Green Synthesis of Silver Nanoparticles and Comprehensive Characterization for Biomedical Applications

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Abstract

This study details the preparation and extraction process of *Origanum onites* plant extract collected from Denizli, Turkey. The leaves were meticulously cleaned, dried, and ground before undergoing extraction in a water-ethanol solution using a Soxhlet Apparatus at 100 ºC for 6 h. The resulting extract was evaporated and stored for future use at + 4°C. Afterwards, the extract was utilized in the green synthesis of silver nanoparticles by adding it to a solution of silver nitrate. The impact of temperature on the synthesis of silver nanoparticles was explored across various temperature values (30 ºC, 60 ºC, and 90 ºC). Furthermore, the influence of reaction time on silver nanoparticle formation was investigated at the determined optimum temperature, with examination periods set at 60, 120, and 180 minutes. The influence of temperature on the antioxidant activity and cytotoxicity of the synthesized nanoparticles has been explored across three distinct temperature values. Characterization of the Silver Nanoparticles (AgNPs) included UV–Vis Spectrophotometry for surface plasmon resonance, Scanning Electron Microscopy (SEM) for morphological analysis, Energy Dispersive X-ray analysis (EDX) for elemental composition, Particle size distribution and Fourier-transform infrared spectroscopy (FT-IR) spectroscopy for interpreting chemical bonds and functional groups.

Introduction

Nanoparticles (NPs), which are an important material class in the field of nanotechnology in recent years with sizes ranging from 1 to 1000 nm, have significant applications in the pharmaceutical industries, biomedical, textiles, wastewater treatment and biosensors [1–4]. Today, researchers' studies on the applications of metal nanoparticles such as silver, copper, selenium, zinc, gold, titanium and magnesium among nanoparticles reach enormous dimensions. Due to their unique morphology, controllable geometric structure, high specific surface area, surface energy, catalytic performance and stability, AgNPs attract a lot of attention among researchers and are applied in many fields from electronics to diagnosis and therapy [3–6]. AgNPs have higher antibacterial activity, lower drug resistance and many improved properties than ordinary silver. The synthesis of AgNPs has a wide variety of sub-methods according to the application area, mainly physical, chemical and biological (green) methods [7]. Traditional methods (physical and chemical methods) for the synthesis of NPs are toxic, non-ecofriendly and expensive [8]. The method of obtaining AgNPs through green synthesis, which has become a subject of intense research in recent years, has low energy consumption and separation units, is environmentally friendly, economical and sustainable, and is highly preferred [8–10]. In this approach, AgNPs are synthesized using raw materials (such as microorganisms, plants, and templates) and reagents suitable for green chemistry [5, 8].

Plants have a wide variety of phytochemical compounds (polyphenols, flavonoids, terpenes, saponins, terpenoids, alkaloids, tannins, saccharides, vitamins, chelating proteins etc.) that have an antioxidant effect [11–12]. Since the first article on the green synthesis of AgNPs via the alfalfa (*Medicago sativa*) plant published by Gardea-Torresdey et al in 2003, the mechanism of silver nanoparticle synthesis via plant extracts remains a mystery [13–14]. Studies have shown that the formation mechanism of silver

nanoparticles depends on the type of plant extract used as a reducing agent, and also that many components present in the plant are responsible for the formation of metal nanoparticles [11, 15]. Also various studies show that silver ions in plant material extracts are formed in plants by reduction via proteins [16], polysaccharides [17], vitamins [18] and secondary metabolites [19]. Sharma et al. reported that secondary amine in the protein was considered responsible for the reduction [16]. Ag + ions adhere to the protein surface and are reduced, forming silver nuclei. The resulting silver nuclei are reduced and grow repeatedly and accumulate in the nuclei, forming AgNPs [14]. In other studies, the presence of some functional groups (such as -C–O–C–, –C = O–, –C–O–, –C = C-), polyol components and water-soluble heterocyclic components in phytochemicals serve as reducing and stabilizing agents in metal synthesis [20–22].

The *Origanum onites*, belonging to the Lamiaceae family, is a group of plants that grow in the Eastern Mediterranean region and are widely used in the treatment of various diseases in traditional medicine [23–24]. *O. onites*, known as Turkish thyme, is a perennial and herbaceous plant species [25]. *O. onites* attracts attention as an important potential in the prevention of antimicrobial, antiviral, antioxidant, anticancer, antidiabetic, anti-inflammatory, antimycotic and neurodegenerative disorders, especially due to its strong phenolic content [26]. Essential oil products of *O. onites* have “Generally Recognized As Safe (GRAS)” (ESO, GRAS—182.20) status [27]. Also; AgNPs synthesis via *O. onites* is limited in the literature. This makes it an environmentally friendly and valuable natural resource that finds many applications, especially in the food and pharmaceutical industries. In 2021, Genc reported the synthesis of AgNPs with methanol extract of *O. onites* for the first time; It has been determined that phytochemicals play an important role in the reduction of silver nanoparticles and that the synthesized approximately 52 nm nanoparticles have high antioxidant activity. The absorption peak was observed in the UV-Vis spectrum at 450 nm. By FTIR analysis, it was observed that secondary metabolites were also present in silver nanoparticles, and by XRD analysis, the synthesized nanoparticles were observed to have a crystal structure [28]. In another study in 2023, Geçer reported the cytotoxic, apoptotic and necrotic processes of AgNPs synthesized by *O. onites*. The absorption peak of AgNPs formation was observed at 433 nm, dimensional analysis of AgNPs was determined at 18.1 nm, characteristic peaks were revealed by FTIR, and the face-centered cubic unit structure was indexed by XRD. Additionally, in the study, cytotoxic activity was performed by MTT test on Capan-1, L929 and Caco-2 cell lines. While AgNPs showed cytotoxic activity in Capan-1 and Caco-2 cell lines, no activity was observed in the L929 cell line [29].

In the present study, the synthesis of silver nanoparticles (AgNPs) through environmentally friendly methods, particularly employing *O. onites* extract, demonstrates a sustainable approach, underscoring the potential versatility of these nanoparticles in various applications. Furthermore, the research extensively investigated the antioxidant properties of the synthesized AgNPs using the DPPH● radical scavenging method, revealing a significant temperature-dependent increase in scavenging capacity, notably at 60°C and 90°C. The observed results imply potential advantages for biomedical and therapeutic applications. Additionally, the evaluation of cytotoxicity on human fetal lung fibroblast (MRC-5) cells demonstrated the biocompatibility of AgNPs over a 48-hour incubation period, further emphasizing their suitability for safe

utilization in medical contexts. In conclusion, the eco-friendly synthesis of AgNPs utilizing *O. onites* extract, coupled with their verified antioxidant activity and biocompatibility, underscores their pivotal role in advancing sustainable nanomaterials for diverse applications.

Material and Methods

Materials

The plant material was collected from Denizli, Turkiye. Silver nitrate ($AgNO_3$) was purchased from Chem Pure (U.S.A). Ethanol was obtained from Isolab (Turkiye). Gallic acid, Folin-Ciocalteu's phenol reagent and sodium carbonate were obtained from Merck KGaA Chemical Co. (Germany). Dimethyl sulfoxide (DMSO) was purchased from Carlo Erba (France). DPPH (2,2-Diphenyl-1-picrylhydrazyl) and ethanol were supplied from Sigma–Aldrich (Germany). MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from Biotium (USA). Antimicrobial Susceptibility Testing discs; Tetracycline, fluconazole and blank were purchased from Bioanalyse (Turkey).

Preparation of Plant Extract

O. onites was collected in March 2022, from Denizli Turkiye. The leaves were washed with distilled water and completely dried plants were ground using mechanical mortar. 20 grams of sample were extracted in water/ethanol solution (30/70) at 100°C in a Soxhlet Apparatus for 6h. The extracted solution was evaporated at 40°C. After solvent was removed, the extract was stored at + 4°C for further use.

