Characterization and Green Synthesis of Silver Nano Particles from Salvia Officinalis Leaf Extract

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Abstract—In this work silver nanoparticles were prepared by green synthesis from salvia L leaves extract. These particles were investigated for its morphology, particles size, chemical composition using Scanning Electron Microscopy (SEM) UV-Vis, FTIR. The result of XRD confirmed that the prepared silver particle size to be 20 nm. It is also retaining the medical values for the antimicrobial activity.

Keywords- Green synthesis; silver nitrate; medicinal plant

I. INTRODUCTION

Among nanomaterials, silver nanoparticles play a major role in the field of nanotechnology and biological medicine due to their attractive physiochemical properties and in the use of nanomedicine [1]. Nanomaterials can be useful in such areas as solar energy conversion, catalysis, medicine and water treatment [2]. There are many approaches to the synthesis of silver nanoparticles, such as thermal decomposition, electrochemical, the use of microwave and green chemistry methods [3]. Green synthesis provides numerous advantages over chemical and physical processes. It's cost-effective, eco-friendly, and simple to scale up for large-scale synthesis. The use of plant extracts to produce nanoparticles is one of the most environmentally friendly green processes [4]. Nanoparticles are produced from plant extracts because of their medicinal properties, which could be used in drugs, targeted drug delivery and cosmetic applications [5]. Salvia comes from the Latin word salvare, which means "to heal," and salvias have been prized for their medicinal and culinary properties for centuries. Salvia, also known as sages, can be found all over the world. \textit{Salvia officinalis} has been used since ancient times for snakebites, increasing women's fertility, and more [6]. It was also used for hair care, insect bites and wasp stings, neurological and mental disorders, oral preparations for mouth, tongue, and throat inflammation, and fever reduction [7]. The present study is carried over by a green synthesis process in the preparation of silver nano particles from \textit{salvia officinalis} leaves using a reflux method [8].

II. MATERIALS AND METHOD

Silver Nitrate (AgNO\textsubscript{3}) was purchased from Spectrum Reagents and Chemical Pvt.Ltd Edayar, Cochin, India. Fresh and healthy leaves of \textit{salvia officinalis}(sage) is collected from The Medicinal Plant Development Area in Dodapetta(MPDA) Nilgiris north forest division Tamil Nadu, India.

2.1 Preparation of Leaf Extract

The collected leaves were washed with distilled water to remove the dust particles. The cleaned fresh leaves were cut into small pieces and put into the round bottomed flask and is refluxed with 200ml of distilled water. The refluxing time was around 2 hours(Figure 2a). In the initial stage the solution was white where the leaves were embedded. After refluxing the color changes to pale yellow color. The extract was filtered with Whatmann no 1 filter paper to collect the sedimented particles. The particles were shade dried and stored in cool place for further characterization studies.
2.2 Synthesis of Silver Nanoparticles

Solution of 0.01mM of (AgNO₃) silver nitrate was prepared using distilled water. Salvia officinalis extract and silver nitrate solution was taken in the ratio of 1:2 respectively. This mixture was stirred at room temperature at 400 rpm around 20 minutes until the color changes. This was then kept at rest until the precipitate completely settled down. The precipitate was centrifuged at 3000 rpm for 30 minutes for further settlement. Using distilled water, the precipitate was collected and cleaned. The precipitate was shade dried until the moisture is gone[9].

2.3 Characterization of Silver Nanoparticles

All the Characterization studies were done at SAIF cochin. The green synthesis of silver Nano particles was confirmed using UV-Visible spectrophotometer. UV-Visible absorption NIR spectrometer Agilent Cary 5000 in the region between 200nm to 3000nm was used to determine absorption of the salvia officinalis leaf extract. The dried salvia officinalis sample were recorded into FT-IR spectrophotometer analyzed and find the functional groups of the given samples using thermo nicolet avtar 370 range between 4000cm⁻¹ to 400cm⁻¹. XRD patterns were recorded from powder X-ray diffractometer (Bruker D8 Advance) operated at 30Kv and spectrum was recorder by cuko radiation with wave length 1.506 Å in the range 200-800nm to calculate the particle size. SEM study was carried out to investigate the shape and size of the AgNPs using joel 6390LA and Energy Dispersive X-ray (EDX) on oxford XMX N operated at 0.5kV to 30kV at magnification 300000 and EDAX Resolution at 136 Ev.
III. RESULT AND DISCUSSION

3.1 Uv–Vis Spectral Studies and Analysis

When the salvia officinalis extract was mixed with the aqueous solution of the silver nitrate, the change in color was noticed from pale yellow to dark brown which is shown figure (3). The change in color is due to the particlesize reduction of silver ion. The silver nanoparticles exhibit some brown color in the aqueous solution [10]. The presence of nanoparticles was confirmed by obtaining a spectrum in the visible range 200nm to 800nm using UV-Visible spectrophotometer. From this analysis specific absorption peak was found at around 431 nm, [11] which is a blue shift and confirms the Ag particles produced are in the nanoscale range figure (4). It is known that when the surface Plasmon vibration in silver nano particles is excited, and the size reduction takes place.

