Research Article

Characterization and Antibacterial Activities of Silver Nanoparticles Synthesized using Black Seed (*Nigella sativa*)

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ABSTRACT

This study examined the structural characteristics and antibacterial activities of silver nanoparticles synthesized from black seed. Aqueous and methanolic extracts of the black seeds were used to synthesize the silver nanoparticles (AgNPs). Synthesized AgNPs were characterized using UV-visible spectroscopy, Transmission Electron Microscope (TEM), X-ray diffraction (XRD), and Energy Dispersive Spectroscopy (EDS). Antibacterial activity of the synthesized AgNPs was tested against *Staphylococcus epidermidis* and *Klebsiella oxytoca*. UV-visible spectra revealed a strong and broad surface plasmon resonance peak between 400-500nm. TEM showed that the AgNPs were spherical in shape and were well separated while XRD showed cubic and hexagonal structures of the AgNPs. EDS spectra showed that AgNPs have a weight percentage of silver as 77.98% and 84.63% for aqueous and methanolic extracts respectively. The AgNPs showed a 14-20mm zone of inhibition against the test organisms. This study showed that AgNPs can be effectively synthesized using black seed and showed moderate antibacterial activity.

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INTRODUCTION
Nanoparticles are particles that have dimensions between 1-100 nm (Kareem et al., 2019). Nanoparticles can serve as building blocks for different physical and biological systems (Sun et al., 2014). Many metals and semiconductors nanoparticles are accorded greater importance based on their electrical, optical, magnetic, and chemical properties. Typically, nanoparticles are used in the field of medicine for therapeutic and diagnostic purposes (Jagtap and Bapat 2013).

In the last few decades, silver nanoparticles have been widely studied by researchers all around the world. Silver nanoparticles possess unique electrical, optical and biological properties, which are responsible for their application in biosensing, imaging, drug delivery, nanodevice fabrication, and medicine (Ibrahim, 2015). There is an increasing research interest in silver nanoparticles as it has been considered to be among the most potent antimicrobial agents (Niraimathi et al., 2013).

Some physical and chemical-based methods include the sonochemical method, chemical reduction, photochemical reduction, metallic wire explosion, and sol-gel method (Ndikau et al., 2017). These methods of synthesizing metal nanoparticles have many limitations when compared with biological methods. Some of the limitations are cost implications, use of toxic chemicals, high energy demand, and the release of hazardous waste products into the environment (Kareem et al., 2019).

The biological method, which involves the use of plants and non-pathogenic microorganisms, is cost-effective, energy effective, and environment-friendly (Ndikau et al., 2017). Although the biological method of synthesis requires a longer period of time for the synthesis of a significant quantity of metal nanoparticles, it requires no capping agents for stabilization of the size of the nanoparticles (Widdatallah et al., 2020).

Black seed is native to North Africa, Southwest Asia, and Southern Europe and is also cultivated in many other countries of the world. It possesses therapeutic potential and biological activities such as antioxidant, diuretic, antidiabetic, antihypertensive, anticancer, antimicrobial, immunomodulatory, anti-schistosomiasis, anti-inflammatory, analgesics, a bronchodilator, spasmyloytic, hepatoprotective, gastroprotective, and renal protective (Mazaheri et al., 2019).

Studies have shown the use of plant extracts for the successful synthesis of silver nanoparticles (Li et al., 2007; Von White et al., 2012; Ndikau et al., 2017). The aim of this study was to synthesize silver nanoparticles using black seed extract and to determine their antimicrobial efficiency against Staphylococcus epidermidis and Klebsiella oxytoca.

MATERIALS AND METHODS
Collection of Samples
Black seeds were purchased from Arisekola Mosque, Ibadan Nigeria and transported to the Department of Microbiology, University of Ibadan. The seeds were then rinsed with water to remove the sand and other particles. It was then air-dried and blended into powdery form.

Preparation of Aqueous and Methanolic Black Seed Extracts
One hundred grams of the powdered black seed was weighed in 1000 mL methanol and 1000 mL distilled water. The mixture was left overnight to allow proper concentration. The yellowish colour extract was filtered using
Whatman No 1 paper. The filtrate was dried in a rotary evaporator to dry and the concentrated sample was stored at 4 °C for further use (Afreen et al., 2020).

