Nigella sativa seed-mediated green biosynthesis of silver nanoparticles and antimicrobial activity

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This work introduces a systematic and efficient approach for producing stable AgNPs utilizing *Nigella sativa* (Ns) seed aqua extract (AE), which exhibit strong antibacterial properties. The characterization of Ns-AgNPs was performed using a UV-visible spectrophotometer (UV-Vis), X-ray diffraction (XRD), Fourier transform infrared (FT-IR) spectroscopy, scanning electronic microscopy (SEM), transmission electronic microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDX). The Ns-AgNPs did not show aggregation, as shown by the results of STEM and XRD. According to the EDX analysis in this research, it was determined that Ns-AgNPs, gave signals in the silver region ($\sim$3 KeV) at 92.3%. Dynamic light scattering (DLS) was used to determine the average particle size and distribution profile of Ns-AgNPs. Ns-AgNPs showed significant antibacterial performance against *Staphylococcus aureous* and Escherichia coli, being effective at low concentrations. Ns-AgNPs may be incorporated into wound dressings, surgical instruments, and medical devices to prevent infections and promote healing.

Keywords: Silver nanoparticles; nanotechnology; green synthesis; *nigella sativa*; antibacterial activity.

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1. Introduction

The emergence and spread of antibiotic-resistant bacteria is an important public health problem that has become increasingly problematic in recent years. Antibiotic resistance occurs when bacteria evolve methods to resist antibiotic actions, making infection treatment more difficult and potentially leading to increased morbidity and mortality [1]. Therefore, natural compounds and materials synthesized from them are being developed to find new drugs and mechanisms of action for resistant microbes that are not affected by existing antibiotics [2,3]. The development of new drugs for resistant microbes is necessary to address the growing problem of antibiotic resistance. There is a need for studies with researchers, clinicians and public health professionals to effectively treat bacterial infections and prevent the spread of antibiotic resistance. The antimicrobial activities of silver nanoparticles (AgNP) have been extensively studied and have shown promising results against various microorganisms such as bacteria, viruses, and fungi [4]. The unique properties of AgNPs, including their large surface to-volume ratio, their large surface charge, and their ability to generate reactive oxygen species (ROS), make them effective antimicrobial agents. The use of AgNPs as antimicrobial agents has several potential advantages over conventional antibiotics [5]. AgNPs have a broad-spectrum activity against a wide range of microorganisms, including antibiotic-resistant strains, and have been shown to have low toxicity to human cells [6]. Furthermore, AgNPs have long-lasting activity and can retain their antimicrobial efficacy even after repeated exposure. In addition to medical applications, AgNPs can also be used in water treatment, food packaging, and environmental remediation [7-9]. Most often, these AgNPs are synthesised in physical, chemical and engineering laboratories using methods that require complex and specialised equipment [10]. A new approach is to use plant extracts as a green and sustainable method to synthesise AgNPs [11]. Plant extracts contain a variety of natural compounds, such as flavonoids, terpenoids, and alkaloids, which can act as reducing agents and stabilisers agents in the synthesis of AgNPs [12]. Plant extracts provide various advantages over traditional synthesis processes, including low cost, environmental friendly, and ease of manufacture. Recent studies have reported the synthesis of AgNPs using plant extracts mixed from a variety of sources, including leaves, stems, roots, and flowers [13]. In the synthesis the plant extract with a silver precursor, such as silver nitrate, and the mixture is heated to initiate the reduction of the precursor and the formation of AgNPs.

Nigella sativa (Ns), also known as black seed, is a flowering plant native to southwest Asia and the Mediterranean region [14]. It has been used for centuries in traditional medicine for a variety of ailments, including respiratory, digestive, and immune disorders. The seeds of Ns contain a variety of bio-active molecules, including thymoquinone, carvacrol, and α-hederin, which have been shown to have antioxidant, antimicrobial, anti-inflammatory and anti-cancer

properties [15]. These compounds are believed to be responsible for the medicinal effects of the plant. Studies suggest that *Nigella sativa* may have a protective effect against a variety of diseases, such as asthma, cancer, diabetes, and cardiovascular disease [16].

