Green Synthesis of Selenium Nanoparticles from Leaf and Stem Extract of *Leucas lavandulifolia* Sm. and Their Application

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1. Introduction

The need for biosynthesis of nanoparticles rose as the physical and chemical processes were costly. Often, chemical synthesis method leads to presence of some of the toxic chemical absorbed on the surface that may have adverse effect in the medical applications [1]. This is not an issue when it comes to biosynthesized nanoparticles via green synthesis route [2].

Selenium and its nanoparticles have an extensive range of applications. Selenium nanoparticles have high biological activity [3], including anti-hydroxyl radical property [4] and a protective effect against the oxidation of DNA [5]. It has also been reported that SeNPs have better bioavailability and less toxicity than other organic and inorganic selenocompounds [6]. Reported remarkable photocatalytic activity of selenium nanoparticles/nanorods for degradation of methylene blue under UV light irradiation, whereas Yang et al. [7] reported that selenium nanoparticles can catalyze the decolorization of congo red efficiently in the presence of UV light. SeNPs has been shown to be an anticancer agent especially for prostate, colon, and lung cancers.

Biological synthesized nanoparticles have upsurge applications in various sectors [8]. Biomolecules present in plant extracts can be used to reduce metal ions to nanoparticles in a single-step green synthesis process. Silver (Ag) and gold (Au) nanoparticles have been the particular focus of plant-based synthesis. Extracts of a diverse range of plant species have been successfully used in making nanoparticles [9]. Green synthesis of selenium nanoparticles (SeNPs) was achieved by a simple biological procedure using the reducing power of fenugreek seed extract. The cytotoxicity of SeNPs was assayed against human breast-cancer cells (MCF-7). It was found that SeNPs are able to inhibit the cell growth by dose-dependent manner. In addition, combination of SeNPs and doxorubicin shows better anticancer effect than individual treatments [10]. An environmentally friendly route has been used for synthesizing selenium nanoparticles using an orange peel extract as both reducing and stabilizing agent. The orange peel extract was found to be more efficient in reducing sodium selenite to selenium nanoparticle of spherical shape. The anti-algal activity of the selenium nanoparticles were tested and found to be effective in inhibiting algal blooms [11].

2. Experimental Methods

Seleniumic, ascorbic acid and ethanol were purchased from Merck and used without further purification. Distilled and deionized water was used in all experimental work.

2.1 Collection and Preparation of Plant Extract

Healthy *Leucas lavandulifolia* leaf was collected from Nallampalli, Dharmapuri district, Tamil Nadu, India. The plant materials were identified and confirmed by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The voucher specimen number (BSI / SRC/5/23/2014-15/Tech.1460).

*Leucas lavandulifolia* leaf was washed several times with distilled water to remove dust particles and then shade dried. *Leucas lavandulifolia* leaf extract was prepared by placing 10 g of dried fine cut in 500 mL glass beaker along with 400 mL of sterile distilled water. The mixture was then boiled for 5 minutes until the color of aqueous solution changed from watery to yellow. Then the mixture was cooled to room temperature and filtered with Whatman No.1 filter paper before centrifuging at 1200 rpm for 2 minutes to remove biomaterials. The extract was stored at room temperature in order to be used for further experiments.

2.2 Synthesis of Selenium Nanoparticles

About 2 mL of plant extract was mixed with 10 mL of 50 mM selenious acid solution, along with 200 µL of 40 mM ascorbic acid which was used as an initiator of reduction reaction. Standard positive control was maintained using selenious acid and 200 µL of 40 mM ascorbic acid for the synthesis of selenium nanoparticles. While plant extract + 200 µL of 40 mM ascorbic acid was used as negative control. The ruby red Se NPs (Fig. 1) were suspended then centrifuged. The powder form of the selenium nanoparticles was used for further analysis.

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The characterization of synthesized selenium nanoparticles are characterized by using following parameter techniques such as UV-Visible spectroscopy, FT-IR, SEM, EDX and examined the antibacterial activity.

2.3 Antibacterial Activity

Four gram-positive bacterial strains Bacillus subtilis (BSuttilis), Enterococcus faedalis (Ef. faedalis), Staphylococcus aureus (S. aureus) and three gram-negative bacterial strains Salmonella typhi (S. typhi), Klebsiella pneumonia (K. pneumonia), Shigella boydii (S. boydii) were used. All the bacterial strains were obtained from clinical laboratories, Salem District, Tamil Nadu. The test organisms were prepared by inoculating a loopful of culture in a 5 mL of Mueller Hinton broth and incubated (37°C) for 14 hours.

The antibacterial activities of the various extracts were evaluated by means of the agar well diffusion assay. The assay was carried out according to the method. Approximately 25 mL of Mueller Hinton Agar (MHA) (HiMedia) were poured into sterile petri dish and allowed to solidify. About 100 µL of bacterial inoculums were poured than swabbed on the MHA media by using sterile cotton swab. In each of these plates four wells (5 nm diameter) were punched in to the agar by using sterile cork borer. Than 50 µL of each extract (50 mg/mL) was separately added into wells and allowed to diffuse at room temperature. Equal volume of DMSO was served as negative control and standard antibiotic (Ciprofloxacin) used as positive control. The plates were incubated of 24 hours at 37 °C and the diameter (in nm) of clear zone of growth inhibition was recorded [12].

