**Soymida febrifuga** aqueous root extract maneuvered silver nanoparticles as mercury nanosensor and potential microbicide

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**ABSTRACT**

The present communication reports a rapid, uncomplicated, sustainable and facile method of eco-friendly synthesis of silver nanoparticles (AgNPs). The pressing need for the development of benign, profitable and eco-friendly alternative routes has inspired researchers to explore plant extracts as safer replacements to hazardous chemicals. In the present study, a benign method of synthesis of AgNPs using *Soymida febrifuga* aqueous root extract has been developed. The characterization studies of synthesized AgNPs revealed spherical morphology and crystalline nature of AgNPs. The average particle size was 21.81 nm. The synthesized AgNPs were employed as mercury nanosensor for the selective and sensitive detection of toxic mercury ions in water and soil samples. The AgNPs showed a marked visual color change and change in surface plasmon resonance band on interaction with mercury ions. The greater selectivity of AgNPs towards mercury ions was observed. The limit of detection of mercury by 100 μL of colloidal AgNPs was found to be $2 \times 10^{-4}$ M visually and $1.332 \times 10^{-5}$ M spectrophotometrically in water samples and $4 \times 10^{-4}$ M visually and $22.3 \times 10^{-5}$ M spectrophotometrically in soil samples. The method makes use of a small quantity of AgNPs for detection of mercury in water and soil samples. The method proposed in the present study provides a rapid, selective and sensitive method for detection of mercury ions in environmental water and soil samples. The synthesized AgNPs were also used as effective microbicidal agents. The microbicidal potential of the synthesized AgNPs was checked against two gram positive and gram negative bacterial strains.
Keywords: Green synthesis, Silver nanoparticles, Soymida febrifuga, Characterization, Mercury nanosensor, Microbicide

1. INTRODUCTION

Nanomaterials are evincing prodigious utilization in the realm of science and technology. They are exhibiting unprecedented applications in diverse products such as cosmetics, paints, packaging materials, biomedical products, biosensors and water filters. Synthesis of nanomaterials is a propitious sector which is extending swiftly. Many routes of synthesis have been devised for the production of nanomaterials. But, regrettably, most of the methods developed till today make use of pernicious organic solvents which render nanoparticles unendurable for human use. Green and sustainable methods with higher efficiency than the conventional methods of nanoparticle synthesis are the need of the hour. These methods involve the use of natural and non-toxic substances for synthesis diminishing the use of hazardous chemicals.

Silver nanoparticles (AgNPs) are the most important metal nanoparticles exhibiting remarkable properties such as antimicrobial activity [1], catalytic activity [2], antioxidant activity [3] and anticancer activity [4]. Silver has been known to be an antimicrobial agent since ancient times [5]. Nanosilver exhibits a notable antimicrobial activity due to increased surface area and a considerable decrease in toxicity to human beings [6]. These prospective properties of AgNPs necessitate the need for the development of novel and safer substitute methods of synthesis which are cost effective and eco-friendly. AgNPs have been synthesized using physical methods [7] and chemical methods [8]. With the advent of green chemistry, there was an upsurge of use of green synthesis procedures to engineer AgNPs. Plant-mediated synthesis is considered most efficient of the methods available as these methods are cost beneficial, make use of innocuous and eco-friendly solvents, can be scalable for large scale production of AgNPs and does not require complex reaction conditions to be maintained during synthesis. Many plants have been exploited for the synthesis of AgNPs as evident from literature. Some of the examples of plant-mediated synthesis of AgNPs involve the use of aqueous root extracts of Catharanthus roseus [9], Delphinium denudatum [10] and Rheum palmatum [11]. Spherical shaped crystalline silver nanoparticles of size 35 – 55 nm, ≤ 85 nm and 121 ± 2 nm were synthesized using aqueous root extracts of Catharanthus roseus root extract [9], Delphinium denudatum [10] and Rheum palmatum [11], respectively. The present study uses the aqueous root extract of Soymida febrifuga in the reduction and stabilization of AgNPs of size 21.81 nm which is smaller than the AgNPs synthesized from the earlier reported results [9–11]. The use of curcumin (turmeric) has also been employed for the green synthesis of AgNPs [12].