This study on the green synthesis of nanoparticles from natural sources using Ns aqueous extracts aimed to evaluate the conversion of silver ions into nanoparticles (Ns-AgNPs) and their antimicrobial activity.

2. Materials and methods

2.1. Plant materials

The research material, *Nigella sativa* (Ns), was collected in Keskin (43,786-56,448), Kırıkkale district/Turkey. First, Ns seeds were washed with tap water, and then twice with deionised water and dried at room temperature. The dried plants were ground in a kitchen mill, obtaining a particle size between 0.50-1.00 mm.

2.2. Plant extract

The maceration method was used for extraction, 5 g of ground plants were taken and mixed with 100 mL of deionised water. The mixture was placed in 500 mL Erlenmeyer flasks and heated in a magnetic stirrer at about 70°C for 30 min. The resulting aqua extract (AE) was filtered through Whatman philtre paper and stored in the refrigerator at +4 for later use.

2.3. Synthesis of Ns-AgNPs

For synthesis of Ns-AgNPs, 20 mL of AE was slowly added dropwise to 100 mL of 1 mmol (0.170 g) $AgNO_3$ solution. The reaction was continued for 2 hours at 50°C on a magnetic stirrer. The yellow colour of the plant extract turned brown after the addition of the silver nitrate ($AgNO_3$) solution. This brown colloidal solution was centrifuged at 10,000 rpm (15 min) to precipitate the Ns-AgNPs. The Ns-AgNPs were washed several times with deionised water and dried [17].

2.4. Characterization of Ns-AgNPs

Characterization of the dried Ns-AgNPs was performed using appropriate spectroscopic analytical methods. UV-vis spectra were performed using the Perkin Elmer Lamda 35 UV/VIS spectrometer, FE-SEM, Tescan Mira3XMU, Brno, Czech Republic, XRD analyses were performed using Emprean, Malvern Panalytical X'pert PRO diffractometer (PXRD), FTIR spectra were performed using Schimadzu FTIR 8400 spectrometer instruments, and dynamic light scattering (DLS) were performed using Malvern, Zetasizer Nano ZS [18-21].

2.5. Antimicrobial test

The antimicrobial activity of Ns-AgNPs was eveluated by the standard microdilution method and reported as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for *E. coli* (ATCC25922), and *S. aureus* (ATCC25923) strains in the microdilution assay. The MBC is the lowest concentration of Ns-AgNPs that kills 99.9% of the bacterial population in the original inoculum [3,22]. In this report, *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were representatives of the gramme-negative and gramme-positive bacteria tested, respectively.

The aqueous extract and the Ns-AgNPs were weighed out to 100 mg, dissolved in 1000 μl of 10% dimethyl sulphoxide, and placed in Eppendorf tubes. The bacteria, *Staphylococcus aureus* and *Escherichia coli*, were grown for 24 hours at 37°C in nutrient broth (Merck Cat. No. 105443), then transferred to nutrient agar and incubated for another 24 hours. In the liquid microdilution test, 100 μl of tryptic soy broth (Oxoid, Basingstoke, and Hampshire, England) was added to all 96 wells of a microplate. The AE was added to the wells and the concentrations were serially diluted twice from left to right over the range of 5 mg/mL to 0.0098 mg/mL. 5 μl of a bacterial suspension of 5×10^5 cfu/mL was added to all wells. One well without AE and Ns-AgNPs was used as a positive control for monitoring bacterial growth and one well with only AE was used as a negative control for monitoring the effects of the plant extract. The whole experiment was repeated 4 times. The microtitre plates were then incubated at 37°C for 24 hours. After the incubation period, the wells without turbidity were identified as minimum inhibitory concentration (MIC). The MBC values of the bacteria were determined from the concentrations above the determined MIC wells by subculturing on agar plates.