**Synthesis of Silver Nanoparticles from Black Seed Extracts**

For the synthesis of silver nanoparticles, 0.1 g of the powdered sample was weighed into 10 mL of dimethyl sulfoxide (DMSO) (0.01 g per 1 ml into 100 mL of Erlenmeyer flask). One millimolar (1 mM) of silver nitrate (AgNO₃) was prepared by weighing 0.2 g of the AgNO₃ in sterile water. Then 1 mL of the extract was mixed into 9 mL AgNO₃ and then exposed to sunlight. The development of brownish color indicated the synthesis of silver nanoparticles (Chahardolia et al., 2017).

**Characterization of Synthesized Silver Nanoparticles**

**Ultra Violet-Visible Spectroscopy**

The optical property of the synthesized silver nanoparticles was determined by UV-Visible spectroscopy using a spectrophotometer. The endpoint of the reaction was evaluated by UV-Visible Spectroscopy. The reduced brown color solutions for aqueous and methanolic extracts were scanned using a Jenway 6405 UV-Vis spectrophotometer operated at a resolution of 1 nm. The ultraviolet-visible spectroscopy was performed at a wavelength of 300-900 nm and distilled water was used as a blank (Kumar et al., 2016).

**Transmission Electron Microscopy**

The morphological feature of the silver nanoparticles was determined with Transmission Electron Microscopy (TEM) using the method of Chahardolia et al. (2017). A drop of aqueous and alcoholic AgNPs sample was loaded on a carbon-coated copper grid, followed by solvent evaporation at room temperature for an hour. Images of the TEM micrograph were recorded and observations of the clear microscopic views were made at different magnifications.

**X-Ray Powder Diffraction**

The silver nanoparticles solution was centrifuged at 2500 rpm for 20 minutes and the pellets were washed thrice using 20 mL of deionized water. The solid residues of silver nanoparticles were washed twice with double distilled water and then redissolved in absolute ethanol and evaporated to dryness at 25 °C to obtain powder. Then the dried powder of silver nanoparticles was used for the X-ray a powder diffraction measurement to determine the formation of silver nanoparticles as described by Jagtap and Bapat (2013).

**Energy Dispersive Spectroscopy**

Energy Dispersive Spectroscopy (EDS) was used to detect X-rays characteristics of the elements present and electrons which lose energy due to their interaction with the black seed extract. Elemental analysis of the AgNPs was conducted using an Energy-Dispersive X-ray detector attached to the SEM machine using the method of Srirangam and Rao (2017).

**Antibacterial Activities of Synthesized Silver Nanoparticles**

The antibacterial activities of the silver nanoparticles were determined using the method of Chahardolia et al. (2017). A 24 hour-old culture of *Staphylococcus epidermidis* and *Klebsiella oxytoca* was swabbed on Mueller Hilton Agar (MHA) plates. Then a sterile cork-borer with a diameter of 7 mm was used to make well on each of the inoculated plates. The silver
nanoparticles, *N. Sativa* seed extracts, and AgNO₃ solutions were then carefully dispensed into the corresponding well using a sterile pipette. A commercial antibiotic (Streptomycin) and dimethylsulfoxide were used as positive and negative control respectively. The plates were then incubated at 37°C for 24 hours after which the diameters of the zones of inhibition were measured accordingly.

**RESULTS AND DISCUSSION**

**Synthesis of Silver Nanoparticles**

The presence of brownish-yellow and dark-brown colouration showed the production of silver nanoparticles. The intensity of the brown colour increase is directly proportional to the duration of exposure to light. The methanolic medium showed a deeper brown colour change compared to the aqueous medium (Figure 1). This might be due to the presence of some compounds extracted by the methanol during extraction. The surface plasmon resonance, which is responsible for the progressive colour change, is affected by the size, shape, and morphology of the synthesized silver nanoparticles (Widdatallah *et al.*, 2020).

![Figure 1: The silver nanoparticles produced from methanolic and aqueous extracts](image)

**Characterization of Synthesized Silver Nanoparticles**

**Ultra Violet-Visible Spectroscopy**

One strong and wide surface plasmon resonance peak was noticed between wavelengths of 400-500 nm throughout the reaction (Figure 2). This indicates that the silver nanoparticles were well dispersed in the aqueous and methanolic solution. Silver nanoparticles usually display maximum ultraviolet-visible absorption spectrum between 400-500 nm. This finding is similar to the results reported by Ali *et al.* (2017).
Figure 2: UV–visible spectrum of aqueous and methanolic synthesized silver nanoparticles