![Figure 4. UV–vis spectra of silver nanoparticles](image)

3.2 F tir Analysis

FTIR spectroscopy was used to characterize and identify the chemical composition of the salvia officinalis extract mixed with silver nitrate solution. The peak at 3550 cm⁻¹ revealed the NH₂ [12] groups and 3415 cm⁻¹ [11,12,13] confirming the OH Absorption 2924 cm⁻¹ there by confirming the CH₃[14]and CH₂ in aliphatic compounds and CH antisymmetric stretching[15],1618 cm⁻¹ showing the C=O alkenes groups [16]. The band 1382 cm⁻¹ shows the SO₂ sulfonyl chlorides and SO₂ antisymmetric stretch [17]615 cm⁻¹ shows the naphthalenes and in plane ring deformation [18]468 cm⁻¹ shows that naphthalenes and out of plane ring bending[19,10].

![Figure 5. FTIR spectra of silver nanoparticles](image)

3.3 Xrd Analysis

Xray powder diffraction was used to determine the phase of the nanoparticles and their crystalline structures. The scherrer formula was used to determine the particle size or grain size of the particles.

\[ d = \frac{0.9 \lambda}{\beta \cos \theta} \]

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where $d$ is the mean diameter of the nanoparticles, $\lambda$ is wavelength of x-ray radiation source, $\beta$ is the angular FWHM of the xrd peak at the diffraction angle $\theta$ [20]. Figure (6) shows the Xrd pattern of silver nanoparticles obtained using *salvia officinalis* leaf extract. The distinct peaks were 38.04, 44.26, 64.44, 77.34, observed [21]. The average crystallite size is calculated using Scherrer equation with the peaks obtained. The average size of synthesized particle is calculated to be 20 nm. (111) [21], (200) [24], (220) [22], (311) [23] indexed angle for the corresponding peaks respectively for the crystalline plane of silver particle obtained.

![XRD spectra of silver nanoparticles](image)

**Figure 6.** XRD spectra of silver nanoparticles

### 3.4 Sem Analysis

SEM technique is employed to determine the surface morphology and the topography of synthesized silver nanoparticles [9]. SEM image exhibited that the biosynthesized silver nanoparticles are mostly spherical in shapes. The size of the nanoparticles was within the range of 20-50nm. It is also noticed that the nanoparticles are in direct contact with each other. The capping agent noticed in the nanoparticles gives the stabilization for the particle. The capping agent may be due to the sediments in the leaf extract.

![SEM analysis](image)

**Figure 7.** SEM analysis of (a) silver nanoparticles in 0.5 µm (b) silver nanoparticles in 0.2 µm (c) silver nanoparticles in 1 µm

### 3.5 Edax

Energy dispersive X-ray spectrometers cash in of the photon nature of sunshine. The energy of one photon is simply enough to supply a quantifiable voltage pulse X-ray in the X-ray range, and the output of an ultra-low noise preamplifier coupled to the low noise is a statistical measure of the associated quantum energy. The EDX graph says (figure 6) that Ag is the main component in the prepared nanoparticles which confirms the Ag nanoparticles. The other particles may be the bio components present in the leaf. Metallic silver nanocrystals generally show typical optical absorption peak approximately at 3 keV due to surface plasmon resonance [25, 26].

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IV. ANTIMICROBIAL STUDIES

4.1 Antibacterial

In the recently revealed writing, it has been accounted for that the silver nanoparticles shows antibacterial activity not simply against Gram positive and Gram-negative microorganisms however additionally against multidrug safe (MDR) microscopic organisms \[27\]. In the present work, the antibacterial adequacy of S.O plant leaves remove integrated silver nanoparticles was concentrated through well diffusion method technique by utilizing the microorganisms, Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli at the fixations 100 µg/ml \[28\]. The microbe’s development was very repressed by this orchestrated silver nanoparticle arrangement unequivocally demonstrative of productive antibacterial activity. Also, it was accounted for that the zone of inhibition of S.O plant leaves extricate intervened incorporated silver nanoparticles was more when contrasted with standard medication (Ciprofloxacin) and S.O plant leaves separate \[29\]. Consequently, the noticed outcomes from the examination obviously showed that the S.O plant leaves remove intervened blended silver nanoparticles perhaps will be used as a possible antibacterial specialist \[30\]. The bacterial activity of synthesized Ag nanoparticles against four bacteria such as E. coli and S- aureus B-subtilis K-pneumonia showed a clear inhibition zone shown in figure(9) and table(1)\[31]\[32]\[33]\[34].