3. Results and discussions

Previous studies have mainly focused on changing certain factors such as metal ion concentration, temperature and pH to produce NPs with desired size and shape and increase the yield [23]. However, the properties of NPs can also be influenced by the chemical composition of the reducing and stabilizing agents used. This means that the composition of the plant extract used in the synthesis process can influence the properties of the resulting metal nanoparticles. The composition of a plant extract can vary depending on the method and conditions of extraction, such as the solvent, temperature, and extraction time. According to studies, extracts from the same plant but prepared using different methods may have variable combinations and quantities of bio-active molecules that can act as reducing and capping agents in the formation of metal nanoparticles. The use of plant materials (seeds, flowers, leaves, peels, roots etc.) for AgNPs has reduced the use of toxic chemicals. Green synthesis, using plant extracts as reducing and capping agents, is simple, inexpensive and environmentally friendly to obtain AgNPs.

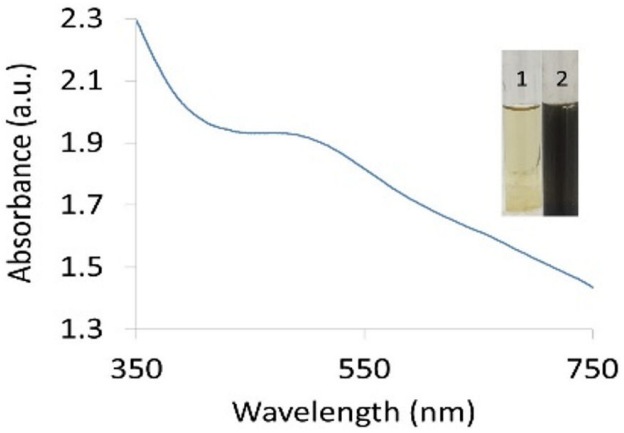

FIGURE 1. UV-Vis absorption spectra of Ns-AgNPs.

From the UV-Vis spectrum, Ag (I) cations were reduced to the neutral Ag (0) type and the band specific for AgNP was detected in the range of 350-500 nm [24,25]. The Ns-AgNPs produced oscillations in the SPR band at about 500 nm wavelength which caused a brown hue. The change in colour from light yellow to brown colloidal solution during the reaction allowed qualitative detection of Ns-AgNPs and the change in colour over time was explained as a result of surface plasmon resonances (SPR) on the AgNPs.

The evaluation of the literature results showed that the synthesis was carried out successfully. Moreover, no band was detected when the same process was repeated for the AE of the plant. According to the results, the Ns-AgNPs showed specific peak at 400-500 nm, indicating the presence of Ns-AgNPs in the solution [26].

It is known that these functional groups are responsible for the stabilisation of AgNPs and reduce the aggregation of nanoparticles in solution. Overall, the study suggests that Ns-AgNPs were successfully synthesised using plant extracts and that the presence of specific functional groups in the plant extracts contributed the stabilisation of Ns-AgNPs.

The results of the FTIR analysis of the Ns-AgNPs, AE and the wavelengths are shown in Fig. 2. The broad band at 3267 cm^{-1} can be attributed to -OH stretching (range of phenol and alcohol). 3004 cm^{-1} and 2911 cm^{-1} represented the -CH stretching vibration. The signals observed at 1643 cm^{-1} can be attributed to the characteristic vibration of the carbonyl group (-CO). The peak at 1469 cm^{-1} represents the symmetrical -CH_2 stretching of alkenes. The peak at 1040 cm^{-1} indicates the participation of -CN in the plane vibration of aliphatic amines. Our report correlated well with the previous report [16]. There was change in peaks in the synthesized Ns-AgNPs, which proposes that functional groups of AE participate in the formation of Ns-AgNPs.

