ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY AND CHARACTERIZATION OF SILVER NANOPARTICLES GREEN-SYNTHESIZED USING SOME MEDICINAL PLANT EXTRACTS

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Abstract. Research on nanoparticles is very important because of their widespread applications in human life, such as in cancer research, food industry, and wastewater treatment. The aims of the current study were to synthesize silver nanoparticles (AgNPs) using different plant extracts and to evaluate their antimicrobial and antioxidant activities. The biosynthesis of silver nanoparticles was confirmed by color changes and characterization techniques such as Fourier transform infrared spectroscopy (FTIR) and ultraviolet–visible (UV–vis.) spectroscopy. The antioxidant properties were evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging methods, and the antimicrobial activities were determined using the well diffusion method. The results confirmed the highest inhibition zone was found in *Syzygium aromaticum*-AgNPs against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*, with 19.0±2.64, 19±2.17, and 19±1.5mm, respectively, followed by *Thymus vulgaris*-AgNPs with 18.0±2.7, 15.0±2.64, and 15.0±2.17mm, respectively. *Artemisia judaica* and *Thymus vulgaris*-AgNPs presented antifungal activity against *Candida albicans* (28.0±2.5 and 25±1.5mm, respectively, compared to 20±2.5 and 13±1.2mm. The AgNPs of *Cinnamomum verum* exhibited the greatest antioxidant activity (92.0%), while the antioxidant activity was only 43.7% for *C. verum* extracts. The green synthesis of silver nanoparticles using plant extracts exhibited antimicrobial and antioxidant activities due to the different functional groups, which will lead to the use of AgNPs in different medical applications in the near future.
Keywords:*nanomedicine, bioactivity, plant extracts, antimicrobial activity*,*radical scavenging*,*FTIR*

Introduction

Recently, nanoscience is attracting increasing attention, and nanostructures have been developed for use in microelectronics as well as medical, and biological studies (Bhagat et al., 2015). Modern research focuses on the designation, synthesis, and development of nanoparticles to be used in pharmaceutical therapy. Nanoparticles can be derived from biological or chemical origins. Microorganisms, enzymes, and plants are considered the foundations of biological nanoparticles. Furthermore, medicinal plants' therapeutic characteristics are adding more value to their derived nanoparticles since many medicinal plants that possess compounds with antioxidant and antimicrobial activities, such as phyto-compounds, show additive potential with these nanoparticles (Pasupuleti et al., 2008; Keshari et al., 2020). Moreover, the use of biological nanoparticles that do not require toxic chemicals during their synthesis is considered advantageous compared to the use of other types of nanoparticles (Forough and Farhadi, 2010; Khali et al., 2014). The distinct properties of metal nanoparticles were, moreover, found to vary depending

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on the method of synthesis (Samat and Nor, 2013). A wide range of metal nanoparticles have been developed and synthesized depending on their intended applications. Au, Ag, Cu, Zn, and many others have been used in metallic nanoparticle synthesis based on the biological method (Samat and Nor, 2013; Bhagat et al., 2015). Silver nanoparticles (AgNPs) are considered a valuable choice due to their toxic potential against causes of disease, such as bacteria, viruses, and fungi, in addition to their unrivaled physical, chemical, electrical, and magnetic characteristics (Bhagat et al.,2015) making them suitable for use in nanomedicine applications such as drug delivery (Praveen et al., 2012), treating cancer (Nayak et al., 2016; Castro-Aceituno et al., 2016), and wound healing (Satyavani et al., 2011; Rigo et al., 2013). The low production yields of AgNPs and the toxicity of their chemical reagents present challenges during the physical or chemical production of nanoparticles. Researchers are also focusing on plant-extract use for AgNP synthesis, thereby avoiding exposure to microorganism pathogenicity. Several extracts derived from plants that are used widely in folk medicine or feature bioactive compounds were employed to synthesize silver nanoparticles such as *Origanum vulgare*, *Brassica nigra*, *Berberis vulgaris* (Salayová et al., 2021), *Azadirachta indica* (Sitaramanjaneya et al., 2018), and *Cestrum nocturnum*. The silver nanoparticles synthesized using *C. nocturnum* extract showed greater potential antioxidant and antibacterial activities against several bacterial strains compared to non-plant-extracted AgNPs (Keshari et al., 2020).

Metabolites found in different plant extracts are responsible for decreasing the particle sizes of nanoparticles because metabolites act as reducing agents (Sitaramanjaneya et al., 2018). Synthesizing nanoparticles from plant extracts can enhance the biological properties of the nanoparticles. Therefore, biological methods for synthesizing nanoparticles using various plant extracts have gained considerable importance in different biological and medical applications (Lalitha et al., 2013;Sitaramanjaneya et al., 2018; Salayová et al., 2021). Recently, silver nanoparticles have been widely used as antioxidant and antimicrobial agents (Asirvatham et al., 2013; Chandra et al., 2014; Bhumi et al., 2015;Sitaramanjaneya et al., 2018). In the present study, *Artemisia judaica*, *Thymus vulgaris*, *Salvia rosmarinus*, *Cinnamomum verum*, *Allium sativum*, and *Syzygium aromaticum were* selected because they used in Jordan as medicinal plants or as food additives and they had not been synthesized previously in Jordan as nanoparticles and test their antimicrobial and antioxidant activity. The main objective of this research was to synthesize silver nanoparticles using different plant extracts and evaluate their antimicrobial and antioxidant activities. The synthesized nanoparticles were characterized via different techniques such as Fourier transform infrared spectroscopy (FTIR) and UV–visible (UV–vis) spectroscopy.