Green Synthesis of Silver Nanoparticles

Briefly, 1 mM $AgNO_3$ was dissolved in 50 ml of distilled water. 10 mg extract was added to the silver nitrate solution under continuous stirring for 3h at 30-60-90°C. At the optimum reaction temperature, the same experimental process was followed for the formation of AgNPs at different reaction times (60-120-180 min). After incubation, the reaction mixture color transformed from light yellow to a dim brown color. After the end of the reaction time, the mixture was allowed to cool and then centrifuged (Hitachi/CR22GII, Japan) at 11000 rpm for 30 minutes. The resulting product was then washed several times with deionized water and centrifuged again under the same conditions (Fig. 1). The resulting black precipitate was kept in an oven at 40°C until completely dry [28, 30].

Characterization

The surface plasmon resonance of NPs was performed using a UV–Vis Spectrophotometer (Thermo Scientific Genesys 150, USA) with a 325–800 nm spectral range. The maximum absorbance was observed at 425 nm. SEM (FEI Company QUANTA 400F Field Emission, USA) was used for morphological analysis. The elemental composition of the obtained samples and the presence of silver in the samples were analyzed by Energy Dispersive X-ray analysis (EDX) combined with FE-SEM during the SEM study. The interpretation of chemical bonds and functional groups was conducted using FT-IR spectroscopy

(Bruker Vertex 80/80v, Germany). Particle size distribution was performed by dynamic light scattering (DLS) using a particle size analyzer. (Zetasizer Nano ZS90, Malvern Panalytical Ltd., UK).

Antioxidant Activity

We investigated the antioxidant activity of the samples using the DPPH● (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method, as developed by Blois in 1958. This assay relies on measuring the scavenging capacity of antioxidants against the stable free radical DPPH●. The odd electron of the nitrogen atom in DPPH● is reduced as it receives a hydrogen atom from antioxidants, forming the corresponding hydrazine. The deep violet color of DPPH● transforms into yellow when mixed with the antioxidants, and the absorbance is measurable at a wavelength of 517 nm [31]. 0.004% DPPH● solution was prepared in ethanol. The prepared DPPH● solution was added to different concentrations of silver nanoparticles in a 1:1 ratio and incubated for 30 minutes in the dark. At the end of incubation, absorbances were measured at 517 nm wavelength (Thermo Scientific Genesys 150, the U.S.A) and percentage DPPH● removal was calculated according to the following formula (Eq. 1) [32]:

% Scavenging = [(AControl - ASample) / AControl] × 100

Antimicrobial Activity

In this study, antibacterial activity of silver nanoparticles obtained by green synthesis on gram positive (Gr+) *S. aureus* ATCC 25923, gram negative (Gr-) *E. coli* ATCC 25922 bacteria and antifungal activity on *C. albicans* ATCC 10231 fungus were investigated. *S. aureus* and *E. coli* were grown in Mueller-Hinton Broth (MHB), *C. albicans* was grown in Sabouraud (2%) Dextrose Broth (SDB). The antimicrobial activity of AgNPs was investigated by the disc diffusion method [33]. Two times activated microorganisms were adjusted to McFarland 0.5 density and smear cultivation was performed by adding 1% to appropriate agar media. Discs were impregnated with 50 µl silver nanoparticles and placed on agar plates and left to incubate for 24 hours at 37°C. At the end of incubation, the zones around the discs were measured and evaluated. Tetracycline (30 µg) and fluconazole (25 µg) antibiotic discs were used as control.

Cytotoxic Activity

Human fetal lung fibroblast (MRC-5) cells were cultured in DMEM supplemented with fetal bovine serum (10%), antibiotic (penicillin–streptomycin solution 1%), and glutamine. The cell cultures were incubated at 37°C in a 5% CO_2 atmosphere. MRC-5 cells were pre-incubated in a 96-well plate at a density of $2x10^5$ cells per well for 24 hours. After incubation, the medium was removed, and DMEM containing nanoparticles was introduced into the wells for testing the activity at 24 and 48 hrs. Cytotoxic activity was determined by the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method. The medium in the wells was removed at the end of the 24th and 48th incubations. MTT solution at 1 mg/ml prepared in DMEM was then added to the wells and kept at 37°C for 2 hours. The production of formazan crystals was analyzed by dissolving the cells with 100 µg DMSO. Subsequently, absorbance was measured at 570 nm, and cell viability was calculated [29].

Results and Discussion

UV–Vis Spectral Analysis

Figure 2 shows the color change of a chemical reaction involving the reduction of Ag^+ ions in the reaction mixture, which manifests itself with a visible color change from light yellow to dark brown.

We used UV-Vis analysis, a widely used technique to follow Ag + ion reduction reactions and confirm the formation of AgNPs. The UV–Vis spectrum of AgNPs exhibits a significant band spanning the visible region from 300 nm to 800 nm, with a particularly sharp surface plasmon peak at 425 nm. This distinctive peak is indicative of the presence of silver nanoparticles, which strengthens the validity of the reduction process. The experimentl design extends to exploring the effect of temperature and reaction time on the Ag + reduction reaction. UV-Vis analysis was performed at three different temperature values (30°C, 60°C, 90°C) at various time intervals (60, 120 and 180 minutes) to obtain a comprehensive data set. The graphical representation of these results in Fig. 3 reveals that the maximum absorbance at 425 nm was obtained if the reaction was carried out for 180 min at 90°C (Fig. 3C).

This meticulous investigation of the temperature and time dependence of the reaction provides critical information for optimizing the synthesis of silver nanoparticles. Specific conditions that provide the highest absorbance at 425 nm are crucial to increase the reproducibility of the Ag^+ reduction process and thus contribute to the advancement of nanoparticle synthesis techniques.

FTIR Analysis

The FTIR spectra of silver nanoparticles synthesized from *O. onites* extract at different temperatures are presented in Fig. 4. A comparison of the FTIR spectra of the extract and silver nanoparticles indicates the formation of silver nanoparticles. The main component of the oil has been determined to be 70.6% carvacrol in the literature [34]. In the FTIR spectrum of silver nanoparticles, a broad band in the range of 3000–3500 cm^{-1} is attributed to OH stretching, observed consistently in all spectra [35]. For the pure *O. onites* extract, a C-H asymmetric stretching band is visible at 2845 cm^{-1}. Peaks corresponding to C–H asymmetric stretching are observed at 2984, 2976, and 2953 cm^{-1} for the a, b, and c spectra, respectively.

Furthermore, the stretching vibration of the C = C functional group appears at 1525 cm^{-1} in the spectrum of *O. onites* extract. This peak is observed at 1543 cm^{-1}, 1551 cm^{-1}, and 1536 cm^{-1} for the a, b, c, and d spectra, respectively. The peak observed at 1070 cm^{-1} in the spectrum of *O. onites* extract (d) can be attributed to the C–O stretching of alcohol. The differences between the extract and nanoparticle spectra are attributed to the oxidation of some molecules in the extract due to the reduction of silver ions.

SEM and EDX Analysis

The morphological form of AgNPs was elucidated using SEM. The distribution of $AgNP_S$ over a large surface area was revealed through SEM images. SEM images were obtained for three different

temperatures (30 ºC, 60 ºC and 90 ºC) values (Fig. 5). The SEM images of *O. onites*-mediated $AgNP_S$ demonstrated the nanoparticles synthesized at all three temperature values were small and spherical in shape and not much aggregated.

The EDX confirmed the formation of silver nanoparticles (AgNPs) (Fig. 6). A prominent, sharp signal at 3 keV distinctly indicates that silver (Ag) constitutes the primary element. Additional signals within the 0–1.0 keV range correspond to the characteristic absorption of carbon and oxygen. SEM images at 300000x magnification (Fig. 6(A)) depicting O. onites-mediated silver nanoparticles revealed the presence of very small and uniformly spherical nanoparticles. Figure 6(B) exhibited a strong signal in the silver region, further confirming the successful formation of AgNPs.