X-ray diffraction (XRD) is a promising technique to observe the structural arrangements in the nanoparticles. Ns-AgNPs were analysed in the 2θ range from 10° to 90° using Cu Kα diffractometer and the obtained results are shown in Fig. 3. The diffraction peaks of the Ns-AgNPs were observed at 38.35°, 46.46°, 64.75° and 77.62°, which correspond to

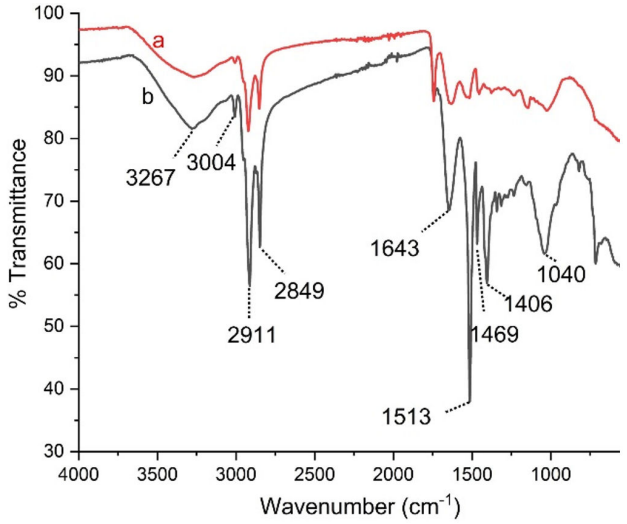

FIGURE 2. FTIR spectrum of a) AE, b) Ns-AgNPs.

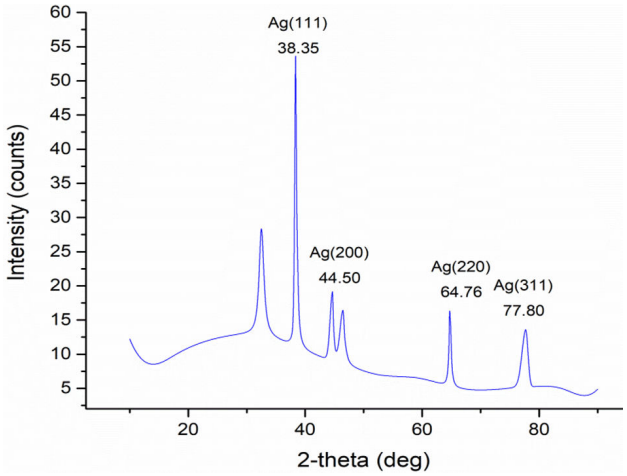

FIGURE 3. XRD pattern of Ns-AgNPs.

the characteristic Bragg diffraction planes (111), (200), (220) and (311) of the face-centered cubic (FCC) structure. These results are in good correlation with an earlier study by Saygi *et al.*, who reported the green synthesis of crystalline Ns-AgNPs with evidence of lattice plans [19].

The Ns-AgNPs did not show aggregation, as shown by the results of STEM and XRD. The size and morphological characteristic of the synthesised Ns-AgNPs were further analysed using scanning transmission electron microscopy (STEM). The image of the Ns-AgNPs is shown in Fig. 4. Ns-AgNPs were within the desired size for efficient drug delivery.

EDX analysis is commonly used to determine the composition of elements in solid state. In EDX analysis, strong signals in the silver region ($\sim$2.983 KeV) are considered as a significant evidence for the formation of Ns-AgNPs (Fig. 5). In various studies, the presence of an $\sim$3 KeV optical absorption peak in the formation of AgNP is believed to be caused

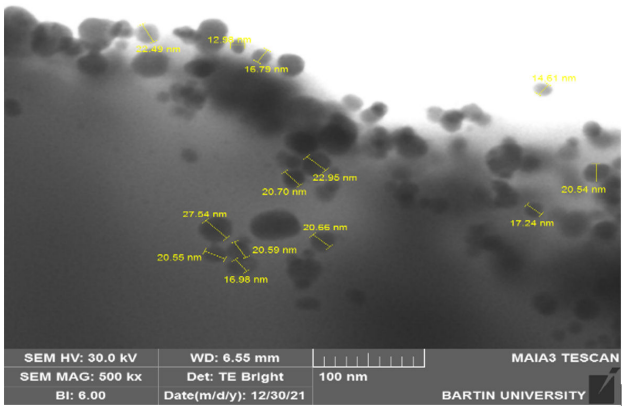

FIGURE 4. STEM image of Ns-AgNPs.