Materials and Methods

Plant Material Collection and Preparation

Aerial parts of *Artemisia judaica, Thymus vulgaris,* and *Salvia rosmarinus* were collected from Al-Mafraq in northern Jordan during March 2021, while *Cinnamomum verum, Allium sativum,* and *Syzygium aromaticum* were purchased as commercial products from Al-Mafraq, Jordan. All plant samples were washed with distilled water, dried in an oven at 60 ˚C, and ground in a blender to achieve a fine powder. Ethanol and distilled water were used for the extraction. Based on the preliminary results, ethanol was used as the extraction solvent in all following experiments.

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Extract Preparation

The extract was prepared using a 500 ml Erlenmeyer flask containing 25 g plant powder and 200 ml ethanol at room temperature for 48 h in the dark. The extract was obtained by centrifuging the mixture at 3500 rpm for 15 min. The obtained extracts were concentrated using a rotary evaporator until completely dried and resuspended in 100 ml ethanol. The extracts were then stored in a refrigerator for further studies (Ashour et al., 2015).

Biosynthesis of Silver Nanoparticles (AgNPs)

To synthesize AgNPs, 0.169 g of silver nitrate was dissolved in 100 ml sterile deionized distilled water (ddw) to form 10 mM $AgNO_3$ solution. Exactly 20 ml of the prepared extract was added dropwise to 80 ml of aqueous $AgNO_3$ (at a ratio of 1:4 (v/v)) at 60 ˚C and stirred continuously with a magnetic stirrer for 2 h. The change in color of the solution indicated the reduction of silver nitrate into AgNPs (*Fig.1*). The solution containing AgNPs was centrifuged at 4500 rpm for 25 min two times, and the resulting pellets were dried in an oven at 90˚C for 48 h. The dried AgNPs were then resuspended in methanol solvent for antioxidant-activity testing and further characterizations (Abdel-Aziz et al., 2014; Sitaramanjaneya et al., 2018; Otunola and Afolayan, 2018; Aritonang et al., 2019; Keshari et al., 2020; Kailas et al.,2020; Deegendra et al., 2020). A visual observation of color changes of the solutions using a laser beam and spectral analysis confirmed the formation of silver nanoparticles. The laser scattered when passing through the nanoparticle solution. However, scattering was not observed when the laser passed through the methanol solvent or extract solutions (*Fig.1*).

(a) (b)

Figure 1.*Image of different solutions facing a laser pointer: (a) AgNPs from the plant extract solution (left) and plant extract (right); (b) AgNP control (left) and methanol solution (right)*

Evaluation of Antimicrobial Properties of Silver Nanoparticles (AgNPs)

Antimicrobial assays of the AgNPs obtained from the plant extracts were carried out according to the agar-well-diffusion method (Alsohaili and Al-Fawwaz, 2014). The antimicrobial activities of AgNPs and plant extracts were tested against two Gram-positive bacterial strains (*Bacillus subtilis and Staphylococcus aureus*), Gram-negative bacteria strain (*Escherichia coli*), and three fungal species (*Aspergillus niger, Penicillium fimorum*, and *Candida albicans*). The bacteria were incubated at 37 ˚C in a nutrient broth before experimental use. The microbial suspensions (overnight bacterial culture) were streaked over the surface of the media using a sterile cotton swab to ensure confluent

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growth of the organism. Wells were then punched in the agar and filled with 75 µl of AgNPs and plant-extract samples. The plates were incubated at 37 ˚C for 24 h, and the fungi were incubated at 28 ˚C for 72 h. Next, the zones of inhibition were observed, and the diameters of the inhibition zones were measured. Then, the plates were photographed. Each test was carried out in three replicates, all values are expressed as the mean±standard deviation (Alsohaili and Al-Fawwaz, 2014; Salayová et al., 2021).

Evaluation of Antioxidant activity of Silver Nanoparticles (AgNPs)

Antioxidant activity was determined by a 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging assay according to Bhakya et al. (2016) with slight modifications (Bhakya et al., 2016; Al-Barri et al., 2021). Briefly, 3.94 mg of DPPH was dissolved in 100 ml methanol to acquire a stock solution. The free radical scavenging activities of the AgNPs, plant extracts, and standard Ascorbic acid were determined using stable radical DPPH by mixing 0.5 ml of the samples (AgNPs or plant extracts) with 2.5 ml of DPPH and allowing the mixture to first stand for 30 min, followed by 60 min at room temperature in the dark. During these times, the absorbance was measured at 517 nm using a spectrophotometer (Jenway 635001-6305 UV/Visible Spectrophotometer). Methanol was used as a blank solution, while ascorbic acid was used as a reference whose efficiency was compared with that of the AgNPs and plant extracts (Al-Barri et al., 2021). The free radical scavenging activity was calculated according to *Equation (1)*:

$$\text{The free radical scavenging activity percentage (\%)} = \frac{\text{Abs DPPH} - \text{Abs Sample}}{\text{Abs DPPH}} \times 100\% \qquad \text{(Eq.1)}$$

where Abs_{DPPH} is the absorbance of the control sample (DPPH solution without the test sample), and $\text{Abs}_{\text{Sample}}$ is the absorbance of the test sample (DPPH solution with AgNPs or plant extracts).

