ginger (*Zingiber officinale*), Garlic (*Allium sativum*), Lemon (*Citrus limon*), Turmeric (*Curcuma longa*) UV spectrophotometer/EVO300 PC – Thermo Fisher Scientific, pH meter (HI 98129), Centrifuge (centrifuge 5810 R), FTIR (CPL-MThermo-FTIR-002), SEM, XRD, *E.coli* (ATCC 25922), *S. aureus* (ATCC 25932), *B. subtilis* (ATCC 6633) and *P. aureginosa* (ATCC 27853).

Collection of samples

Mountain honey (Sider), cultivar honey (Abu Twaiq), turmeric, lemon, garlic and ginger were collected from Muscat, Rustaq and Bahlain Sultanate of Oman.

Synthesis of silver nanoparticles

Exactly 15 mL of 2% honey (Sider and Abu Twaiq) was mixed with 20 mL of AgNO₃ (0.1 M and 10 mM) respectively. The reaction mixture was kept at room temperature and the progress of the reaction was observed by a colour change and the surface plasmon resonance absorption was measured using a UV Vis spectrophotometer in the scan range of 200-800 nm.

Optimization of honey concentration

Exactly 15 mL of 3- 6% honey (Sider and Abu Twaiq) was transferred to 50mL volumetric flask. The reduction reaction was initiated by taking 20 mL of 10 mM AgNO₃ in varying concentrations and optimized by adding deionized water¹².

Optimization of Silver Nitrate concentration.

Exactly 15 mL of 5% cultivar and 6% mountain honey was taken in 50 mL volumetric flask and 20

mL of varying concentrations (2, 4, 6, 8, 10, 20, and 30) of AgNO₃ was added to both the types of honey and optimized by adding deionized water until 50 mL mark¹².

Synthesis of nanoparticles at different pH

Exactly 15 mL of 5% Sider and 6% Abu Twaiq honey was transferred to 50 mL volumetric flask. The reduction reaction was initiated by transferring 20 mL of 20 mM AgNO₃ to both the types of honey and the final volume was made up to the mark with deionized water. The pH of the reaction mixtures was adjusted to 6.5,7, 7.5,8 and 8.5 using 0.1 M HCL and 0.1 M NaOH¹³.

Synthesis of nanoparticles at different temperatures

Exactly 15 mL of 5% sider and 6% Abu Twaiq was transferred to 50 mL volumetric flask. To which 20 mL of 20 mM AgNO₃ was added to both the types of honey and the final volume was made up to the mark using deionized water. The reaction mixtures were incubated at temperatures ranging from 45 to 75 °C, at 10 °C intervals in boiling water bath and one set was incubated at room temperature for 10 minutes¹³ as control.

Incubation duration and primary analysis

The reaction mixture for silver nanoparticle synthesis from honey was kept at room temperature and the progress of the reaction was noted by a change in colour and measuring characteristic surface Plasmon resonance absorption band using UV Vis spectrophotometer (Shimadzu Corporation, Tokyo, Japan) for a period of 24 hours in the scan range 200-800 nm with a resolution of 1 nm at a scan speed of 300 nm/min.

Characterization of silver nanoparticles by FTIR, XRD and SEM.

Dried silver nanoparticles from synthesized nanoparticles mixture were prepared through repeated centrifugation and drying using oven and vacuum. Silver nanoparticles were separated from 5% sider honey and 6% Abu Twaiq honey prepared nanoparticles mixture by centrifugation at 10000 rpm for 10 minutes. The supernatant was removed and deionized water was replaced and the process of centrifugation followed by the collection of pellets was repeated thrice. Finally, the silver nanoparticles remaining in the pellet suspension were dried in an oven at 50 °C, then centrifuged and parched material containing nanoparticles was taken for X-Ray

diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) analysis and scanning electron microscope (SEM). X-ray diffraction (XRD) pattern of dry nanoparticle powder was obtained using Siemens D5005 X-ray diffractometer with Cu- K radiation ($\lambda = 0.1542$ nm).

