Investigate the Structural Properties of Silver Nanoparticles Produced by Bio Production of Green Spinicia Oleracea Leaf Extract and their Influence on Antibacterial Activity

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Abstract
This study used Green Spinach Leaf Extract for the green synthesis (monocolors) of silver nanoparticles (AgNPs). It focuses on the optimization of synthesis by evaluating the impact on each of the size distribution and activity of antibacterial activity against the bacteria (Escherichia coli) of the green spinach leaf extract volume percent. The characterization of AgNPs was carried out utilizing UV-Visible spectrophotometers, size examination of particles, transmission electron microscopes, energy spectrometry with dispersion X rays, Fourier infrared spectrometer transform, and x-ray scanning methods. The experimental data showed that AgNPs have been produced effectively and that the size of particle is controlled by the amount of green spinach leaf extract. With the size reaching (5 nm, polydispersity indices = 0.063 nm), the smaller volume percentage creates AgNPs with a spherically formed reach of 20%. The synthesized AgNPs by the spinach leaf extract had a good antibacterial activity, especially at the concentration of (500µg/ml) in which the inhibition zone reached 35mm against gram negative bacteria Escherichia coli.

Introduction
Because of The use of plants' extract for the production of metal nanoparticles is an emerging technology that has extensively been explored in the past several years with a view to replacing dangerous and non-renewable chemicals. The green production of nanoparticles in recent years has been an intriguing subject [1]. The fundamental concept of synthesis is the capacity to reducing metal ion precursors by flavonoids and alkaloids. Certain metal and metal oxide nanoparticles,
for example silver nanoparticles [2], gold nanoparticles [3,4], zinc oxide nanoparticles (ZnONP), [5] and platinum nanoparticles (PtNPs), are created via the reduction of plant extract [6].

AgNP green synthesis production is intriguing since several researches have shown synthesized nanoparticles' features as a function of the plant extract type, content, and synthesis methods. The antibacterial, anti-fungal, anticancer, and antioxidant effects of green synthesized AgNPs are claimed to have occurred. AgNPs’ activity, physical, and chemical characteristics are influenced by their shape and form, which are influenced by the plant extract employed, composition, and synthesis technique [7].

Some research has shown that the biological/chemical activities of AgNPs are closely linked to the physicochemical properties of AgNPs. The present research looks at using Green Spinicia oleracea leaf extract as a reducing agent in the manufacture of AgNPs. This research focuses on the extraction, optimization, synthesis, and assessment of antibacterial properties of produced AgNPs.

Materials and Methods

Materials

The analytical grade of all reactants in this investigation is utilized without any additional purification. Silver nitrate (AgNO3) was purchased from (Avonchem limited UK) and Merck-Millipore has supplied the distilled water (Germany). Spinicia oleracea green leaf extract was collected in Baghdad, Iraq, from a local market. The soaking process was used to create Spinicia oleracea leaf extract (GSE). Overnight, 50 g dry green spinach leaves were roughly soaked in water (100 ml) solvent. GSE was extracted from the combination by filtering the solution. The soaking extract was then dried in an oven at 50°C, and 1 gram of the powder was produced after drying was weighed and analyzed in 50 ml of distilled water to dilute the extract with diluted silver nitrate in a molar ratio specified for the purpose of research.

Synthesis of AgNPs

Silver nanoparticles have been synthesized by mixing AgNO3-10-2 M with GSE at a constant volume ratio of 20%. The blend was handled for 5 hours at room temperature to guarantee a reduction reaction between Ag + and Ag0 [8]. The decline study was confirmed by UV-Visible spectroscopy. Analyzer of particle size, infrared microscope, and transmission-electron microscope (TEM) Fourier transform was used to further investigate the AgNPs. For these tests, the Particle Size Analyzer HORIBA and the JEOL TEM apparatus were used with a dynamic light dispersion system operating at 120 kV. In order to ensure that the single phase of Ag is acquired from synthesis, the Perkin–Elmer spectrometer equipment was used and x-ray diffractors (XRD) analysis was carried out using the Shimadzu X6000 equipment, which was operated as a radiation source with the Ni filtered CuKα.

Antibacterial activity test of AgNPs

Using the well diffusion experiment, the antibacterial activity of produced AgNPs was determined. Synthesized AgNPs with stock concentration (500µg/ml) and dilutions (250, 125 µg/ml) was used to detect the antibacterial activity. The bacterial culture in the McFarland turbidity tube was activated for 18 hours in nutritional broth at 37 pounds in a McFarland turbidity tube with a concentration of 1.5*108 cells/ml. At 18hr, pathogenic bacteria (Escherichia coli) were
active at 37 LC. Pathogenic bacteria utilizing cotton swab have been injected on sterilized nutrient agar plates. The wells were cut off by using a sterile pipette of a pasture after 5-10 minutes. At each concentration, agNPs solution (100μL) has been applied to the well and incubated for 24 hours at a temperature of 37 0C. Inhibition areas were measured in mm after the incubation period [9].