Characterization of Silver Nanoparticles (AgNPs)

The synthesized Silver Nanoparticles AgNPs were characterized using the following methods. (a) UV–visspectroscopy (Specord S 600-Molecular Spectroscopy-UV Vis Diode-array Spectrophotometers, Germany). In this method, the reduction of silver nitrate was monitored at different time intervals(30, 60, 90, 120, 150, 180, and 210 min) by measuring the UV–visible spectrum of the reaction mixture at a scanning speed of 280–680 nm with a quartz cuvette and methanol as a reference. (b)Fourier transform infrared spectroscopy (FTIR)(Bruker Vertex 70 FTIR, wavelength range of 4000-400 cm^{-1}, Germany). FTIR of the biosynthesized AgNPs was used to determine the presence of different functional groups in the sample. The silver nanoparticle (AgNP) solution was evaporated in an oven at 65 °C for 48h to obtain the AgNPs in a powdered form and then loaded into a sample holder using the KBr method. Next, background screening of potassium bromide (KBr) was performed, and a potassium bromide pellet was produced with silver nanoparticles and scanned again. The spectra showed different frequencies, which were analyzed using infrared and Raman spectroscopy. All measurements were carried out in the range of 400–4000 cm^{-1} at a resolution of 4 cm^{-1} to identify the presence of functional groups on the synthesized silver nanoparticles (Deegendra et al., 2020).

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Results

Biosynthesis of Silver Nanoparticles (AgNPs)

In the present study, silver nanoparticles (AgNPs) were synthesized from medicinal plants that are well-known in Jordan and used in folk medicine. The antimicrobial and antioxidant activities were investigated, and then the AgNPs were characterized using UV–vis spectroscopy and FTIR. The formation of AgNPs started after mixing the plant extracts with the silver nitrate solution (*Fig.1*).

Antimicrobial Activity of AgNPs

Tables 1 and *2*outline the antimicrobial results obtained using the well-diffusion method. The ethanolic extract of *S. aromaticum* was active against all tested microorganisms, and *Candida albicans* was the most sensitive. The highest inhibitory activity was seen against *Candida albicans* (30±2.5mm) using the *S. rosmarinus* extract, while the weakest activity was demonstrated against *E. coli* using the *Allium sativum* extract. On the other hand, the aqueous extracts of most plants used in this research presented no inhibitory activities against the tested microorganisms. *Candida albicans* was the most susceptible tested organism to the aqueous extract (22±1.75mm) when using the *S. aromaticum* extract, which also showed inhibitory activity against all tested microorganisms except for *Penicillium fimorum.* The ethanol extracts exhibited better antimicrobial results than the aqueous extracts, so the ethanolic extracts was used in the biosynthesis of silver nanoparticles in the following experiments.

Table 1.*Antimicrobial activities of different ethanol plant extracts using the well-diffusion method*

	Escherichia coli	***Staphylococcus aureus***	***Bacillus subtilis***	***Aspergillus niger***	***Penicillium fimorum***	***Candida albicans***
Artemisia judaica	13±2.17 mm	12±0.25 mm	12±1.5 mm	13±1.73 mm	0	24±2.64 mm
Thymus vulgaris	13±2.64 mm	12±0.5 mm	12±0.75 mm	0	11±0.75 mm	15±2.0 mm
Salvia rosmarinus	12±0.5 mm	14±1.5 mm	14±2.5 mm	16±2.5 mm	0	30±2.5 mm
Cinnamomum verum	11±0.75 mm	12±0.75 mm	0	0	0	10±1.5 mm
Allium sativum	10±1.73 mm	11±1.5 mm	11±0.75 mm	0	12±1.5 mm	26±2.17 mm
Syzygium aromaticum	16±2.0 mm	15±2.64 mm	14±0.25 mm	11±1.5 mm	13±0.5 mm	25±1.73 mm

Table 2.*Antimicrobial activity of different aqueous plant extracts using the well-diffusion method*

	Escherichia coli	***Staphylococcus aureus***	***Bacillus subtilis***	***Aspergillus niger***	***Penicillium fimorum***	***Candida albicans***
Artemisia judaica	0	0	0	0	0	11±1.5 mm
Thymus vulgaris	0	0	0	0	0	16±2.0 mm
Salvia rosmarinus	0	0	0	0	0	0
Cinnamomum verum	0	0	0	0	0	0
Allium sativum	0	0	0	0	0	0
Syzygium aromaticum	17±0.75 mm	11±0.5 mm	11±1.5 mm	12±0.75 mm	0	22±1.75 mm

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Antibacterial Activity of AgNPs

The antibacterial activities of the plant extracts and AgNP solutions were evaluated against one Gram-positive (*Escherichia coli)* and two Gram-negative (*Staphylococcus aureus and Bacillus subtilis*) bacterial species. As shown in *Fig. 2*, the results revealed that the antibacterial activities of AgNPs against *E. coli* were higher than the activities of all plant extracts. Moreover, the antibacterial activities of AgNPs against *B. subtilis* and *S. aureus* were higher than the antibacterial activities of all plant extracts, except for *Cinnamomum verum* and *Artemisia judaica*. The zone of inhibition confirmed that the AgNPs offered greater antibacterial activities than the plant extracts.

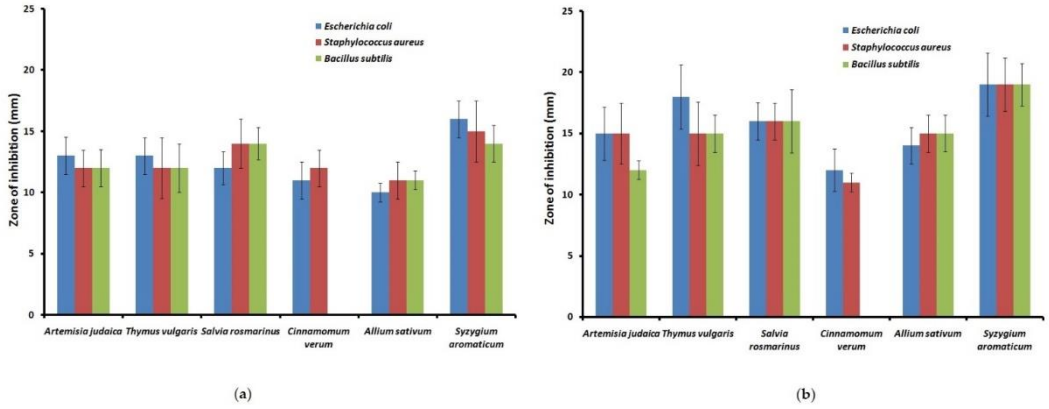

Figure 2. *Antibacterial activities of (a) plant extracts and (b) AgNP–plant against the tested bacterial species for 24 hours. The results represent the means of three replicates, and the error bars represent the standard deviation*