Antimicrobial activity of silver nanoparticles

The test microorganisms were collected in the form of pure stock cultures from microbiology lab, Higher College of Technology, Muscat, Oman. The organisms used are *E.coli* (ATCC 25922), *S aureus* (ATCC 25932), *B subtilis* (ATCC 6633) and *P. aureginosa* (ATCC 27853). The inoculum was prepared by transferring the pure colonies of bacteria from the mother stock plates with a sterile loop into the broth and incubated at 37 °C in incubator shaker for 48 hours. The colloidal solution of the 5% (w/v) silver nanoparticles was prepared by dissolving in dimethyl sulfoxide and sonicating at 30 °C for 15 minutes, while the food supplement extracts (crude) were mixed with the nanoparticle solution in 1:1 (v/v) concentration ratio. Agar well diffusion method was performed to study the antimicrobial study. Four wells of 3 mm size were made in each nutrient agar plates with a sterile cork borer. The wells for each triplicate on single bacteria were loaded with 100 μ L of silver nanoparticles synthesized from sider and Abu Twaiq honey respectively. Prior to the making of wells, the sterile nutrient agar plates were inoculated with 100 μ L of respective O/N bacterial broth. DMSO, AgNO₃ (20 mM) was used as negative control while ampicillin (2 mg/mL) was used as a positive control. Simultaneously a 5% honey from each type was used. All the plates were incubated at 37 °C for 48 hours and the zone of inhibition were measured in mm (diameter) after 48 hours. The measurements were subjected to statistical analysis to determine the significance of nanoparticles as antimicrobial agents.

Results and Discussion

UV-Vis spectroscopy of AgNPs

UV Vis spectroscopy in the scan range of 200-800 was used to follow the formation of silver nanoparticles by reduction of the aqueous silver ions during the exposure to the different concentrations of sider and Abu Twaiq honey. The colourless aqueous silver nitrate solution became yellowish brown a few minutes after the addition of Honey samples. The optimal absorption peak was observed at 450 nm

which confirms the presence of silver nanoparticles. It is well known that the change in colour was due to the free electrons which get excited when nanoparticles are at the surface following a band formation in Plasmon resonance absorption band due to combined vibration of electrons of silver NPs in resonance with light wave¹⁴. Among the different honey concentrations tested with the aqueous solution of 10 mM AgNO₃, the formation of nanoparticles was observed within 30 minutes with 5% sider honey and 6% Abu Twaiq honey at an absorption maxima of 450 nm.

Optimization of Silver nitrate concentration for the synthesis of silver nanoparticles

The formation of silver nanoparticles using a different concentration of silver nitrate (2, 4, 6, 8, 10, 20, and 30) with optimized concentrations of honey it was observed that 20 M concentration gave the narrowest peak than other concentrations at 450 nm in UV-visible characterization. This is due to the availability of more complex functional groups in honey. While with 30 mM concentration the reduction was less because aggregation of nanoparticles occurred due to a competition between silver ions and functional groups¹².

Synthesis of silver nanoparticles using honey with different pH

pH is considered as a physical factor that accelerates the formation of nanoparticles in a short time. It was observed that at lower pH (pH 6.5) the formation of nanoparticles and change in colour to dark brown consumed more time and gave low absorption values. Approximately similar results were obtained with pH 7 and 7.5, while with pH 8 and 8.5 the formation of nanoparticles was observed within 1 minute. This difference information of nanoparticles may be due to the reduction potential of precursors present in honey. In an alkaline environment, honey extracts have more negatively charged functional groups and are capable of sufficiently binding and reducing silver nitrate into nanoparticles, while under the acidic condition there is an increased aggregation of nuclei instead of formation and dispersion of particles¹⁵.

Synthesis of silver nanoparticles using honey of different temperature

Temperature is another physical factor which plays a significant role in the formation of nanoparticles. As per the reaction time is taken to colour change, it was observed that the increased temperature caused the formation of nanoparticles rapidly between 25–45 °C,

but at the higher temperature (55 and 65 °C), oxidation of silver nanoparticles¹⁶ was observed. It was reviewed in many reports that high temperature is conducive to nucleation while the low temperature is conducive to growth in the field of wet-chemical nanoparticle synthesis. Increasing reactive temperature leading to decreased particle size¹⁷. While nanoparticles with larger size can be obtained at a relatively lower temperature.