Zeta potential analysis

Particle size distribution curves for AgNPs prepared at different reaction times at 90 degrees are presented in Fig. 7. When the particle distribution of AgNPs prepared at three different reaction times is examined, it is observed that AgNPs with very similar particle sizes are formed. At 90 ºC, the average particle sizes for reaction times of 60, 120, and 180 minutes were determined as 171 nm, 169 nm, and 152 nm, respectively.

Antioxidant Activity

The antioxidant activity of *O. onites*-mediated AgNPs was evaluated using DPPH● (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay (Fig. 8). The synthesized AgNPs had free radical Scavenging activity. The cleaning ability increased in a temperature-dependent manner. In our study, antioxidant experiments were conducted on extraction solutions from three-hour (60, 120, and 180 minutes) processes at all three temperatures (30, 60, and 90 ºC) values. It is evident that samples at 60 ºC and 90 ºC exhibit DPPH● removal efficiency across all concentration levels for 180 min., whereas samples at 6.25 and 12.5 mg/ml concentrations for 30 ºC do not demonstrate DPPH● removal efficiency (Fig. 8A). Figure 8A illustrates that samples at 90 ºC exhibit consistently high DPPH● removal efficiency across all concentration values. For this reason, antioxidant measurements were performed for AgNPs synthesis at 90 ºC for 60, 120, and 180 minutes (Fig. 8B). It was observed that the nanoparticles synthesized for 3 hours displayed the highest DPPH● removal efficiency.

Origanum species are known to be strong antioxidants due to the terpenes, phenols, phenolic acids, flavonoids, carvacrol, thymol etc. compounds they contain [36]. In recent studies, it has been reported that silver nanoparticles obtained from Origanum species are also strong antioxidants and can be used in medical, cosmetic and food industries due to their natural and environmentally friendly nature. Genç et al. (2020) reported that AgNPs (60 ºC, 2 h) obtained from *O. onites* collected from Tokat/Turkey had high DPPH removal (IC_{50},18.42 ± 0.34 µg/mL) and therefore could be used as antioxidant agent in food industry. In another study, DPPH● removal of AgNPs obtained by biosynthesis with *O. majorana* extract at 70 ºC for 2 hours was investigated. As a result of the study, it was reported that the obtained AgNPs

had a high DPPH● removal activity (IC_{50}, 12.25 µg/mL) and could be used in food and pharmaceutical applications [37].

Antimicrobial Activity

AgNPs obtained by green synthesis were found to have antimicrobial activity on all microorganisms studied (Fig. 9, Table 1). Antibacterial activity was found to be higher than antifungal activity. The antimicrobial activity of AgNPs on *E. coli* (Fig. 9B) and *S. aureus* (Fig. 9A) was found to be almost similar. When the 180 minute antimicrobial activities of 30, 60 and 90 ºC temperatures were compared, a low activity was observed at 30 ºC, while high antimicrobial activity was found at 60 ºC (*E.coli* and *S. aureus*: 10 mm, *C. albicans*: 6.5 mm) and 90 ºC (*E.coli*: 10 mm, *S. aureus*: 9.5 mm and *C. albicans*: 7 mm). The antimicrobial activity of nanoparticles synthesized at three different times of 90 ºC temperature (60, 120 and 180 minutes) was found to cause inhibition on microorganisms at all three times. The highest activity was observed at 120 and 180 minutes (Table 1).

Diseases and deaths caused by pathogenic microorganisms are a serious problem worldwide [38]. *Escherichia coli* is a Gram-negative (Gr-) coliform bacteria. It can cause various potentially fatal infectious diseases, including colitis, acute renal failure [39], diarrhea [38]. It has been reported that more than 2 million people die each year from *E. coli*-related diseases [39]. *Staphylococcus aureus* is an important opportunistic pathogen normally found on human skin and nasal mucosa. It is known to be responsible for nosocomial infections [40]. It can also cause pneumonia, endocarditis, meningitis and infection in wounds [41]. *Candida albicans* is a fungus that survives on upper respiratory tract, gastrointestinal and genital mucosal surfaces and skin in humans [42]. *C. albicans* is responsible for intestinal intolerance, vaginal fungi, wound and hospital infections. It has also been reported that it can cross the body barrier and enter the bloodstream and affect the central nervous system, liver, spleen, heart and kidneys [43–44]. Antimicrobial resistance has become a global health problem [45–46]. The World Health Organization (WHO) (2019) report states that at least 700 thousand people die from drug-resistant diseases every year [47] and bacterial antimicrobial resistance caused 1.27 million deaths directly and 4.95 million deaths indirectly in 2019 [46]. Plants stand out as alternative antimicrobial sources. Studies with *O. onites* indicated that the extract [24] and essential oil [47–48] of this species have high antimicrobial activity on resistant pathogens. It is known that silver nanoparticles show very strong antimicrobial activity on resistant microorganisms [49]. Within the scope of the study, silver nanoparticles were obtained at different temperatures and times with *O. onites*, which has known antimicrobial properties, and their antimicrobial activities were compared. The best activity was detected at 180 minutes of 60 and 90 ºC temperatures. There is no literature on the antimicrobial activity of AgNPs from *O. onites*. However, studies with *O. vulgare* are available. Sankar et al. (2013) reported that they obtained a 10 mm zone of inhibition against *E. coli* with silver nanoparticles synthesized with *O. vulgare*, similar to our study [50]. Benedec et al. (2018) reported that the gold nanoparticles synthesized with *O. vulgare* resulted in a high zone on *S. aureus* and *C. albicans*, but did not cause inhibition in *E. coli* [51].

Hambardzumyan et al. (2020) reported that silver nanoparticles from *O. vulgare* were effective on *S. aureus* and *E. coli* [52].

Table 1
Antimicrobial activity (mm) for AgNPs synthesized at 180 minutes at 30 ºC, 60 ºC, 90 ºC and at 90 ºC for 60, 120, and 180 minutes.

	E. coli ATCC 25922	*S. aureus* ATCC 25923	*C. albicans* ATCC 10231
30ºC 180 min	6	8	5
60ºC 180 min	10	10	6.5
90ºC 180 min	10	9.5	7
90ºC 120 min	8	9	6
90ºC 60 min	7.5	8	6
Tetracycline*	21	24	-
Fluconazole**	-	-	18
*tetracycline: antibacterial disc, ** fluconazole: antifungal disc			

Cytotoxic Activity

It was observed that AgNps obtained with *O. onites* were not toxic on healthy cells and even increased healthy cell growth. At 60, 120, and 180 minutes for 90ºC, the viability was 95% and 98% at the 60th minute, but the viability increased at the 120th and 180th minutes. When the 180-minute cytotoxicity of 30, 60 and 90 ºC temperatures was compared, it was observed that the number of living cells increased depending on time. The highest viability was detected at temperatures of 60 and 90 ºC (Fig. 10). Silver has been used in biomedical fields for many years because it is a powerful antioxidant and antimicrobial agent. However, the safety of its use is still controversial. For this reason, it is important that the NPs to be obtained show high biological activity and do not harm healthy cells [29, 53–54]. It is known that the factors used in the synthesis of NPs (temperature, time, method, etc.) affect the size and shape of the nanoparticle to be obtained and this can directly affect the properties of the NPs [51]. In our study, it was observed that different temperatures and times changed the activity of AgNPs and none of the AgNPs obtained did not harm healthy cells. It has been shown that silver nanoparticles obtained from *O. onites* leaves show cytotoxic activity on cancer cells, human pancreatic adenocarcinoma cell lines (Capan-1), and human colon adenocarcinoma cell line (Caco-2), while they do not harm healthy cells, mouse normal fibroblast cell lines (L929). In fact, at some doses it has been shown to increase cell growth, similar to our study [29]. In another study, it was stated that silver nanoparticles obtained by plant-derived green synthesis caused cytotoxicity in MCF-7 (breast cancer cell lines) and Hela (cervical cancer cell line) cancer cells, but did not harm healthy cells NIH-3 T3 (mouse embryonic fibroblast) [55]. In another study

conducted with MCF-7 and healthy L-929 cell lines, it was stated that green synthesis AgNPs selectively inhibited cancer cells but did not harm healthy cells. It is suggesting that cancer cells have an abnormal metabolism and high proliferation rate, making them vulnerable, and AgNPs show superior cytotoxicity against cancer cells due to the high uptake of nanoparticles by these cells rather than healthy cells [56].