Figure 2 shows that the antibacterial activities of the AgNPs using *T. vulgaris* and *C. verum* against *E. coli* were higher than those against *S. aureus*, whereas the activities of *S. rosmarinus* extracts against *E. coli* were less significant than those against *B. subtilis* and *S. aureus.* When AgNPs of *Allium sativum* and its extracts were tested for antibacterial activity, *B. subtilis* and *S. aureus* showed higher activity than *E. coli*. In both tested *C. verum* solutions, no antimicrobial effect was observed against *B. subtilis.* The results indicated that the plant extract alone exerted less significant antimicrobial effects than those of the plant-based AgNPs, which exhibited a broad spectrum of antibacterial action on the tested Gram-negative and Gram-positive bacteria.

Antifungal Activity of AgNPs

The antifungal activities of the AgNPs and plant extracts were tested against 3 fungi (*Aspergillus niger*, *Penicillium fimorum*, and *Candida albican*), and the results are presented in *Fig. 3*. The results show that the antifungal activity of the AgNP–plant solution was higher than that of plant extracts against *A. niger*, whereas *C. verum* and *A. sativum* showed no activity in either solution (AgNP–plant and plant extracts) against *A. niger.* The maximum zone of inhibition against *A. niger* was17±2.17 mm, exhibited by the AgNPs of*S. rosmarinus* compared to16±2.64 mm exhibited by *S. rosmarinus* extracts. The antifungal activities of AgNPs using *Artemisia judaica*, *Thymus vulgaris*, *Allium sativum*, and *Syzygium aromaticum* against *Penicillium fimorum* were higher than the activities of the related plant extracts. However, there was no antifungal activity

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observed for *S. rosmarinus* and *C. verum* extracts or AgNP solutions against *Penicillium fimorum*. *Candida albican* was susceptible to all plant AgNPs and extracts, the antifungal activities of AgNPs using *Artemisia judaica*, *Thymus vulgaris*, and *C. verum* against *C. albicans* were higher than those of the plant extracts, whereas the antifungal activities of the *S. rosmarinus*, *Allium sativum*, and *Syzygium aromaticum* extracts were higher than those of the plant AgNP solutions.

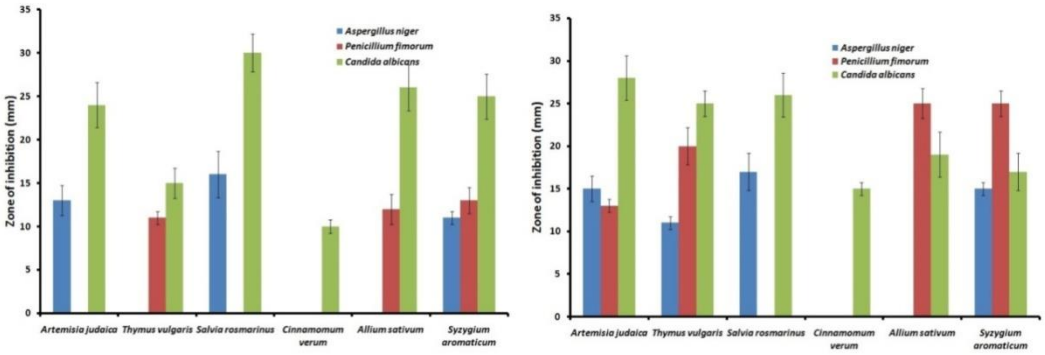

Figure 3. *Antifungal activities of (a) plant extracts and(b) AgNP–plant solutions against the tested fungal species for 24 hours. The results represent the means of three replicates, and the error bars represent the standard deviation*

Antioxidant Activity of AgNPs

The antioxidant properties of plant extracts and AgNPs were evaluated using DPPH scavenging and compared to standard ascorbic acid. As shown in *Fig. 4*, differences were observed between the values obtained. The results confirmed that the plant extracts and AgNPs have antioxidant activities. However, ascorbic acid presented greater antioxidant activity than that of the AgNPs or plant extracts. The recorded value for the lowest antioxidant activity was 18.0% for AgNPs of *Allium sativum*, while the highest antioxidant values were found for AgNPs using *Cinnamomum verum* (92.0%) and plant extract of *Salvia rosmarinus* (92.0%), which is close to the value of standard ascorbic acid (97.0%).

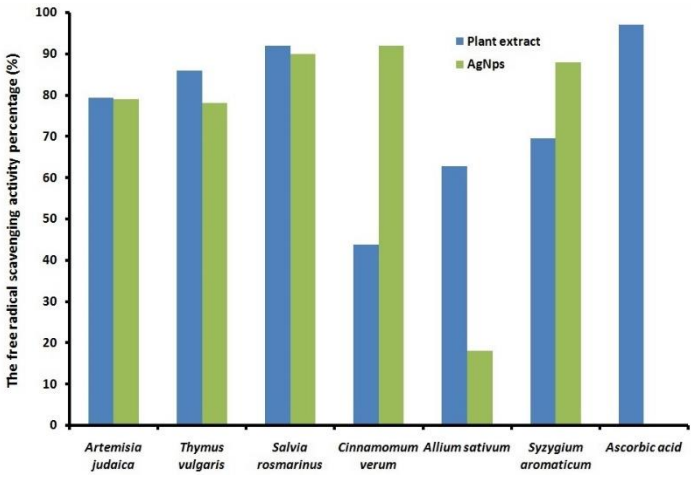

Figure 4. *The percentage of Antioxidant (DPPH) scavenging activity of silver nanoparticles and ascorbic acid*