FTIR analysis

The peaks were observed at varying frequencies in the FTIR spectroscopy. The peaks corresponded to the presence of an amino group of proteins, aromatic compounds, and aldehydes, unsaturated compounds like alkenes, primary amines, amides and stretching of amines. The above spectrum depicts the presence of active functional groups in the infrared spectrum of different AgNPs obtained from mountain honey

(Fig. 1 and 2). Presence of amine and amide group, phenolic compounds, carbonyl group(-coo-) in silver nanoparticles from both the honey play an important role in reduction while, presence of phenolic compounds O-H support the antimicrobial activities¹⁸ in silver nanoparticles from Abu Twaiqhoney. The results of FTIR indicated the importance of honey constituents in maintaining the stability of the produced nanoparticles within the matrix. The binding of honey proteins to AgNPs using both the free amine groups and carboxylate ion of the amino acid residue was previously reported¹³.

XRD analysis

The biosynthesized silver nanoparticles from honey were further confirmed by the characteristic peaks observed in the XRD image as shown in Fig. 3 and 4. The XRD pattern showed 6 intense peaks in the whole spectrum of 2θ value ranging from 0–70 nm. Silver

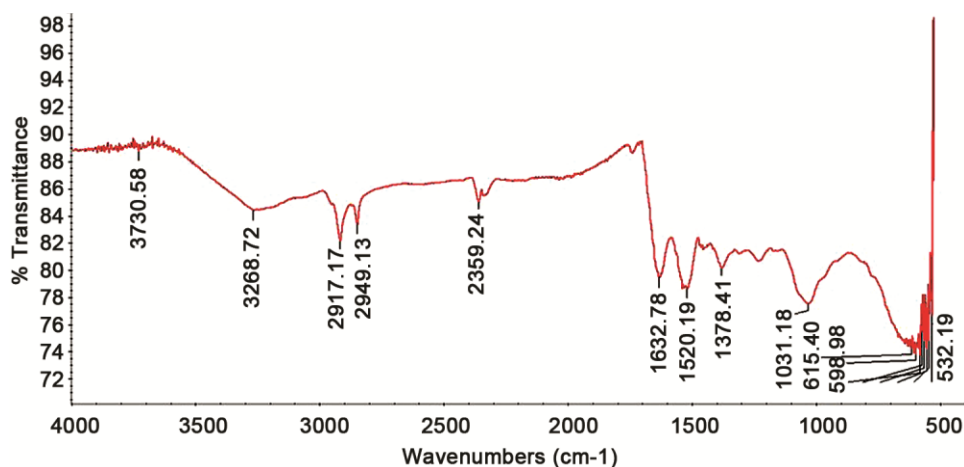


Fig. 1 — FTIR spectra of Sider-AgNPs

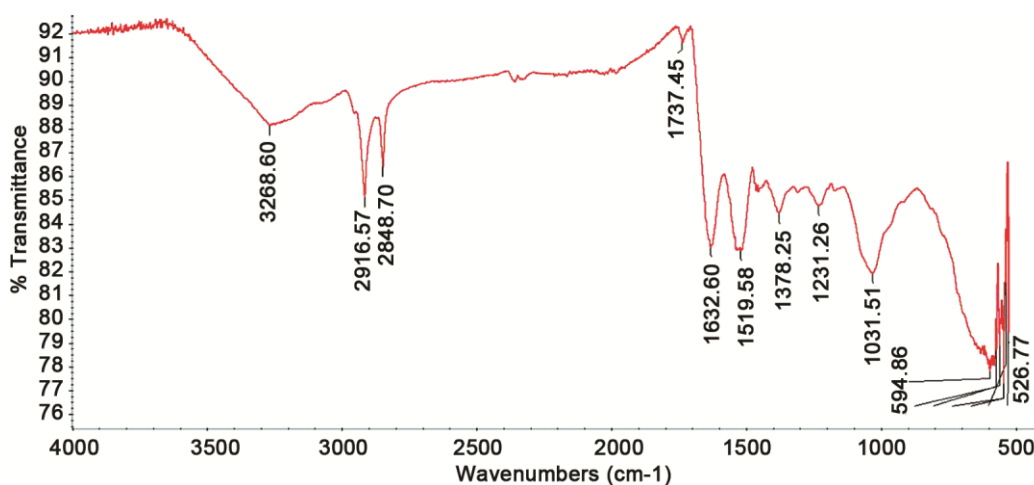


Fig. 2 — FTIR spectra of Abu Twaiq-AgNPs

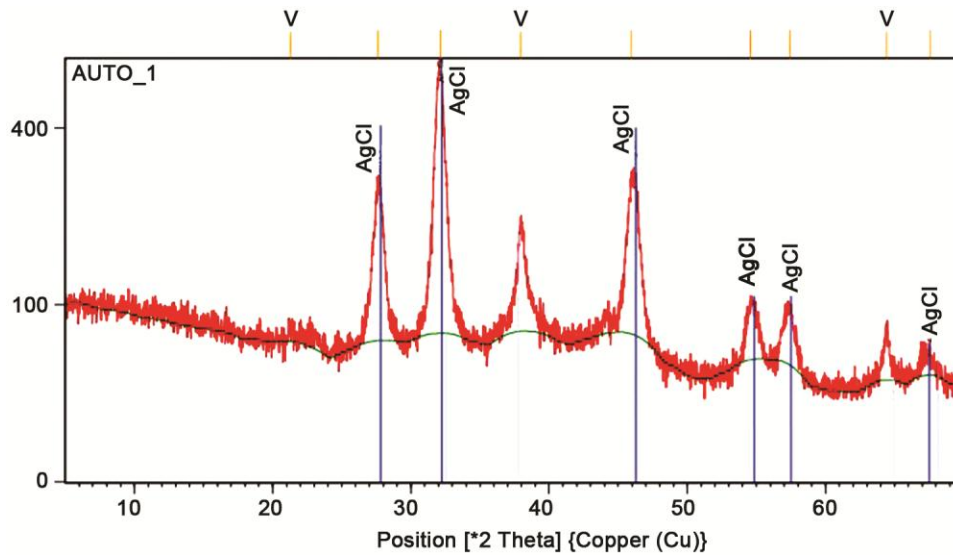


Fig. 3 — XRD data of Sider-AgNPs

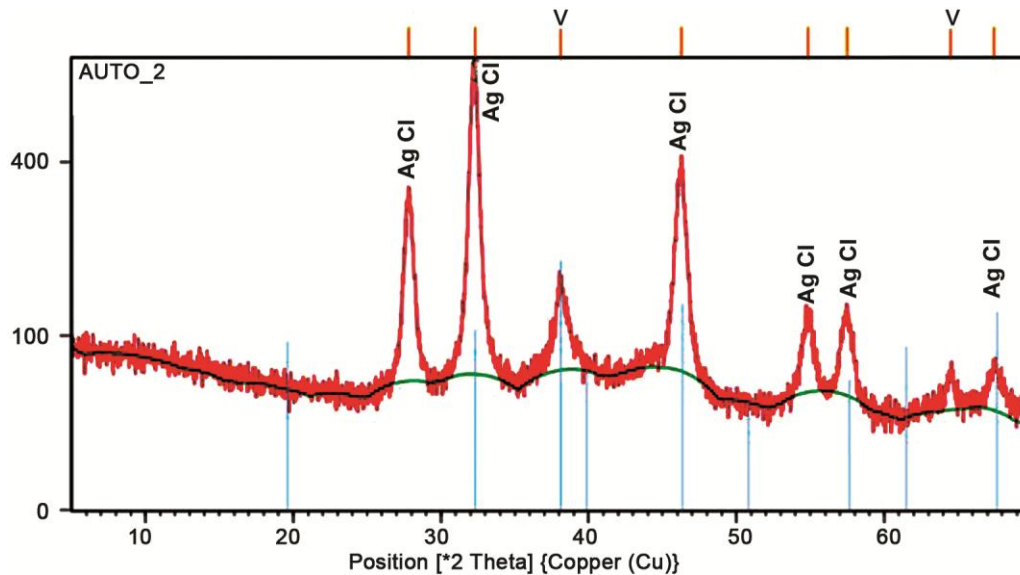


Fig. 4 — XRD data of Abu Twaiq-AgNPs

crystals showed peaks at 27.61, 32.13, 45.98, 54.56, 57.44 and 67.61 could be attributed to the 111, 200, 220, 311, 222, 400 crystallographic planes. The XRD patterns of Ag indicated that the structure of silver nanoparticles is face-centred cubic¹⁹. The average crystalline size of silver nanoparticles was found to be around 20- 90 nm in both the types. The sizes were calculated by the full width at half maximum (FWHM) using Scherer formula. Earlier workers reported similar results for Ag nanoparticles²⁰⁻²². Unassigned peaks found in the XRD might be due to unreacted contaminants with nanoparticles, needs more attention during purification.