Conclusions

In conclusion, this study delineates a comprehensive investigation into the preparation, extraction, and applications of *O. onites* plant extract-derived silver nanoparticles (AgNPs) obtained through a green synthesis approach. The meticulous preparation of the *O. onites* extract involved cleaning, drying, and grinding of leaves, followed by extraction in a water-ethanol solution using a Soxhlet Apparatus. The resulting extract was utilized for the green synthesis of AgNPs, with the impact of varying temperatures (30 ºC, 60 ºC, and 90 ºC) on nanoparticle formation investigated.

The synthesized AgNPs were characterized using UV–Vis Spectrophotometry, Scanning Electron Microscopy, and FT-IR spectroscopy, providing valuable insights into their surface plasmon resonance, morphological features, and chemical composition. The antioxidant activity of the AgNPs was evaluated through the DPPH radical scavenging method, demonstrating a temperature-dependent enhancement in scavenging capacity, particularly at 60 ºC and 90 ºC. Furthermore, the antimicrobial properties of the AgNPs were explored against Gram-positive (*S. aureus*), Gram-negative (*E. coli*) bacteria, and the fungus *C. albicans*. The AgNPs exhibited significant antibacterial activity, surpassing their antifungal effects, with the highest efficacy observed at 60 ºC and 90 ºC synthesis temperatures. Notably, the antimicrobial activity was dependent on both temperature and synthesis duration, with longer durations at 90 ºC demonstrating the highest inhibitory effects.

The study investigated the cytotoxicity of AgNPs on human fetal lung fibroblast (MRC-5) cells, revealing their biocompatibility over a 48-hour incubation period. Additionally, the study found that AgNPs exhibited non-toxicity on a healthy cell line and human fetal lung fibroblast (MRC-5) cells, demonstrating their biocompatibility over the same 48-hour incubation period. Results highlight the potential of O. onites-mediated AgNPs as multifunctional nanoparticles with antioxidant and antimicrobial activities. The temperature-dependent variations observed in their properties open avenues for tailored applications, emphasizing the importance of synthesis conditions in optimizing the desired characteristics.

Performance Evaluation of AgNPs obtained with Origanum onites extract

The performance evaluation of the Green Synthesis of AgNPs presented in this study is commendable, reflecting a meticulous and comprehensive approach to both preparation and characterization. The utilization of *O. onites* plant extract, collected from Denizli, Turkey, adds a unique and environmentally friendly dimension to the synthesis process.

The study systematically investigates the impact of various parameters, such as temperature and reaction time, on the synthesis of AgNPs. The exploration of different temperature values (30 ºC, 60 ºC, and 90 ºC) and reaction times (60, 120, and 180 minutes) provides valuable insights into the controllable factors influencing nanoparticle formation. This attention to detail enhances the reproducibility of the synthesis method and contributes to a more nuanced understanding of the process.

Characterization techniques including UV–Vis Spectrophotometry, Scanning Electron Microscopy, Energy Dispersive X-ray analysis, Particle size distribution, and Fourier-transform infrared spectroscopy contribute to a thorough understanding of the properties of the synthesized AgNPs. The examination of surface plasmon resonance, morphological features, elemental composition, and chemical bonds provides a robust foundation for evaluating the nanoparticles' potential applications.

The study goes beyond mere characterization and delves into the functional aspects of the AgNPs. The evaluation of antioxidant activity using the DPPH radical scavenging method showcases a temperature-dependent enhancement in scavenging capacity. This not only highlights the multifunctional nature of the AgNPs but also emphasizes the importance of synthesis conditions in tailoring their desired characteristics.

The exploration of antimicrobial properties against both bacteria (S. aureus, E. coli) and fungus (C. albicans) reveals significant inhibitory effects, with a noteworthy temperature-dependent variation. The correlation between synthesis temperature and duration with antimicrobial efficacy further underscores the careful consideration of these parameters in optimizing the performance of the AgNPs. The cytotoxicity evaluation on human fetal lung fibroblast (MRC-5) cells is a crucial aspect of the study, demonstrating the biocompatibility of the AgNPs over a 48-hour incubation period. This finding is particularly promising for potential biomedical applications.

The study provides a thorough and well-executed investigation into the Green Synthesis of AgNPs using *O. onites* plant extract. The detailed exploration of synthesis conditions, comprehensive characterization, and functional assessments contribute to the understanding of the AgNPs' properties and potential applications. The temperature-dependent variations observed underscore the importance of synthesis conditions in tailoring the nanoparticles for specific purposes.

Declarations

Competing interests:

The authors declare no competing interests.

Author Contribution

Sema Yiyit Doğan: Project administration, Validation, Conceptualization, Methodology, Data Collection, Writing-original draft, Formal Analysis, Investigation, Visualization, Seçil Kaya: Project administration,

Validation, Conceptualization, Methodology, Data Collection, Writing-original draft, Formal Analysis, Investigation, Visualization, Ebru Kondolot Solak: Supervision, Conceptualization, Visualization, Writing-review & editing.