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The values were 92.0% and 88.0% for *Cinnamomum verum* and *Syzygium aromaticum*–AgNPs, respectively, indicating that silver nanoparticles of these plants exerted higher scavenging activity than did the plant extract alone (43.7%and 69.5%, respectively). However, the antioxidant activity of *Thymus vulgaris* and *Allium sativum* extracts (85.9 and 62.7%, respectively) represented higher scavenging activity than the 78.0% and 18.0% values for AgNPs when using *Thymus vulgaris* and *Allium sativum*.

UV–vis Spectra Analysis of AgNPs

Preliminary characterization of the biosynthesized silver nanoparticles (AgNPs) was carried out using the ultraviolet–visible (UV–vis) spectrum, which is the simplest and most indirect technique able to indicate the formation of metal nanoparticles, provided that SPR (surface plasmon resonance) exists for the metal nanoparticles. Color changes of the extracts confirmed the synthesis of AgNPs. Spectrophotometric analysis of the produced colored solution through a spectra range of 280–680 nm using a Shimadzu UV-vis spectrophotometer showed an SPR hike between 410 and 460 nm, which confirmed the formation of AgNPs (*Fig.5*). The UV–vis spectrum of plant–silver nanoparticles was recorded as a function of time at different time intervals (30, 60, 90, 120, 150, 180, and 210 min). The AgNPs of the plant *Cinnamomum verum* exhibited a peak at 429 nm after 210 min under UV–vis spectroscopy. The formation of AgNPs was monitored by observing the increase in intensity overtime, which also indicated an increase in the amount of nanoparticles formed.

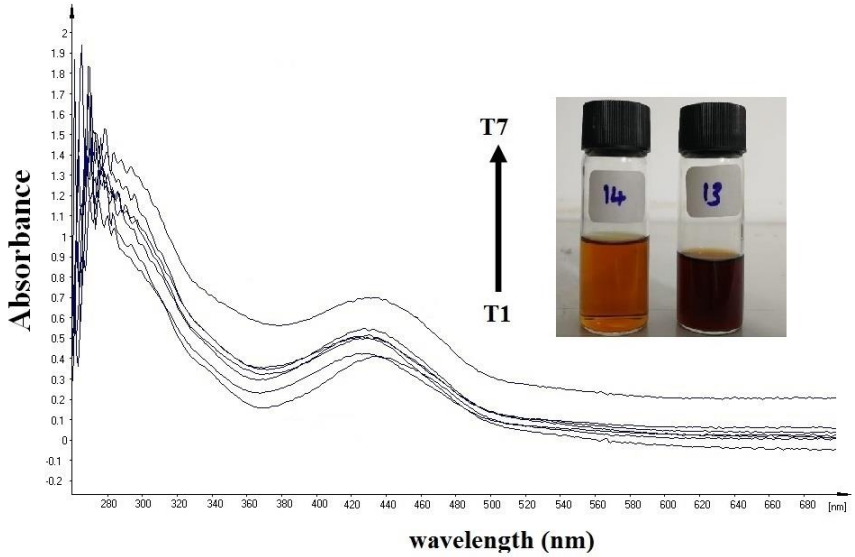

Figure 5.*Ultraviolet–visible (UV–vis) spectra of silver nanoparticles using Cinnamomum verum extract as a function of time at intervals of 30, 60, 90, 120, 150, 180, and 210 min, with transformation the reaction mixture's color*

Fourier Transform Infra-Red (FTIR) Spectra Analysis of AgNPs

Fourier Transform Infrared FTIR spectroscopic analysis was applied to identify the functional groups present on the surfaces of the bioactive compounds and responsible for the reduction of AgNPs from the plant samples. FTIR data for the AgNPs revealed different peaks assigned to different functional groups of bioactive compounds. FTIR

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analysis of the AgNPs was performed in the range of 4000-400 cm^{-1}, as shown in *Figs. 6* and *7*. The FTIR analysis revealed different stretches of bonds at different absorption peaks for each of the bioactive compounds.