AgNPs are also known to act as potential sensors in the detection of heavy metals and other chemical compounds [12]. Mercury is a heavy metal which is potentially detrimental to human health and is a hazardous environmental pollutant. The World Health Organization considered mercury as one of the top ten chemicals or group of chemicals of major public health concern. The presence of mercury in massive quantities in environmental samples such as soil and water samples is leading to bioaccumulation of mercury in the ecological food chain. This leads to a devastating effect on human population and other living organisms.
Hence, a need to identify sensors for monitoring, sensing and detecting mercury in environmental samples is much-needed technology today. Despite the availability of several instrumental techniques, they have limitations. The conventional methods have poor selectivity for mercury, involve the use of a complicated procedure, require complex and expensive instruments and are not suitable for on field testing. Novel methods which can overcome the limitations of the conventional sensing methods need to be devised for screening, sensing and quantifying the mercury present in a sample. Being tiny in size, AgNPs have an enhanced surface area which can be exploited in the detection of mercury present in environmental samples.

The unique properties exhibited by AgNPs may be attributed to their small size morphology. The AgNPs synthesized from aqueous root extract of *Soymida febrifuga* being smaller in size when compared to their counterparts synthesized from various other plant sources [9–11] can offer a large surface area ending up as a potential mercury sensor.

In the present study, the use of aqueous root extract of *Soymida febrifuga*, an Indian medicinal plant belonging to the family, Meliaceae has been reported. The use of *Soymida febrifuga* root has not yet been exploited for AgNP synthesis. The method developed makes use of very small amounts of the plant material and silver nitrate for the effective synthesis of AgNPs. The method is simple, fast, cost-effective and environment-friendly. To obtain particulars about size, shape and morphology of the synthesized AgNPs, the characterization of AgNPs was accomplished employing assorted instruments such as UV-Visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffractometer (XRD) and Transmission Electron Microscope (TEM). The synthesized AgNPs have been employed as a simple and selective nanosensor for rapid sensing of mercury present in water and soil samples.

The colorimetric sensing of mercury using AgNPs is simple, cost-effective, quick, environmentally benign and highly active. The presence of divalent mercury in water and soil samples can be detected both visually and spectrophotometrically. The synthesized AgNPs were evaluated for their antibacterial efficacy against two gram positive and two gram negative bacterial strains.

2. EXPERIMENTAL

2.1. Materials

Silver Nitrate (AgNO₃) was purchased from Sigma-Aldrich, India. Sodium sulphate (Na₂SO₄), Potassium chloride (KCl), Barium chloride (BaCl₂·2H₂O), anhydrous Calcium chloride (CaCl₂), Magnesium sulphate heptahydrate (MgSO₄·7H₂O), Cobalt (II) acetate tetrahydrate (Co(CH₃COO)₂·4H₂O), Copper sulphate (CuSO₄), Manganese sulphate monohydrate (MnSO₄·H₂O), Mercuric sulphate (HgSO₄), Lead nitrate (Pb(NO₃)₂) and Zinc acetate dihydrate (Zn(CH₃COO)₂·2H₂O) were purchased from SD Fine Chemicals Ltd., Mumbai, India.

Nutrient agar was procured from HiMedia Laboratories, Mumbai, India. Double-distilled water was used for the experiments.

2.2. Collection of plant material

Healthy roots of *Soymida febrifuga* were collected from the Nallamala forest area, Nagarkurnool district, Telangana, India. The roots were thoroughly washed with tap water...
followed by double distilled water several times in order to remove the dust particles and were shade-dried to remove the residual moisture. The roots were finely chopped into small pieces and then powdered using a conventional blender. The dried root powder was then sieved and stored in amber colored airtight container for further use.

2. 3. Preparation of aqueous root extract of *Soymida febrifuga*

The aqueous root extract of *Soymida febrifuga* used to reduce silver ions to silver nanoparticles was prepared by adding 0.1 g of dried root powder to 100 mL of double distilled water and the contents were heated at 60 °C for 15 minutes. The extract was cooled to room temperature and filtered through Whatman No. 1 filter paper. The filtered extract was stored in refrigeration for further use in the synthesis of AgNPs.