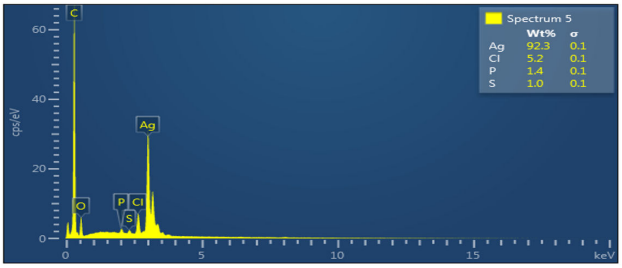

FIGURE 5. Ns-AgNPs EDX analysis.

by surface plasmon resonance of AgNPs [27,28]. According to the EDX analysis in this research, it was determined that Ns-AgNPs, gave signals in the silver region (∼3 KeV) at 92.3%. These findings suggest that Ns-AgNPs, has sharp signals in the silver region (∼3 KeV) and weak signals from other elements are likely caused by enzymes or proteins found in the structure of the plants [29,30].

The average particle size/size distribution of synthesized Ns-AgNPs was determined by DLS (Fig. 6). Compared to the sizes that were acquired from STEM measurement of Ns-AgNPs was determined to be 150 nm. This value is greater than the obtained. DLS measurements are used to analyze Ns-AgNPs that have a hydrated layer that is composed of swelling organic moieties on the surface of AgNPs [27].

Recently, a number of pathogenic microorganisms have evolved resistance to the commercial antibiotics and antifungal medications currently on the market, and they also have a negative impact on human health. New antimicrobial drugs that are active and secure are therefore needed. This study once again examined Ns-AgNPs' antibacterial properties (Table I). The antimicrobial effects of AE and Ns-AgNPs forms were tested using *E. coli* and *S. aureus* strains using broth microdilution test [31,32]. Dilution tests are used to determine the minimum concentration required to inhibit or kill the growth of a microorganism. In this study, broth microdilution test was applied. Tube dilution can be applied in two ways, "macro" and "micro". The principle of both methods is the same. In macro dilution, test tubes are used, while

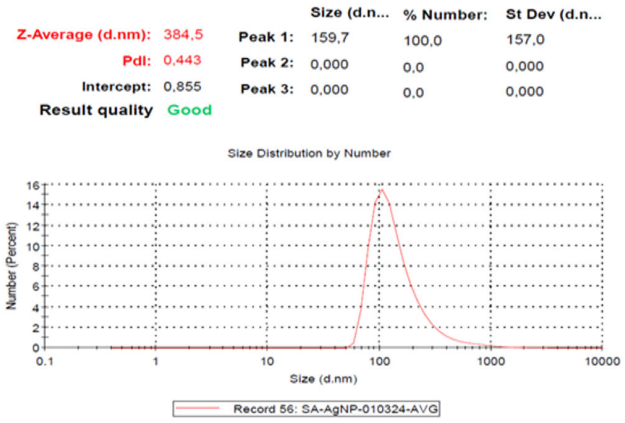

FIGURE 6. DLS size distribution of Ns-AgNPs.

TABLE I. MIC (μg·mL^{-1}) and MBC (μg·mL^{-1}) results of AE and Ns-AgNPs.

	AE[a]		Ns-AgNP[a]	
Bacteria	MIC	MBC	MIC	MBC
Staphylococcus *aureus*	0.125	0.5	0.007	0.015
Escherichia *coli*	0.0625	0.25	0.007	0.007

in microdilution, "U" or "V" based "microplates" are used. Mueller-Hinton broth supplemented with cations (calcium and magnesium) was used as the medium in the microdilution method. AE and Ns-AgNPs tested were first prepared in special solvents and then diluted doubly in this liquid medium. A standard inoculum of the microorganism (1×10^5 CFU/ml) was prepared and 5 μl were added to the wells containing various dilutions of the extracts. In addition, to show bacterial growth, a control well without an extract, a medium only, and a well containing extract was prepared, and after an overnight incubation at 35°C, the medium was examined for bacterial growth with turbidity. The lowest concentration that prevented bacterial growth without visible turbidity was considered the minimum inhibitory concentration (MIC).