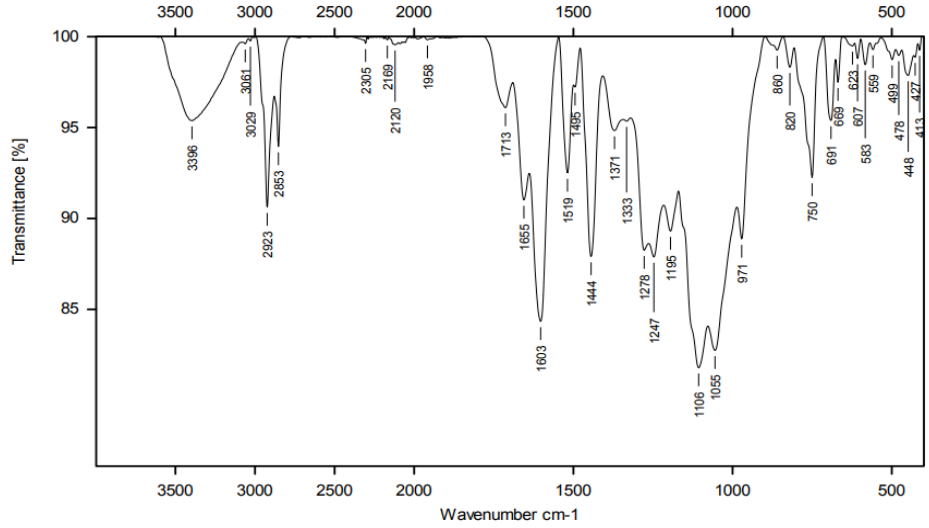

Figure 6. *The IR spectra of Thymus vulgaris plant extract (4)*

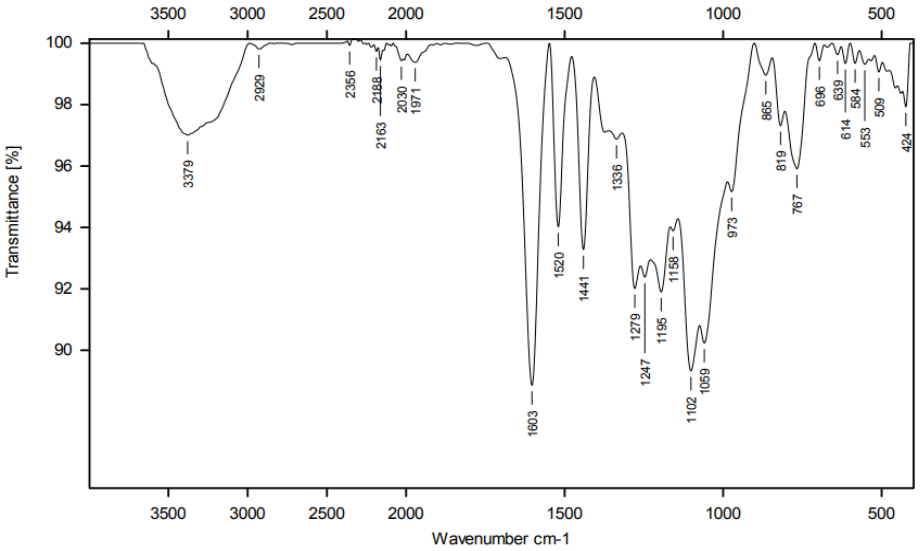

Figure 7. *The IR spectra of AgNPs mediated by Thymus vulgaris plant extract (10)*

Discussion

In the present study, silver nanoparticles were synthesized from the medicinal plants *Artemisia judaica, Thymus vulgaris, Syzygium aromaticum, Cinnamomum verum, Allium sativum,* and *Salvia rosmarinus*, which are used locally as medicine for different types of illness. The biosynthesis of silver nanoparticles has emerged as an alternative to other methods because the biological synthesis of nanoparticles is considered eco-friendly, cost-efficient, and suitable for biomedical and pharmaceutical applications (Sharma et al., 2009; Kumar et al., 2012;Rajan et al., 2015;Ahmed et al., 2016;Mussin et al., 2021).

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Silver nanoparticles using plant extracts were investigated for their antimicrobial and antioxidant activities and were characterized using FTIR and UV–visspectroscopy. Several previous reports in the literature reported that silver nanoparticles can be synthesized by different plants such as *Chenopodium murale*, *Eucalyptus hybrid*, *Gliricidia sepium*, *Carica papaya*, *Azadirachta indica*, and *Capsicum annuum* (Shankar et al., 2004; He et al., 2007; Bar et al., 2009; Dubey et al., 2009; Jha and Prasad, 2010; Raut et al.,2010;Abdel-Aziz et al., 2014; Anand et al., 2020).

Silver ions in the plant extract were reduced to silver NPs after mixing with the plant extract for 3 hours. The plant extracts transformed into a wide range of different colors (dark amber, light amber, reddish brown, and pale yellow). This change of color confirmed the reduction of silver nitrates into silver nanoparticles (Anand et al., 2020). Color change was also observed in several previous reports. These reports suggested that the color changed due to the surface plasmon resonance (SPR) of deposited silver nanoparticles (Vigneshwaran et al., 2006; Saxena et al., 2010; Khandelwal et al., 2010;Abdel-Aziz et al., 2014). The intense peak of the SPR absorption of silver nanoparticles in the region of 250-300 nm was caused by scattering laser due to the Tyndall effect and the AgNPs present in the solution. According to the Tyndall effect, when a laser beam is allowed to pass through the solution, the path of the beam becomes illuminated. This illumination occurs because the laser beam is scattered when it hits the nanoparticles in the solution. When the particles in the solution are less than 1nm, the light passes through without scattering (Goncharova et al., 2019).

Medicinal plant extracts are usually prepared by water or organic solvents to determine their activities and efficacy. In ancient times, people could not use organic solvents, so they instead used water to extract plants. Usually, this process does not extract all the active compounds. Consequently, the produced extracts may not contain all the pharmacologically active compounds (Hussain et al., 2015). In this study, the ethanol extracts of all plants, except for those of *Syzygium aromaticum*, showed greater antimicrobial activity than those of water extracts. All bacterial and fungal species used in this study, except for *Candida albican*, were found to be more resistant against aqueous extracts than ethanolic extracts. Similar results have been reported by Hussain et al. (2015), Nair et al. (2005), Nisha et al.(2013), and Shetty et al.(2016). Antimicrobial activity differs according to the type of solvent used, which may be due to differences in the active ingredients of the plant extracts. Most antimicrobial compounds already identified in plants are reportedly aromatic or saturated organic molecules that can be easily extracted with organic solvents (Cowan, 1999; Nair et al., 2005; Shetty et al., 2016). Thus, in our experiments, ethanolic extracts were used for the biosynthesis of silver nanoparticles.