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Figures

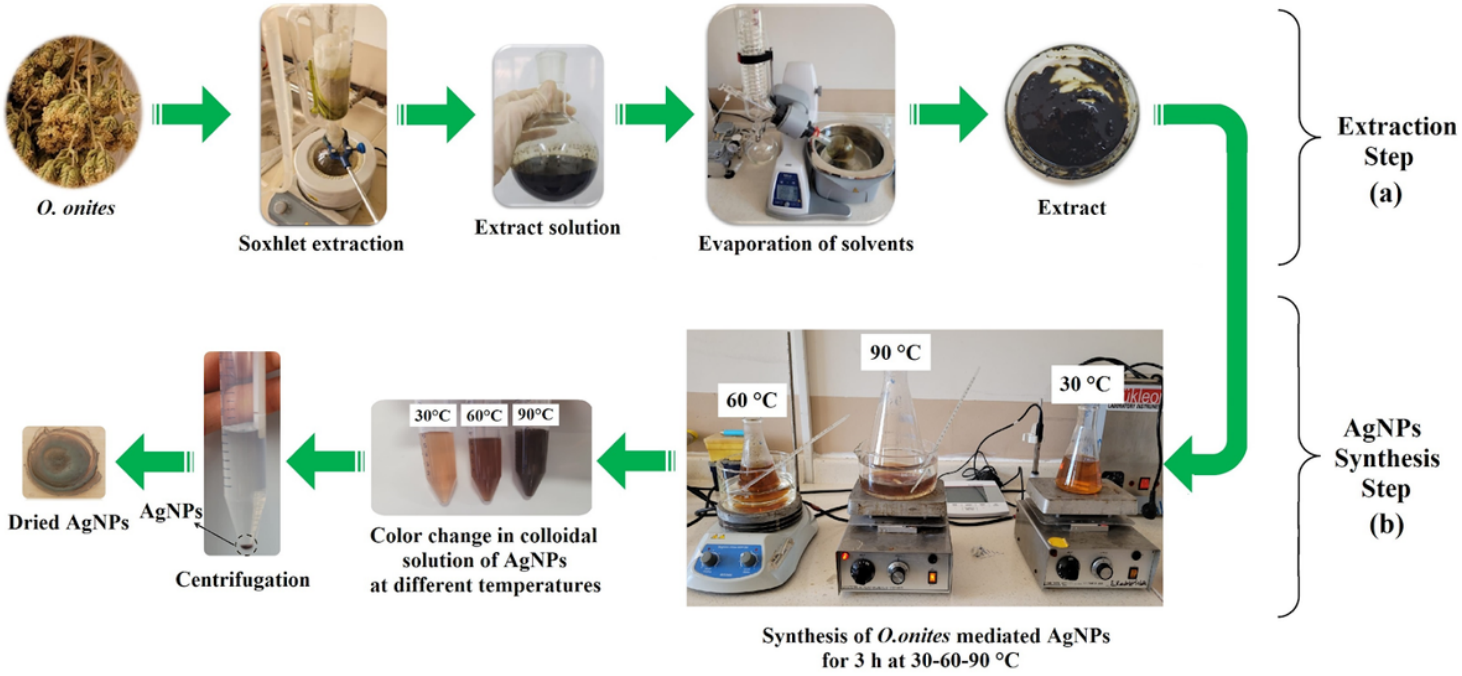


Figure 1

Schematic representation of the preparation of silver nanoparticles from *O. onites*extract

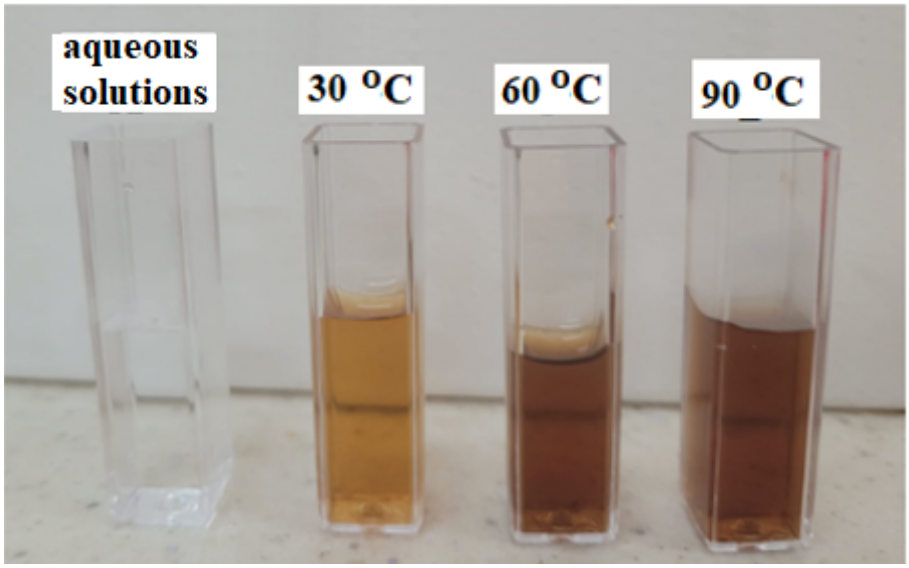


Figure 2

The color changes during the green synthesis of AgNPs at different temperatures.

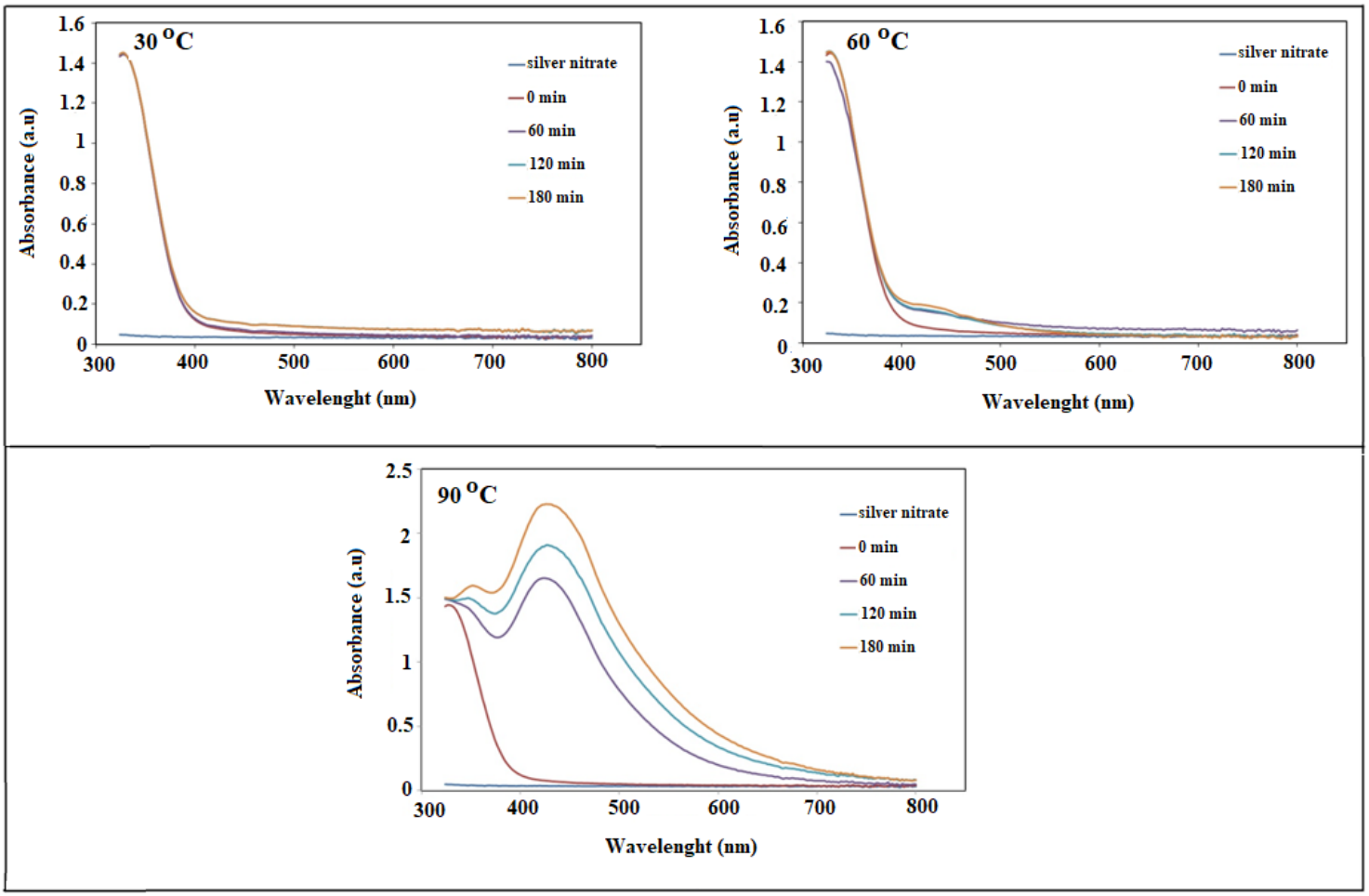


Figure 3

UV-Vis absorption spectra.