Figure 8. EDX Spectrum of synthesized AgNPs with 2 mL salvia officinalis leaf extracts solution

Figure 9. Bacterial activities of synthesized Ag nanoparticles against

(a) S. aureus (b) Bacillus subtilis (c) Klebsiella pneumonia (d) Escherichia coli
Table 1. The results of antibacterial activity with zone of inhibition.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Control</th>
<th>SO</th>
<th>Ciprofloxacin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Zone of inhibition in mm</td>
</tr>
<tr>
<td>1. Staphylococcus aureus</td>
<td>-</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>2. Bacillus subtilis</td>
<td>-</td>
<td>22</td>
<td>40</td>
</tr>
<tr>
<td>3. Klebsiella pneumoniae</td>
<td>-</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>4. Escherichia coli</td>
<td>-</td>
<td>20</td>
<td>16</td>
</tr>
</tbody>
</table>

4.2 Antifungal

The salvia officinalisAgNPs were tested for antifungal activity by disc diffusion method against the test organisms Aspergillusniger and Aspergillus flavus and Candida albicans Penicillium sps.[35]. The Sabouraud dextroseagar (SDA) medium plates were sterilised, and the overnight grown C. albicans culture was dispersed using a sterile cotton swab.A. flavus cultures were spread on potato dextrose agar (PDA)[36]. The antifungal activity was also evaluated against the silver nitrate (1 mM) and salvia officinalis leaf extract as control and antibiotic Ketoconazole (25 lg) as a standard. Sterile discs of 6 mm diameter were soaked in SNPs, dried plant extract and silver nitrate solution (1 mM)[37]. Discs were distributed over the surface of the inoculated agar plate after the test organisms had been disseminated on plates. Each disc was pressed down to ensure full contact with the agar surface[38]. The plates were incubated at 27°C after the placing of discs. Each plate was inspected for normal growth after an overnight incubation. The diameter of the zones of total inhibition, including the diameter of the disc, was measured using the naked eye. Zones were measured to the nearest whole millimeter, using sliding calipers, which is held on the back of the inverted petri plates for the measurement[39].

(a) (b)(c)(d)

Figure 10. Antifungal activity against (a) A. niger (b) A. flavus and (c) C albicans. (d) Penicillium sps
Table 2 The results of antifungal activity with zone of inhibition

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Microorganisms</th>
<th>Control</th>
<th>SO</th>
<th>Ketoconazole</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Zone of inhibition in mm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Aspergillus niger</td>
<td>-</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>2.</td>
<td>Aspergillus flavus</td>
<td>-</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td>3.</td>
<td>Candida albicans</td>
<td>-</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>4.</td>
<td>Penicillium sps</td>
<td>-</td>
<td>18</td>
<td>11</td>
</tr>
</tbody>
</table>

V. CONCLUSION

Green synthesis of stable silver nanoparticles using salvia officinalis leaf extract at room temperature was reported in this study. Synthesis was shown to be efficient in terms of reaction time and nanoparticle stability when no external stabilizers or reducing agents were used. It proves to be an environmentally friendly, quick green approach to synthesis, as well as a cost-effective and efficient way to make silver nanoparticles. Plant extract for synthesis has the advantages of being energy efficient, cost effective, and safeguarding human health and the environment, resulting in less waste and safer goods. This environmentally friendly technology could be a viable alternative to traditional physical/chemical methods for silver nanoparticle manufacturing, and hence has the potential to be utilized in biomedical applications. XRD reveals that the particles produced were in the average size of 22nm. UV analysis also supports the reduction of particles into the nano size. FTIR gives the vibration, stretching and stability in the formation of AgNO₃ nanoparticles. SEM shows the shape of AgNO₃ and EDAX shows its composition. The silver nanoparticles have been shown to have strong antibacterial and antifungal action against the bacteria and fungi that have been examined. Our results confirm that biosynthesized AgNPs has given excellent antifungal activity against salvia officinalis silver nanoparticles.

VI. ACKNOWLEDGEMENTS

The authors gratefully acknowledge the Department of Physics Government Arts College Ooty the Nilgiris Tamil Nadu, India for providing all the laboratory facilities and. The authors are also grateful to Sophisticated Test & Instrumentation CentreCochin University of Science and TechnologyCochin, Kerala, India. For analytical facility.

REFERENCE


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