SEM study

Surface morphology of Abu Twaiq honey showed in Fig. 5, with large size and irregular shape of silver nanoparticles, were observed and are dependent on the interference of phytochemicals. While sider honey nanoparticle showed in Fig. 6, demonstrated particles of size 100nm with undefined shape. Similar results were also reported for phyto-synthesised silver nanoparticles²³. Amounts of silver nanoparticles with spherical size were very few. The aggregation of particles due to the presence of cell components on the surface of silver nanoparticles as a capping agent and the spherical size of AgNps provide the ability to

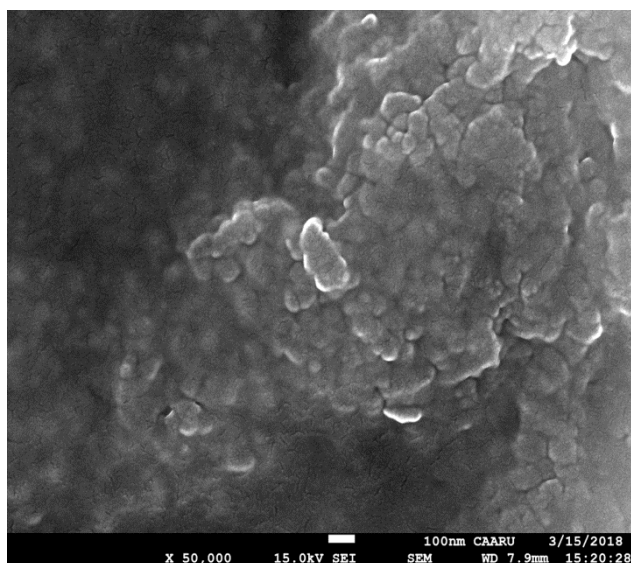


Fig. 5 — SEM image of AbuTwaiq-AgNPs

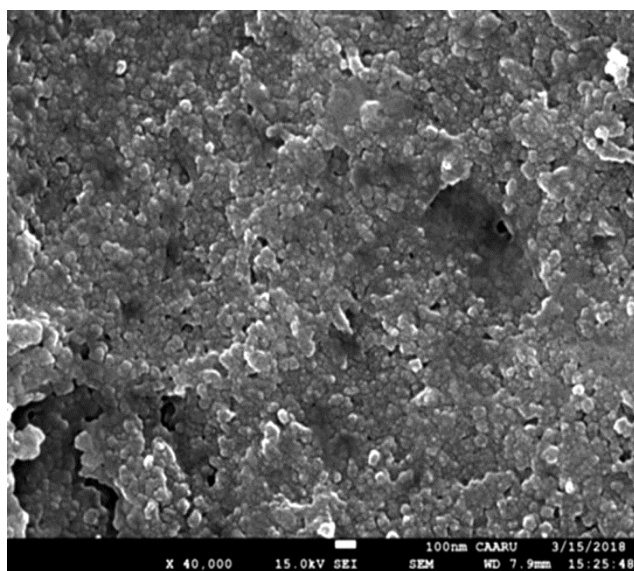


Fig. 6 — SEM image of Sider-AgNPs

penetrate into microbial cells and carry out their bactericidal properties. This result strongly confirms that Honey might act as a reducing and capping agent in the production of silver nanoparticles.