2. 4. Synthesis of AgNPs

The filtered aqueous root extract of *Soymida febrifuga* was treated with 1 mM silver nitrate solution in the molar ratio of 1:2 respectively. The mixture was heated in a water bath at 100 °C for 15 minutes. A color change of the solution from an orange color to reddish brown color demonstrated the formation of AgNPs. The experiment was repeated thrice for confirmation of AgNP formation.

2. 5. Characterization of AgNPs

The scan of optical absorbance between 200 – 800 nm with a Shimadzu double beam UV-Visible spectrophotometer UV-2600 was performed to investigate the reduction of silver ions to AgNPs by the aqueous root extract of *Soymida febrifuga*. SHIMADZU IR Prestige21 Fourier Transform Infrared spectrophotometer was used to obtain the infrared spectra of the synthesized AgNPs and the root extract using KBr pellet method. FTIR spectra were recorded from wave number of 250 – 4000 cm\(^{-1}\). X’pert Pro X-Ray diffractometer [Pan-alytical B. V., The Netherlands] operating at 40 kV and a current of 30 mA at a scan rate of 0.388 min\(^{-1}\) using CuK\(\alpha\) radiation [\(\lambda = 0.154 \text{ nm}\)] over a 20 range of 20-80° with a step size of 0.02° was used for recording the diffraction pattern of the synthesized AgNPs. The particle size and surface morphology were confirmed using the Transmission Electron Microscope [TEM]. The ultrasonicated nanoparticle suspension was cast onto a carbon-coated copper grid and the sample was allowed to dry at room temperature. The grid with the AgNPs was then loaded into the TEM holder. The TEM holder was then loaded into a Tecnai G2 (FEI, The Netherland) TEM operating at a voltage of 200 kV.

2. 6. Nanosensing of mercury (Hg\(^{2+}\)) in water and soil samples using AgNPs

The nanosensing of mercury (Hg\(^{2+}\)) in water and soil samples was done using *Soymida febrifuga* aqueous root extract primed AgNPs as colorimetric probes and nanosensor. To evaluate the propensity of AgNPs in detection of metal ions, eleven disparate metal salts, i.e., Sodium sulphate (Na\(_2\)SO\(_4\)), Potassium chloride (KCl), Barium chloride (BaCl\(_2\)·2H\(_2\)O), anhydrous Calcium chloride (CaCl\(_2\)), Magnesium sulphate heptahydrate (MgSO\(_4\)·7H\(_2\)O), Cobalt (II) acetate tetrahydrate (Co(CH\(_3\)COO)\(_2\)·4H\(_2\)O), Copper sulphate (CuSO\(_4\)), Manganese sulphate monohydrate (MnSO\(_4\)·H\(_2\)O), Mercuric sulphate (HgSO\(_4\)), Lead nitrate (Pb(NO\(_3\))\(_2\)) and Zinc acetate dihydrate (Zn(CH\(_3\)COO)\(_2\)·2H\(_2\)O) have been used. For the nanosensing of mercury (Hg\(^{2+}\)), 100 μL (0.01 mg/L) of the synthesized AgNP colloidal solution was added to 10 mM
stock solutions of different metal ions, shaken well and allowed to stand for 5 minutes of interaction to assess the selectivity of the synthesized AgNPs towards mercury ions (Hg\textsuperscript{2+}). The UV-Visible absorption spectra of the resulting solutions were measured. In another set of experiment, various concentrations (0.001 μM to 10000 μM) of mercury (II) ions were subjected to the colloidal suspension of AgNPs synthesized from aqueous root extract of *Soymida febrifuga*, shaken well and allowed to stand for interaction of 5 minutes to determine the sensitivity or limit of detection of Hg\textsuperscript{2+} by the synthesized AgNPs. The UV-Visible spectra of the resulting solutions were recorded.

2. 7. Preparation of water and soil samples

To demonstrate the practical applicability of AgNPs as colorimetric probes and potential nanosensor, water and soil samples collected from Osmania University, Hyderabad were spiked with different metal ion solutions and different concentrations of mercury (II) ion solutions as described in the general protocol given above. To determine the selectivity and sensitivity of the synthesized AgNPs in the detection of mercury (II) ions, the water samples were directly exposed to colloidal AgNPs and the metal ions in the soil samples were extracted using warm water, filtered through Whatman filter paper and then subjected to colloidal AgNPs.