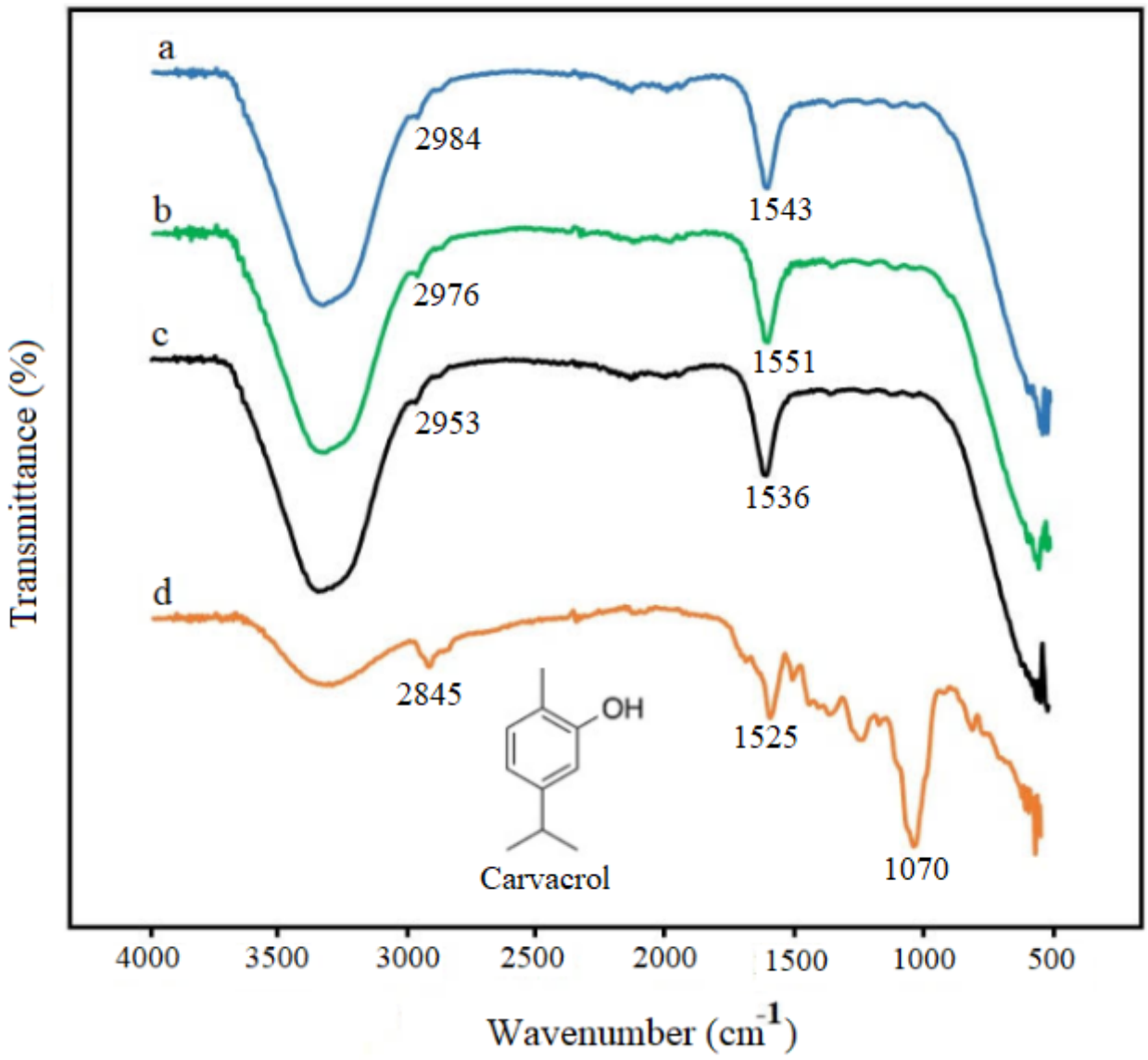


Figure 4

Transmittance spectrum of AgNPs prepared at different temperatures (a) 30 ºC, (b) 60 ºC, (c) 90 ºC, (d) *O. onites* extract.

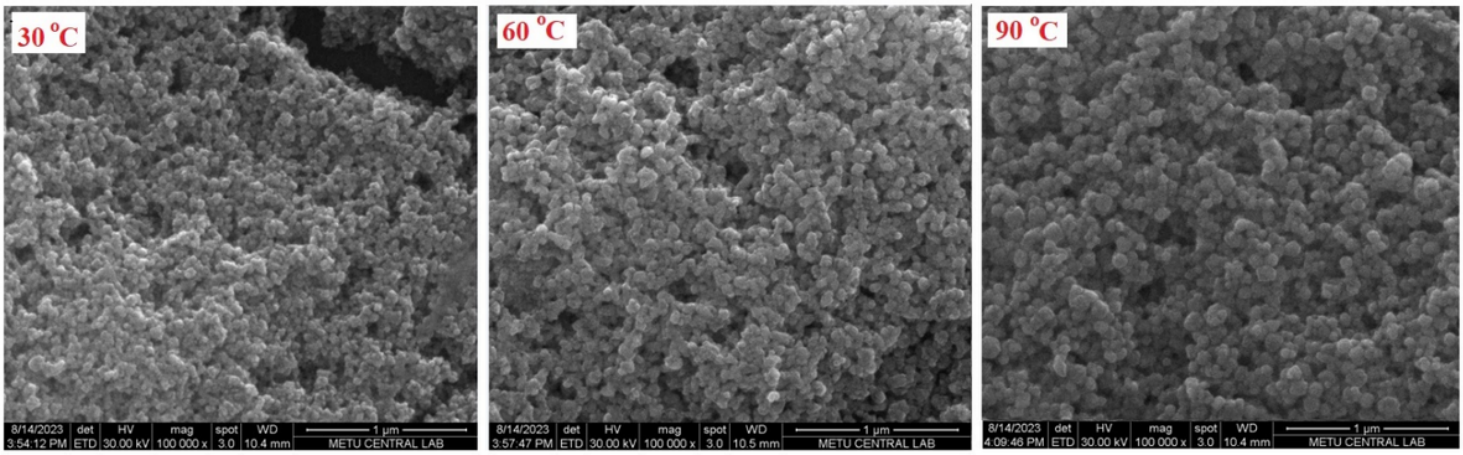


Figure 5

SEM image of AgNPs at 100000x magnification at different temperatures.

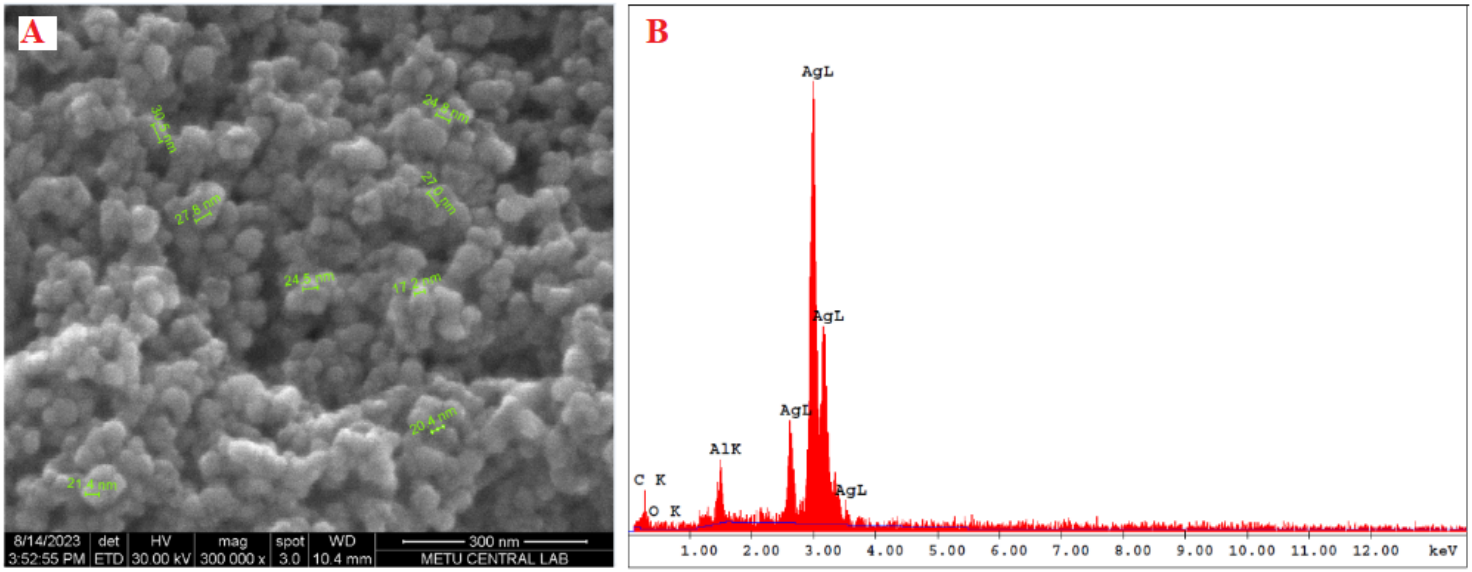


Figure 6

SEM image of AgNPs at 300000 magnifications at 90 ºC (A) and EDX spectrum of AgNPs (B).