2.4 Statistical Analysis

Statistical analyses were conducted using SPSS software (16.0 version). Analysis of Variance (ANOVA) in a completely randomized design and corresponding to the selenium nanoparticles. The small peak observed in the UV region may be due to the small organic molecules present in reaction mixture. The UV data may support to further characterization of Leucas lavandulifolia leaf extract mediated selenium nanoparticles.

3.2 FT-IR Analysis

The major absorption band appeared at 3420, 3250, 3025, 3660, 2361 ± 0.57 is due to O-H stretching H-bonded alcohols and phenols. The band at 2350 cm-1 is due to stretching of O-H groups in water, alcohol and phenols and N-H stretching in amines [16]. The band at 3025 cm-1 corresponds to O-H stretch carboxylic acids. The band at 2660 cm-1 is due to N-H stretching of amino acid. The band at 2361 cm-1 is due to C-H stretching of aryl acid. The band at 1654 cm-1 attributed to the C=C stretch in aromatic ring. N-H bending in amine and C=O stretch in polyphenols. The C-N stretch of amide-I in protein gives the band at 1362 cm-1. The band at 1224 cm-1 is due to the C-O stretching of ether, C-O stretching in amino acid causes a band at 1078 cm-1. Finally the weak band at 724 cm-1 is the result of C-H out of plane bending [17, 18].

The FT-IR results imply that the Se-NPs were successfully synthesized and capped with bio-compounds present in the Se-NPs aqueous extract by using a green method.

3.3 Field Emission Scanning Electron Microscopic (FESEM) Analysis

Scanning electron microscope is employed to analyze the shape of the synthesized selenium nanoparticles. Fig. 4 shows the FE-SEM image of selenium nanoparticles. Majority of the particle were spherical in shape with diameter range 56-75 nm. These particles were well distributed with good aggregation. From these studies, the synthesized selenium nanoparticles may efficient applications in pharmacology.

3.4 Antibacterial Studies

The antibacterial effect of Leucas lavandulifolia leaf aqueous extract mediated used Se-NPs were examined by disc diffusion method against to gram positive bacteria like Staphylococcus aureus, Staphylococcus epidermidis and gram negative bacteria like Escherichia coli, Salmonella typhi and sample was taken in three different concentration to display its antibacterial potential. The-SeNPs zones of inhibition against to selected pathogenic strains are shown in Fig. 5. The zone of inhibition is measured in diameter (mm) for all organisms are shown in Table 1. Leucas lavandulifolia leaf aqueous extract mediated Se nanoparticles is exhibited efficient zone of inhibition against to all organisms. The higher zone of inhibition (15.33 ± 0.57) is observed specifically to E. coli and S. epidermidis than other two organisms. The high antibacterial activity of selenium nanoparticles is due to their extremely large surface area, which provides better contact with microorganisms [18]. Moreover, selenium nanoparticles act as effective antibacterial agent. The antibacterial study exposed the bacterial
resistance potential of green synthesized SeNPs is highly potential and the selenium nanoparticle may use to kill the deleterious bacteria for preventing from their mediated diseases.

Table 1 Antibacterial activity of Leucas lavandulifolia leaf aqueous extract mediated Se-NPs

<table>
<thead>
<tr>
<th>S. No</th>
<th>Organisms Name</th>
<th>Diameter of zone of inhibition (in mm)*</th>
<th>MA-L/Se-NPS</th>
<th>Positive control#</th>
<th>Negative control@</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>E. coli</td>
<td>15.33 ± 0.57</td>
<td>22.00 ± 0.81</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>2</td>
<td>S. aureus</td>
<td>13.33 ± 1.15</td>
<td>22.50 ± 1.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>3</td>
<td>S. epidermidies</td>
<td>15.33 ± 0.57</td>
<td>35.00 ± 2.16</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>4</td>
<td>S. typhi</td>
<td>12.66 ± 1.15</td>
<td>31.50 ± 1.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Fig. 5 Antibacterial activity of Leucas lavandulifolia leaf aqueous extract mediated Se-NPs. Streptococcus aureus, Staphylococcus epidermidies, Escherichia coli and Salmonella typhi

4. Conclusion

Selenium nanoparticles are synthesized through green method using aqueous extract of Leucas lavandulifolia leaf. The extract of polyphenol components and the water-soluble heterocyclic components such as alkaloid and flavones were principally responsible for the reduction of selenium ions and the stabilization of the nanoparticles. FT-IR spectra revealed the presence of reducing groups in extract for Se NPs synthesis. The synthesized selenium nanoparticles shows spherical shape with average diameter range is 56 nm – 75 nm. Green synthesized selenium nanoparticles could be a potential antibacterial agent to treat diseases caused by bacteria.

References


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