2. 8. Antibacterial activity

*Soymida febrifuga* aqueous root extract primed AgNPs were experimented with four human disease-causing bacteria to examine the microbicidal effectualness of the synthesized AgNPs. Disc diffusion method was used to evaluate the microbicidal activity of the synthesized AgNPs against two gram positive bacterial strains (*Bacillus subtilis* and *Staphylococcus aureus*) and two gram negative bacterial strains (*Escherichia coli* and *Pseudomonas putrida*) in Mueller-Hinton agar plates. The bacterial cultures were incubated for 24 hours at 37 °C on nutrient agar medium followed by storage under refrigeration at 4 °C. The bacterial strains were then inoculated and grown on Mueller Hinton agar plates to obtain an equable bacterial growth. Sterile filter paper discs were cut and saturated with different concentrations of 5 μL (0.54 μg/L), 10 μL (1.08 μg/L), 15 μL (1.62 μg/L) and 20 μL (2.16 μg/L) of the colloidal AgNP solution. The sterile discs saturated with AgNPs were then placed on the petriplates with equable bacterial growth. A standard antibiotic, Ampicillin [10 μg/10 mL] was used as a positive control. The plates were then incubated for 24 hours at 37 °C. After incubation, the diameter of the zones of inhibition was measured.

3. RESULTS AND DISCUSSION

3. 1. Visual observation

The successful synthesis of AgNPs from the aqueous root extract of *Soymida febrifuga* was primarily confirmed by the visual color change from orangish red color to dark brown color exponentially with time as evident from the inset image in Figure 1. This phenomenon may be an outcome of excitation of the SPR during the synthesis of AgNPs [13]. Similar results were reported in the synthesis of AgNPs using aqueous leaf extract of *Azadirachta indica* where the colour of the solution changed from yellow to reddish brown colour [14].
3. 2. UV-Visible spectral analysis

UV-Visible spectroscopy has been a widely used technique in the characterization of the synthesized AgNPs. The UV-Visible spectra as shown in Figure 1 have shown a single strong characteristic absorption peak at 421 nm. This absorption peak corresponds to the Surface Plasmon Resonance (SPR) peak of AgNPs. The formation of a single narrow SPR band at shorter wavelengths indicates the formation of small sized spherical AgNPs without agglomeration. Similar results were reported in AgNPs synthesized from agricultural waste Annona squamosa peel extract [15].

3. 3. Fourier Transform Infrared Spectroscopy

FTIR spectra of Soymida febrifuga aqueous root extract and AgNPs thus synthesized were recorded as clearly seen in Figure 2. The bands at 3415 and 3342 cm\(^{-1}\) in FTIR spectrum of Soymida febrifuga aqueous root extract correspond to the stretching vibrations of OH bond indicating the presence of phenol. The bands observed in the region of 2926 cm\(^{-1}\) and 1739 cm\(^{-1}\) correspond to the stretching vibration of C-H bond in aromatic compound [16] and to the stretching vibration of C=O carbonyl group respectively. The peaks at 1616 cm\(^{-1}\), 1516 cm\(^{-1}\) and 1438 cm\(^{-1}\) may be attributed to the stretching vibrations of C=C of the phenyl rings of flavonoids [17]. The peak at 1319 cm\(^{-1}\) may be assigned to the C-H bond in an aromatic
hydrocarbon [18]. The FTIR peaks at 1261 cm\(^{-1}\), 1107 cm\(^{-1}\) and 1033 cm\(^{-1}\) correspond to stretching vibration of the C-O bond, an ester group and to the stretching C-OH vibrations of carbohydrates and glucosyl moieties respectively. The FTIR spectrum of aqueous root extract of *Soymida febrifuga* revealed the presence of flavonoids. The decrease in peak intensities and shift of the peaks indicated the participation of the OH groups in the reduction of silver ions to nanosilver and the participation of flavonoids as stabilizing and capping agents in the AgNP synthesis.