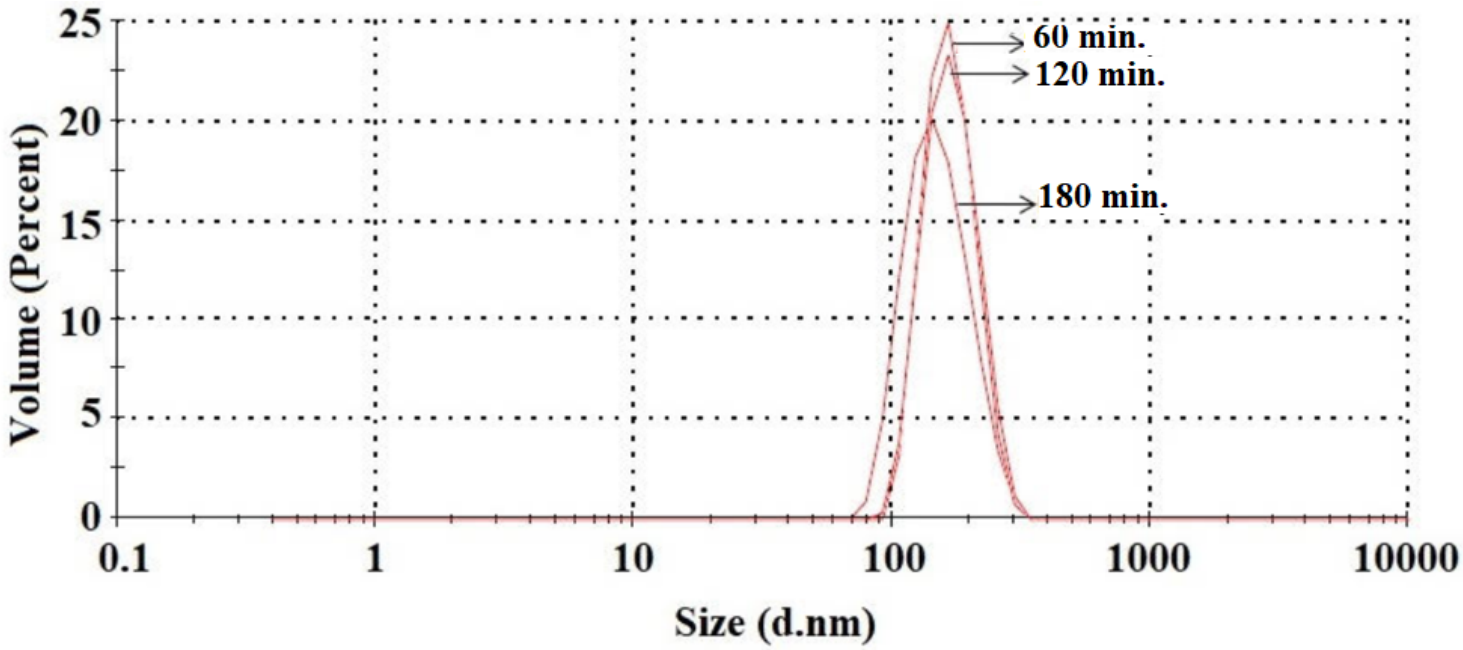
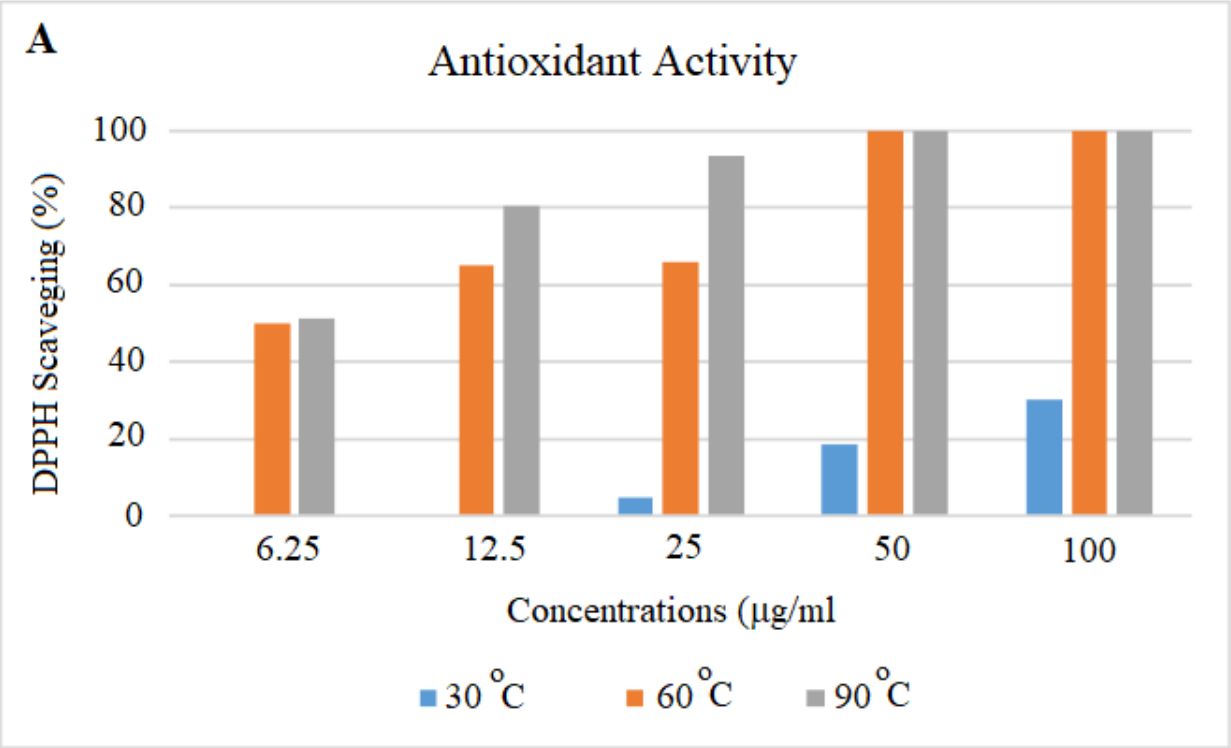


Figure 7

Particle size distribution of AgNPs for different reaction times.