In recent years, the research and development of new antimicrobial drugs have increased due to an increase in resistant strains (Tacconelli et al., 2018;Mussin et al., 2021). In response, nanoparticles and medicinal plants have been used as promising alternatives for the treatment of various infections (Ahmed et al., 2016; Tacconelli et al., 2018;Amparo et al., 2019; Mussin and Giusiano, 2020;Mussin et al., 2021). The traditional Indian medicine Ayurveda was perhaps the first to use metallic herbal extracts. These extracts are used to treat many diseases. The medicinal properties of silver-extract preparations have been known since the 17^{th} century BC. These preparations were created by using a series of physico-chemical processes to obtain metal extracts after exposure to metallic silver in the presence of medicinal plants (Mukkavalli et al., 2017;Mussin et al., 2021).

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The enhanced antimicrobial activity of biosynthesized nanoparticles is due to the synergistic effect between the natural compounds present in plant extracts and nanoparticles (Duran et al., 2016; Kailas et al., 2020). Plants and their parts contain various compounds that act as reducing agents to produce nanoparticles from metal salts. These compounds include proteins, nucleic acids, fats, carbohydrates, pigments, and several types of secondary metabolites (Khwaja et al., 2018). Various studies reported that AgNPs have shown excellent antimicrobial effects against a wide range of microorganisms (Siddiqi and Husen, 2016a,b;Khwaja et al., 2018).

In the present results, the AgNPs exhibited promising antimicrobial activities against both bacteria (Gram-positive and Gram-negative bacteria) and fungi. The extent of the inhibitory effects on bacterial growth was observed on selected bacterial strains, among which *E.coli* was found to be more sensitive when *Syzygium aromaticum*-AgNP solution was used followed by *Staphylococcus aureus and Bacillus subtilis* strains. Moreover, the bacterial membranes of Gram-positive and Gram-negative bacteria show differences in their structures. The most distinctive difference is the double lipopolysaccharide layer in Gram-negative bacteria and the thick peptidoglycan layer in Gram-positive bacteria. According to a series of reports, Gram-negative bacteria are more susceptible to silver nanoparticles because the positive charges of silver ions interact with the negatively charged lipopolysaccharide of the cell membrane with greater affinity when compared to Gram-positive bacteria; these activities disable cell membrane functions due to holes in the cell membrane (Sondi and Salopek-Sondi, 2004; Gogoi et al., 2006; Otunola and Afolayan, 2018). However, even in Gram-positive bacteria, which have relatively thick and continuous peptidoglycan, cell walls could restrict the entry of bare silver nanoparticles, while interactions of teichoic acid with bioreductive capping agents such as phenol, proteins, esters, etc. of the silver nanoparticles may facilitate their possible entry into Gram-positive bacteria species (Jain et al., 2015; Otunola and Afolayan, 2018).

The antimicrobial activity of silver nanoparticles was previously reported by (Rai et al., 2009; Jha and Prasad, 2010; Khandelwal et al., 2010; Govindaraju et al., 2010;Abdel-Aziz et al., 2014). In the present study, silver nanoparticles produced by different plant extracts were found to be effective against *Candida albican* followed by *Penicillium fimorum* and *Aspergillus niger*, similar to previous reports that showed a zone of inhibition when the synthesized NPs were tested against *Aspergillus niger* and *Aspergillus flavus* (Govindaraju et al., 2010; Abdel-Aziz et al., 2014). Different theories were proposed to explain the antimicrobial actions of colloidal silver solutions, such as membrane damage due to free-radical generation Kim et al. (2007), cell membrane permeability alterations Sondi and Salopek-Sondi(2004) and the release of membrane proteins and lipopolysaccharides (Amro et al., 2000; Abdel-Aziz et al., 2014).

The results of the present study demonstrated the antioxidant activities of plant extracts and AgNPs, compared to those of standard ascorbic acid, using a DPPH scavenging assay. A difference was observed between the plant extracts and AgNPs, while ascorbic acid presented greater antioxidant activity than both solutions (AgNPs and plant extracts). The values recorded for both *C.verum* and *S. aromaticum* nanoparticles (92.0% and 88.0%, respectively) indicated that the silver nanoparticles of these plants possessed higher scavenging activity than that of the plant extract alone (43.7% and 69.5%, respectively). If the plant extract presents some antioxidant activity, the whole solution may exhibit antioxidant activity, but the nanoparticles alone are incapable of having this property (Khwaja et al., 2018). Previous research demonstrated that the antioxidant activities of biosynthesized AgNPs can be attributed to the integration of existing functional groups

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on the surfaces of silver nanoparticles originating from the plant extract (He et al., 2017; Kailas et al., 2020). Similarly, proteins, enzymes, and biosurfactants may act as reducing agents and could be used as stabilizing agents (Khwaja et al., 2018).

However, the antioxidant value*s* of *T. vulgaris* with plant extract alone was higher in scavenging activity than silver nanoparticles. Similar results were also found in *A. sativum extract,* the same results were obtained by other researchers using grapefruit pomace extract/AgNPs and *Thymus vulgaris* and *Thymus citriodorus* (Maliar et al., 2017;Habashy et al., 2018;Kumar et al., 2019; Saratale et al., 2020) these results may indicate the effect of the synthesis of nanoparticles in different plant extract have different trend.

Previous research demonstrated that the antioxidant activities of biosynthesized AgNPs can be attributed to the integration of existing functional groups on the surfaces of silver nanoparticles originating from the plant extract (He et al., 2017;Kailas et al., 2020). Similarly, proteins, enzymes, and biosurfactants may act as reducing agents and could be used as stabilizing agents (Khwaja et al., 2018).

Earlier studies on the antioxidant activities of biosynthesized AgNPs from *P. pinnata* showed considerable free radical scavenging potential (Priya et al., 2016). Moreover, phyto synthesized silver nanoparticles using *Elephantopus scaber* exhibited strong antioxidant activity in terms of DPPH radical scavenging (Kharat and Mendhulkar, 2016). Similarly AgNPs synthesized using plant extracts showed significant antioxidant potential (Nagaich et al., 2016;Roy et al., 2019;Kailas et al., 2020).