![FTIR spectra](image)

**Figure 2.** FTIR spectra of aqueous root extract of *Soymida febrifuga* and AgNPs

3. 3. 1. Probable mechanism of AgNP formation

The change in peak intensities and peak positions as evident from the FTIR spectra clearly indicate the participation of flavonoids in reduction and stabilization of the synthesized AgNPs. Flavonoids are a group of polyphenolic compounds which actively chelate and reduce silver ions into nanosilver. The tautomeric transformation of flavonoids from enol-form to keto-form may release a hydrogen atom that can reduce the metal ions to form nanoparticles. The probable mechanism of nanoparticle formation can be explained in stages of initiation of nanoparticle formation or nucleation and further aggregation [19]. The mechanism of AgNP formation primed by the aqueous root extract of *Soymida febrifuga* has been explained in Scheme 1.
Scheme 1. Probable mechanism of AgNP formation and stabilization by flavonoids present in aqueous root extract of *Soymida febrifuga*.

Figure 3. XRD pattern of AgNPs synthesized from *Soymida febrifuga* aqueous root extract.

The X-Ray diffraction pattern obtained for dried AgNPs is as shown in Figure 3. The indexing process of the diffraction pattern obtained was done and the Miller indices (hkl) have been assigned to each peak corresponding to AgNPs. The Table 1 shows the peak indexing of the AgNPs synthesized from aqueous root extract of *Soymida febrifuga*. The $d_{\text{calculated}}$ and $d_{\text{observed}}$ values were also in close agreement with each other as seen from the Table 1. The XRD
pattern of the synthesized AgNPs displayed the presence of intense peaks at 20 values of 37.69°, 45.81°, 64.00° and 77.04° representing the (111), (200), (220) and (311) lattice plane reflections of face centered cubic lattice structure of silver respectively. These diffraction angles and d-spacing values of peaks corresponding to fcc structure of silver in AgNPs were compared with the standard powder diffraction card of Joint Committee on Powder Diffraction Standards (JCPDS) File No.: 04-0783 corresponding to silver and were found to be in agreement [16]. The details of the same have been depicted in Table 2. The XRD analysis confirmed the formation of FCC AgNPs. The peak broadening of diffraction peaks indicated the small crystallite size of AgNPs [20]. The high intensity peak for FCC materials is generally [111] reflection which is seen in the sample along with other peaks whose intensities have reflected the high degree of crystallinity of the AgNPs synthesized from the aqueous root extract of *Soymida febrifuga* [20]. This feature adds to the special antibacterial properties of AgNPs [21].

**Table 1.** Peak indexing with d\textsubscript{calculated} and d\textsubscript{observed} values and crystallite sizes with lattice strain values of *Soymida febrifuga* aqueous root extract primed AgNPs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>2(\theta)</th>
<th>d\textsubscript{calculated} (Å)</th>
<th>d\textsubscript{observed} (Å)</th>
<th>hkl</th>
<th>FWHM (β) in radians</th>
<th>Crystallite size D (nm)</th>
<th>Lattice parameter a (Å)</th>
<th>Lattice strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>37.6954</td>
<td>2.38390</td>
<td>2.38640</td>
<td>111</td>
<td>0.0055</td>
<td>27.85</td>
<td>4.129</td>
<td>0.0040</td>
</tr>
<tr>
<td>2.</td>
<td>45.8150</td>
<td>1.97821</td>
<td>1.98060</td>
<td>200</td>
<td>0.0082</td>
<td>19.08</td>
<td>3.956</td>
<td>0.0049</td>
</tr>
<tr>
<td>3.</td>
<td>64.0017</td>
<td>1.45302</td>
<td>1.45479</td>
<td>220</td>
<td>0.0082</td>
<td>20.72</td>
<td>4.109</td>
<td>0.0033</td>
</tr>
<tr>
<td>4.</td>
<td>77.0434</td>
<td>1.23633</td>
<td>1.23681</td>
<td>311</td>
<td>0.0201</td>
<td>9.2</td>
<td>4.100</td>
<td>0.0063</td>
</tr>
</tbody>
</table>