Almatroudi *et al.* have determined the antibacterial activity of Ns-AgNPs [16]. When compared with this study, our results revealed that Ns-AgNps exhibited excellent antibacterial activity and was better than the conclusion of the previous study.

Therefore, it has been emphasized that Ns-AgNPs can be considered as a potential option in medical applications, alternative treatments, designing anti-biofilm agents, and treating multi-drug resistant bacterial infections. Additionally, it has been found that Ns-AgNPs derived from Nigella sativa AE exhibit a greater antibacterial effect than their AE. As a result, similar to the literature, Ns-AgNPs may be used in the prevention and termination of antibacterial agents in the application of various medical treatments [15]. This is consistent with previous studies and suggests that the low likelihood of side effects provides an additional advantage for biomedicinal use, in addition to their antioxidant and antimi-

crobial properties. When evaluating these results, it was determined that Ns-AgNPs is more successful in preventing the antimicrobial activity.

The antibacterial activity of AgNPs synthesized from the same plant was tested by Vijayakumar *et al*. However, our study showed antibacterial activity in the very lowest concentrations [33,34].

The antimicrobial properties of AgNPs have been described; however its mechanism of action is still unclear. It's action can be explained by changes on cellular function such as generation of reactive oxygen species (like süper oxide anions and hydroxyl radicals), and the morphological changes of bacteria. Moreover, AgNPs may interact with cell's proteins which direct to de-naturation of proteins in the bacteria. AgNPs were attached to the outer surface of the bacteria, can damage cell membranes, celluler proteins, DNA, and finally bacteria cells death.

Different susceptibility profiles have been detected between Gram-positive and Gram-negative bacteria. This difference might be attributed to their different cell wall components. Gram-negative cell walls contain three components that lie outside of the peptidoglycan layer (its thickness ranged 7-8 nm,very thin layer): lipoprotein, outer membrane, and lipopolysaccharide.

On the other hand Gram-positive cell walls contain considerable amounts of teichoic asit and teichuronic acids (its thickness ranged from 20-80 nm, very thick layer). Due to this change, Gram-negative bacteria are thought to be more sensitive than Gram-positive bacteria [35]. Besides the size of AgNPs also plays an important role in the antibacterial effect. The antimicrobial activity of AgNPs is influenced by dimensions of the particles and concentration. Studies have found that smaller particles have a greater antibacterial effect [36].

4. Conclusions

The research aimed to investigate the green synthesis of silver nanoparticles (AgNP) and their antimicrobial effects using AE from *Nigella sativa*. One possible approach is to use green chemistry principles to develop a sustainable and environmentally friendly method that avoids the use of toxic chemicals. The study found that *Nigella sativa* showed a maximum absorbance at about 500 nm (UV-Vis). FTIR analysis of Ns-AgNPs revealed the presence of polyphenols and amino groups, aliphatic groups, terpenoids, flavonoids, and carbohydrate structures. The average particle size of Ns-AgNP was determined as 20.88 nm. SEM and XRD results showed that there was no aggregation in the AgNP. EDX analysis found that Ns-AgNPs had a 92.3% silver content. The antibacterial activity of Ns-AgNP and AE on bacteria was determined, and it was observed that Ns-AgNP form had a stronger antibacteriel effect. As mentioned above, it is known that there may be multiple mechanisms that can explain the antibacterial effect and the difference among bacteria. In this study, only the antibacterial effect of AE and Ns-AgNPs was evaluated, and factors that could explain this effect were not investigated. This is one of the limiting factors of our study. More studies are needed on this subject.

Author contributions

All authors: Conceptualization, Methodology, Software, Formal analysis, Validation, Writing-original draft, Writing-review & editing,

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Declarations

Competing interests

The authors report no declarations of interest.

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