Antibacterial activity of silver nanoparticles

The antimicrobial activity of silver nanoparticles in combination with different supplements was studied against two gram-positive (*B. subtilis*, *S. aureus*) and two gram-negative bacteria (*E. coli*, *P. aurigenosa*). The synthesized silver nanoparticles from two types of honey showed varied inhibition zone with supplements against the tested microorganisms. Among all the combinations studied against tested microorganisms, the maximum zone of inhibition was confirmed against *B. subtilis*. Lemon in combination with MH-AgNPs and Turmeric in combination with CH-AgNPs against G+ve *B. subtilis* showed the highest zone of inhibition (16.5 ± 0.70) showed in Table 1. Ginger in combination with MH-AgNPs showed the highest zone of inhibition against *S. aureus* (15.5 ± 0.70). Overall the antimicrobial activity of silver nanoparticles showed the highest zone of inhibition against G+ve bacterial stains than the G-ve stains, due to the thick cell wall, the zone of inhibition was less against G-ve bacteria. Based on these findings it is clear that the supplements have no significant role with the synthesized AgNPs against different bacteria tested. Also, the impendent study in this project revealed that honey alone has no antimicrobial effect, so only AgNPs considered to be having antimicrobial activity because of the size of silver nanoparticles have high surface area leads to effective interaction and penetration. In addition, the nano size of silver is capable to attack bacteria and penetrate easily inside the cell and make a severe effect on biomolecules²⁴. AgNPs can be used as

Table 1 — Antibacterial activity of different combinations of nanoparticles with food additives

S. No.	Combinations of nanoparticles/ test organisms	<i>E. coli</i>	<i>B. subtilis</i>	<i>P. aurigenosa</i>	<i>S. aureus</i>
1	T+ MH-AgNPs	11.5±0.70	14.5±0.70	10±1.41	13±0.00
2	L+ MH-AgNPs	10.5±0.70	16.5±0.70	15.5±0.70	13±1.41
3	G+ MH-AgNPs	0.55±0.07	11±1.41	12±0.00	11.5±0.70
4	G*+ MH-AgNPs	10±0.00	11.5±0.70	10.5±0.70	15.5±0.70
5	T+ CH-AgNPs	10±0.00	16.5±0.70	10.5±0.70	13±1.41
6	L+ CH-AgNPs	10.5±0.70	12.5±0.70	13.5±0.70	11.5±0.70
7	G+ CH-AgNPs	9.5±0.70	11.5±0.70	11.5±0.70	13.5±0.70
8	G*+ CH-AgNPs	10.5±0.70	13.5±0.70	12±1.41	12.5±0.70
9	Ampicillin	10.5±0.70	14.5±0.70	26±1.41	28±1.41

Note: MH: Sider-AgNPs, CH: Abu Twaiq-AgNPs, G: Garlic (*Allium sativum*), G*: Ginger (*Zingiber officinale*), L: Lemon (*Citrus limon*), T: Turmeric (*Curcuma longa*). The zone of inhibition expressed in the table is the mean of triplicate in mm.

effective antibiotics against *E. coli*, *Salmonella typhimurium*, *Staphylococcus epidermidis* and *Staphylococcus aureus*²⁵. The effect of AgNPs as antimicrobial substance depends on the shape of nanoparticles, studied against *E. coli* and found that triangular-shaped nanoparticles are the most effective shape²⁶.

Conclusion

The reduction of the silver ion into silver nanoparticles was optimized with 5% of mountain honey (sider) and 6% of cultivar honey (*Abu Twaiq*) with 20 mM silver nitrate. The formation of silver nanoparticles was confirmed using UV-Vis spectroscopy in 200-800 nm range where maximum wavelength was noticed with silver nanoparticles at around 450 nm. The size of silver nanoparticles was determined through SEM revealed to be less than 100 nm with varied shape, FTIR spectrum showed all functional groups like amines, phenolic and carboxylic acids and are responsible as capping agent with varied biological function like antimicrobial etc. XRD pattern indicated that the structure of silver nanoparticles is face-centred cubic where the average crystalline size of silver nanoparticles was found to be around 20–90 nm in both types of nanoparticles. The antimicrobial study of nanoparticles in combination with food additives proved that nanoparticles serve as potent carriers to deliver the supplements to the target site more effectively and they could be considered as a potential drug delivery carrier.

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