Transmission Electron Microscopy

The TEM images of the silver nanoparticles revealed that the nanoparticles were spherical in shape, with a size ranging from 20 nm to 100nm in which few nanoparticles were agglomerated (Figures 3). The TEM images further showed that the silver nanoparticles were not in direct contact and were separated by constant space with little deviations. Similarly, Shameli et al. (2012) also reported spherical shaped silver nanoparticles synthesized through green synthesis.
Figure 3: TEM micrograph of silver nanoparticles synthesized from aqueous and methanolic extract of *Nigella sativa* at 20nm, 50nm and 100nm

**Key:** B.S (A)- Aqueous extract; B.S (M)- Methanolic extract

**X-Ray Powder Diffraction**

The XRD peaks at 20°, 12.1°, 26.3, 45.5 and 59.7° can be indexed to the (111), (200), (220) and (311) planes for aqueous synthesized nanoparticle and 10.0°, 20.1°, 25.7°, and 63.3° can be indexed to the (111), (200), (220) and (311) planes for methanol synthesized nanoparticles as shown in Figure 4. These Bragg’s reflections indicated the presence of (111), (200), (220) and (311) sets of lattice planes and further on the basis that they can be indexed as face-centred-cubic (FCC) structure of silver nanoparticles formed in this present synthesis are crystalline in nature.

This showed that the structure of silver nanoparticles in the sampled black seed is cubic and hexagonal. It indicates that the silver nanoparticles are biphasic in nature. Jayaseelan *et al.* (2013) reported a similar XRD pattern in silver nanoparticles synthesized using *Tribulus terrestris* and *Prosopis juliflora* extract.

Figure 4: XRD spectrum of synthesized silver nanoparticles from aqueous and methanolic extract of *Nigella sativa*
**Energy Dispersive Spectroscopy**

Energy Dispersive Spectroscopy (EDS) spectra recorded from the silver nanoparticles were shown in Figures 5 and 6. From the EDS spectra, it was clear that the percentage weight of silver in the synthesized silver nanoparticles was 77.98% and 84.63% for aqueous and methanolic synthesized silver nanoparticles respectively.

![EDS analysis of synthesized silver nanoparticles from aqueous extract of *Nigella sativa*](image1.png)

**Figure 5:** EDS analysis of synthesized silver nanoparticles from aqueous extract of *Nigella sativa*

![EDS analysis of synthesized silver nanoparticles from methanolic extract of *Nigella sativa*](image2.png)

**Figure 6:** EDS analysis of synthesized silver nanoparticles from methanolic extract of *Nigella sativa*
Antibacterial Activity

The results, presented in Table 1, showed that the AgNPs exhibited moderate antibacterial activity against the test organisms. The most sensitive bacterium against aqueous and methanolic synthesized nanoparticles was *Staphylococcus epidermidis* with inhibition zones of 20 and 16 mm respectively. In a similar study, Loo *et al.* (2018) reported a substantial antibacterial activity of green synthesized silver nanoparticles against *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* spp. using extract of pu-erh tea leaves. Studies have indicated the antibacterial activity of AgNPs by attachment to the bacterial cell wall, or the formation of free radicals (Yu *et al.*, 2013; Jasusja *et al.*, 2014). Silver nanoparticles can adhere to the bacterial cell wall and cell membrane to interrupt respiration and permeability (Ranjan *et al.*, 2013).

Table 1: Zone of Inhibition of Synthesized silver nanoparticles using *N. sativa* against selected bacteria

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gram Reaction</th>
<th>DMSO</th>
<th>Streptomycin</th>
<th>AgNO₃</th>
<th>Extract(aq)</th>
<th>Extract(m)</th>
<th>AgNPs(aq)</th>
<th>AgNPs(m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>Positive</td>
<td>-</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>6</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td><em>Klebsiella oxytoca</em></td>
<td>Negative</td>
<td>-</td>
<td>20</td>
<td>2</td>
<td>12</td>
<td>10</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

CONCLUSION

This study revealed that green synthesis of silver nanoparticles can be achieved using aqueous and methanolic extracts of *Nigella sativa*. The integrity of the synthesized silver nanoparticles was confirmed by analytical techniques like UV-VIS spectra, TEM, XRD, and EDS. The synthesized AgNPs from both aqueous and methanolic extracts showed moderate antibacterial activity against *Staphylococcus epidermidis* and *Klebsiella oxytoca* hence could be used as an antibacterial agent for certain bacterial infections.
CONFLICT OF INTEREST

No conflict of interest declared

REFERENCES