Phenolic compounds in plants are strong antioxidants with high reduction capacity. Higher total phenolic content in plants facilitates the reduction of silver ions to nanoscale-sized silver particles due to the electron donating ability of these phenolic compounds. It is well documented that phenolic compounds may contribute to antioxidant activities. However, antioxidant activities are likely attributable to the phenolic contents in plants due to their redox properties, which allow these nanoparticles to act as reducing agents, hydrogen donors, and singlet oxygen quenchers (Pietta, 2000;Chang et al., 2001; Awika et al., 2003; Nsimba et al., 2008). Moreover, the increase in the antioxidant activity of AgNP extracts, compared to that of some plant extracts, suggests that the plant extract itself is responsible for the majority of antioxidant activity, whereas silver nanoparticles contribute little to antioxidant activity (Abdel-Aziz et al., 2014). The FTIR results confirmed the various functional groups present on the surfaces of the bioactive compounds. The DPPH assay confirmed that the AgNPs have antioxidant scavenging activities. These properties of silver nanoparticles emerge due to the presence of functional groups on the surfaces of silver nanoparticles (Anand et al., 2020).

The primary method for detecting AgNPs produced through the bioreduction of Ag^+ was by a visual color change and confirmed by UV–vis spectral analysis (Salayová et al., 2021). In the present study, a single peak between 410 and 460 nm in the UV–visible region, with an absorption peak at 429 nm, was recorded via surface plasmon resonance (SPR), which confirmed the formation of silver nanoparticles (Kailas et al., 2020). The intensity of the color increased as incubation time increased. Previous studies reported that silver nanoparticles give an absorption peak at 420–450 nm as a result of the SPR characteristics of the nanoparticles (Mohanta et al., 2016; Nayak et al., 2016; Mohanta et al., 2017). Other reports have shown that AgNPs exhibit an absorption peak in the range of 410–450 nm based on SPR (Yazdi etal., 2019; Mohammad et al., 2020; Heikal et al., 2020). The SPR band in the UV–vis spectrum is due to electron oscillation around the surfaces of the nanoparticles. Moreover, SPR is dependent on the size, shape, and

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agglomeration of silver nanoparticles, which are reflected by the UV–vis spectra (Mohammad et al., 2020). However, the lack of LSPR suggests the formation of ultra small silver nanoparticles or silver clusters containing a small number of atoms (Santos et al., 2015; Anand et al., 2020).

The IR spectra of AgNPs derived from *Artemisia judaica* 1, *Cinnamomum verum* 2, *Allium sativum* 3, *Thymus vulgaris* 4, *Syzygium aromaticum* 5, and *Salvia rosmarinus* 6 extracts were determined to identify the functional groups responsible for the stabilization of silver nanoparticles. The IR data of the AgNPs mediated by plant extracts were analyzed and compared to the IR data observed for the extract control before reacting with $AgNO_3$.

The IR spectra of extracts 1-6 showed very strong absorption peaks in the region of 3300-3428 cm^{-1}, whereas the IR spectra of the AgNPs mediated by the plant extracts (7-12) showed that the absorption of the OH peaks shifted to a lower wave number and was only observable in the range of 3284-3415cm^{-1}. For example, the absorption peak of the hydroxyl group of *Thymus* plant extract 4 appeared at 3396 cm^{-1} (*Figure 6*), while the peak at 3408 cm^{-1} revealed O–H stretching vibrations, which were induced by the presence of alcohol and phenol (Raut et al., 2010; Deegendra et al., 2020). The absorption for the same group shifted to a lower frequency and appeared at 3379 cm^{-1} for the AgNPs mediated by *Thymus* plant extract 10 (*Figure 7*).

Moreover, the IR spectral data showed that the absorption peaks of carbonyl groups were lower for the AgNPs mediated by the plant extracts compared to the absorption peaks of the plant extracts, indicating the interaction of the silver cation (Ag^{+}) with the carbonyl group. For example, the absorption peak of the carbonyl group of *Thymus* plant extract4 appeared at 1713 cm^{-1} (*Figure 6*), where absorption of the carbonyl group disappeared for AgNPs mediated by *Thymus* plant extract 10 (*Figure 7*). The IR spectra revealed the absorption of many groups responsible for the stabilization of nanoparticles acting as capping or stabilizing agents. Therefore, these bioextracts may have been involved in the reduction, capping, and stabilization of the produced AgNPs (Otunola and Afolayan, 2018; Sitaramanjaneya et al., 2018).

Conclusions

Compared to other metallic nanoparticles, silver nanoparticles (AgNPs) are considered among the most vital and attractive nanomaterials and are used in different applications of human life. In the present work, green synthesis of silver nanoparticles was performed by using organic solvent extracts of some medicinal plants traditionally used in Jordan. Plant extracts have bioactive compounds that are responsible for the reduction and capping of $AgNO_3$ into AgNPs. The synthesized silver nanoparticles exhibited antimicrobial activities against both Gram-positive and Gram-negative bacteria, as well as some fungi. The AgNPs also displayed high antioxidant activities against free radicals. Silver nanoparticles were further characterized by UV–vis. spectroscopy and FTIR analysis. These AgNPs could be used as antimicrobial agents in medicine, health care, and biotechnology in the future because they are non-toxic, cost effective, and eco-friendly. The long-term effects of silver nanoparticles and other metalNPs on human health and crops, however, remains unclear. Therefore, further studies are needed to fully characterize the cytotoxic activities of silver nanoparticles and other metal NPs against anticancer cells and the effects of these particles on the environment.

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