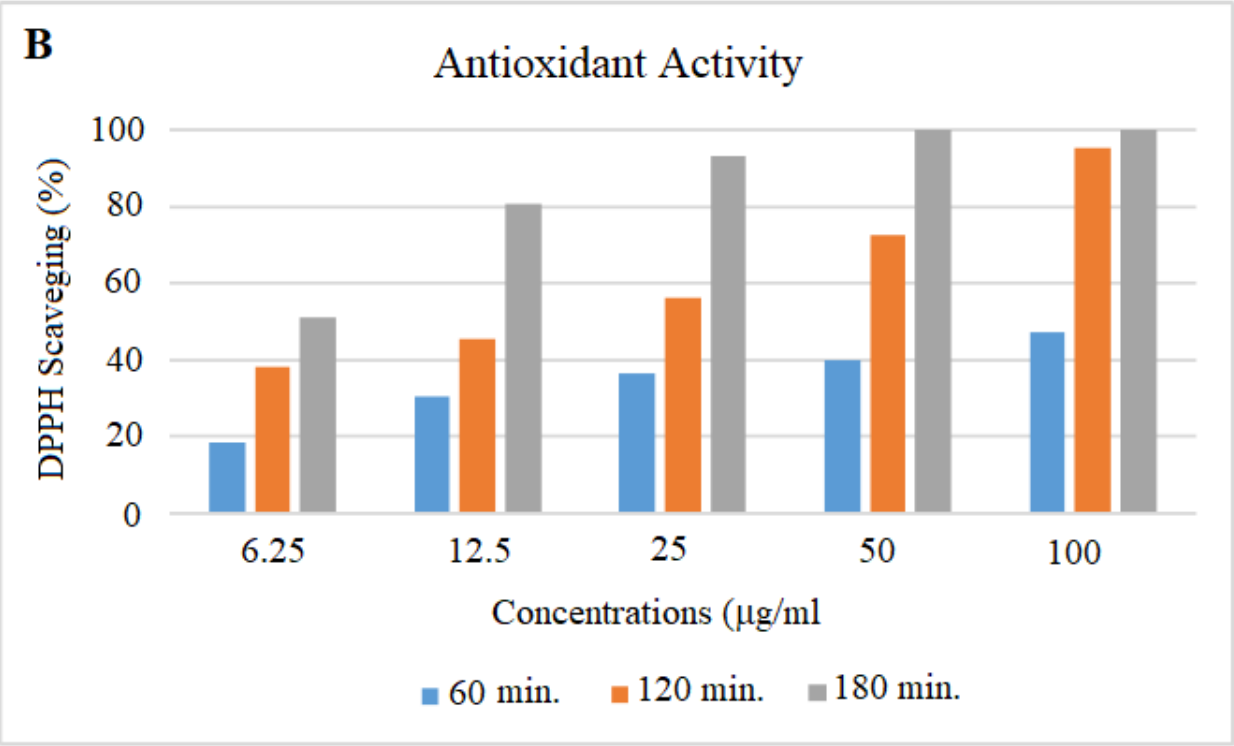


Figure 8

Antioxidant activity assay (DPPH) at different concentrations (A) for AgNPs synthesized at 180 minutes, (B) for AgNPs synthesized at 90 ºC.

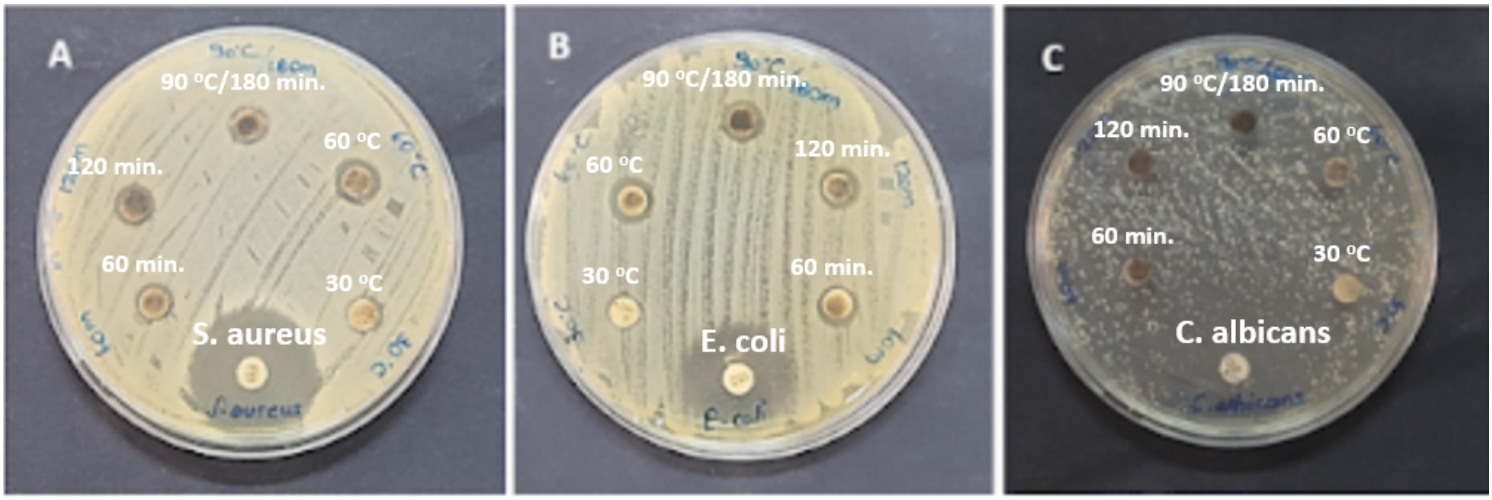


Figure 9

Antimicrobial activity assay for AgNPs synthesized at 180 minutes at 30 °C, 60 °C, 90 °C and at 90 °C for 60, 120, and 180 minutes against (A) *S. aureus*, (B) *E. coli*and (C) *C. albicans*.

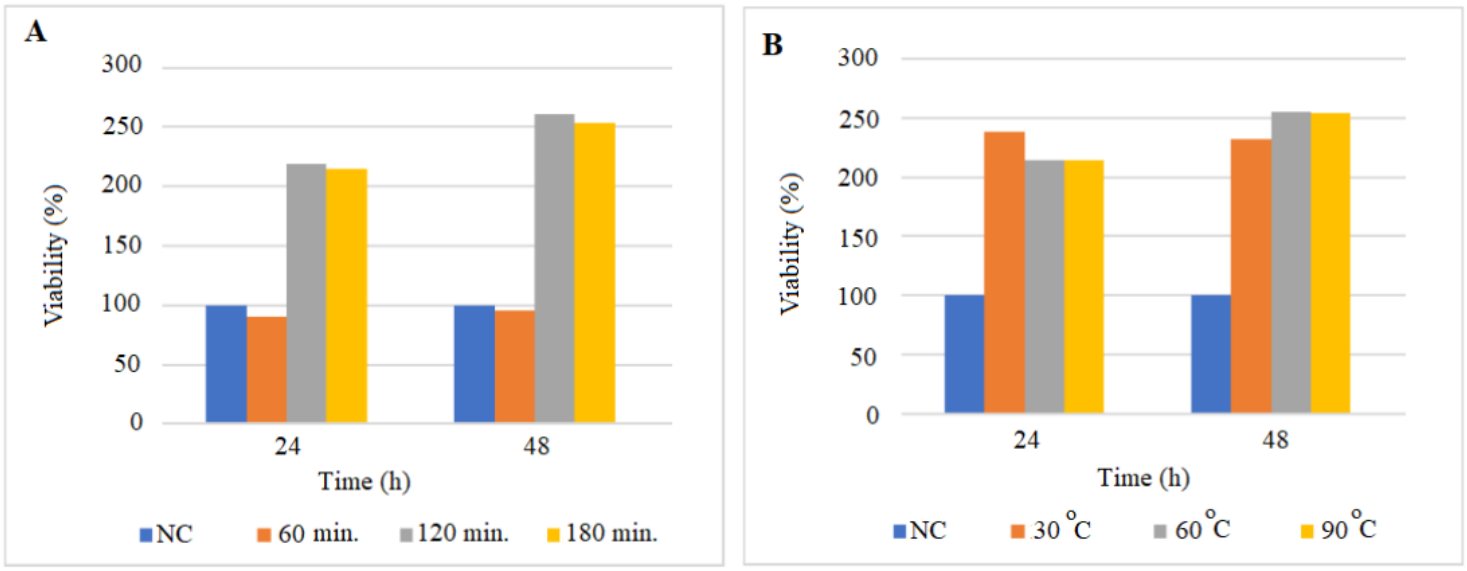


Figure 10

Cell viability over increasing time against MRC-5 cell line (A) for nanoparticles synthesized at 90 °C, (B) for nanoparticles synthesized at 180 minutes.