Results and discussion

Depiction of the UV-visible spectra of produced AgNP at various GSE volumes are shown in Figure 1. GSE which shows specific wavelengths in the range 300–350 nm and strong spectrum 406 nm. These spectrums are connected to the presence of RSE anthocyanine and phenolic compounds, which is consistent with previous research [10]. All AgNPs have a maximum wavelength of between 390 and 430 nm, which indicates surface resonance (SPR).

In order to indicate the surface plasm resonance (SPR) absorption range, AgNPs shows a maximum wavelength in the 390–430 nm region. Given that each AgNP sample demonstrates a single SPR band, the nanoparticles are projected to be spherical, whereas the nanoparticles correspond to the anisotropic molecules, as there are two and more SPR bands [11]. AgNPs-2 produced the most intense spectrum which is associated with SPR production. The examination of particle size ensures the average distribution of particle size and particle sizes. Figure 2. shows the spherical form of nanoparticles between 2–41 nm in the image of the spinach extract with AgNP. The findings fit into the distribution of particulate size and UV-visional AgNP spectrum that indicates the nanoparticles' size range. The organic cap material aids in the stabilization of nanoparticles. The existence of organic material has been also shown by the X-ray spectrum energy dispersion of AgNPs, which displays Ag, C and O existence. As capping AgNPs, the C and O signals derive from organic GSE molecules. There is no N signal that indicates the lack of AgNO3 since the Ag+ production is completely reduced.
Figure 2. TEM image of AgNPs synthesized.

The reflex spectrum of filtered AgNPs is shown in Figure 3. Four strong peaks are shown in the XRD pattern with 2θ values between 30 and 70. Intense peaks at 2θ point values are in the range of 31.9, 43.31, 60.4 and 63.4 (111), (200), (220) and (311) according to JCPDS, silver file 04-078. [12].

Figure 3. Image of XRD pattern of AgNPs synthesized.

GRE AgNPs have been studied for their antibacterial effects, and the results were presented in Table 1, which included a comparison of the inhibition areas in the samples for GRE, as well as with amoxicillin as a positive control and with water: ethanol (1:1) as a solvent GRE, where the obtained area that indicates that the maximum antibacterial activity is what was extracted and prepared from a sample of GRE AgNPs.
Table 1. Inhibition zone of antibacterial activity test of synthesized GRE AgNPs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Measurement 1 (mm)</th>
<th>Measurement 2 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GRE AgNPs</td>
<td>32.1</td>
<td>31.7</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>19.6</td>
<td>19.3</td>
</tr>
<tr>
<td>Water</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>

The antibacterial activity increases with the proportion of GRE, which is likewise consistent with the rising particle size average. The volume ratio of silver nanoparticles concentration has no effect on the inhibition zones because it works in principle on the size of the obtained nanoparticles with the effect of the extract on the sizes of these particles, this means that the nanoparticles have no effect on the antibacterial activity at different GRE percentages. These results show that particle size has an influence on antibacterial activity, with smaller particles being more effective [13]. The smaller size aids in more efficient penetration of the bacterium cell membrane for subsequent degradation of sulfur- and phosphorus-containing complexes such as, DNA and causing cell death [11].

As depicted in figure 4, the efficacy of the GRE plant extract with AgNPs on the bacteria used, as well as the antibacterial activity of the GRE AgNPs depending on the size of the silver nanoparticles, which were finally formed with the extract to give a efficacy of bacterial inhibition due to the small size of these nanoparticles and their penetration into the innovative cell membrane, with an effective diameter of up to 32 mm.

![Figure 4: The antibacterial activity of synthesized AgNPs by GRE plant extract against E. coli. Inhibition zones according to concentration of synthesized AgNPs; a: 35mm at 500µg/ml, b: 32mm at 250 µg/ml, c: 30mm at 125µg/ml.](image)

**Conclusion**

The use of green spinach leaf extract as a bio reduction was effectively produced in silver nanoparticles (AgNPs). The analysis of UV-visible Spectrophotometry, FTIR and XRD indicate that AgNPs were produced from the full Ag+ reduction of AgNO₃ nano-size precursor. The volume percentage of GSE is shown to impact the average and distribution of different particle sizes. The effective capping and stabilizing characteristics of the AgNPs were shown in the FTIR, EDX and SEM analyzes. In addition, the synthesized AgNPs showed antibacterial activity against E. coli, with the tendency to enhance antibacterial activity as a consequence of smaller particle size.
Disclosure statement
No potential conflict of interest was reported by the authors.

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