From the XRD analysis, the average particle size has been calculated using the Debye-Scherrer’s formula as seen in Table 1. The average crystallite size of the synthesized AgNPs calculated using the Debye Scherrer’s formula was found to be 19 nm. The crystallite size values were found to be within the range of the particle size obtained by TEM analysis. The synthesized AgNPs were found to be smaller in size than AgNPs synthesized from leaf extracts of *C. circinalis*, *F. amplissima*, *C. benghalensis* and *L. nodiflora* [22]. The lattice constant ‘a’ depicts the unit cell edge of fcc silver crystal. Theoretically the lattice constant ‘a’ value for silver is calculated to be 4.07Å. Experimentally the lattice constant values have been depicted in the Table 1. The experimental lattice constant ‘a’ calculated from the various Bragg reflections of AgNPs was found to be 4.0735 ± 0.117Å. The theoretical and experimental lattice constant ‘a’ values were found to be in close agreement with each other and also with the lattice constant value from the JCPDS File No. 04-0783 which was found to be 4.086Å. The XRD parameters of the synthesized AgNPs are reported in Table 3. Unassigned peaks at 20 diffraction angles of 27.32°, 31.76°, 43.77° and 54.43° may imply the presence of biological capping by phytochemicals of the aqueous root extract of *Soymida febrifuga* on the surface of AgNPs [23]. The diffraction peak at 31.76° indexed with [101] plane in biosynthesized AgNPs was observed by Amutha *et al.* [24] and Marslin *et al.* [25]. Similar results have been observed in the AgNPs synthesized from aqueous root extract of *Soymida febrifuga*. 
Table 2. Comparison of the experimental diffraction angle and d-spacing with the standard JCPDS Silver File No. 04-0783

<table>
<thead>
<tr>
<th>S. No.</th>
<th>hkl</th>
<th>Soymida febrifuga root extract primed AgNPs</th>
<th>Standard JCPDS Silver: 04-0783</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Experimental diffraction angle</strong></td>
<td><strong>d-spacing (Å)</strong></td>
</tr>
<tr>
<td>1.</td>
<td>111</td>
<td>37.6954</td>
<td>2.38640</td>
</tr>
<tr>
<td>2.</td>
<td>200</td>
<td>45.8150</td>
<td>1.98060</td>
</tr>
<tr>
<td>3.</td>
<td>220</td>
<td>64.0017</td>
<td>1.45479</td>
</tr>
<tr>
<td>4.</td>
<td>311</td>
<td>77.0434</td>
<td>1.23681</td>
</tr>
</tbody>
</table>

Table 3. XRD parameters of Soymida febrifuga aqueous root extract mediated AgNPs

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Structure</th>
<th>Average crystallite size</th>
<th>Bond angle</th>
<th>Lattice parameters</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>VALUES</td>
<td>FCC</td>
<td>19 nm</td>
<td>α = β = γ = 90°</td>
<td>a = b = c = 4.0735 ± 0.117Å</td>
<td>67.59 Å³</td>
</tr>
</tbody>
</table>

3.5. Transmission Electron Microscope [TEM]

The shape and size of the resultant AgNPs were elucidated with the TEM analysis as evident from the Figure 4a. The morphology of the AgNPs synthesized from the aqueous root extract of Soymida febrifuga was predominantly spherical with a smooth surface. The TEM micrographs have displayed that the edges of the particles were lighter than the centers suggesting the capping of the AgNPs by biomolecules of the plant extract [26]. The particles were well dispersed. Similar results were reported by Das et al., 2015 [27]. The histogram showing the particle size distribution of AgNPs is shown in Figure 5. This suggests that a maximum number of particles were in the size range of 20 - 25 nm. The average particle size was found to be 21.81 nm. The particle size obtained is smaller than the AgNPs synthesized from aqueous root extracts of Catharanthus roseus root extract [9], Delphinium denudatum [10] and Rheum palmatum [11].

The TEM study confirmed that the AgNPs were not agglomerated and were well-dispersed. Figure 4b depicts the selected area electron diffraction (SAED) pattern of AgNPs synthesized from aqueous root extract of Soymida febrifuga. The AgNPs were crystalline in nature as evident from the SAED pattern. The diffraction rings were assigned lattice planes similar to the diffraction peaks obtained from XRD analysis. Mollick et al., 2015 reported similar findings [4].

-93-
3.6. Nanosensing of mercury (Hg\textsuperscript{2+}) in water and soil samples using AgNPs

3.6.1. Selectivity of AgNPs towards mercury (II) ions in water and soil samples

The AgNPs synthesized from the aqueous root extract of *Soymida febrifuga* were found to possess properties of a colorimetric probe and a nanosensor in the detection of mercury (II) ions in environmental water and soil samples. On addition of colloidal solution of AgNPs as small as 100 μL (0.01 mg/L) to solutions of different metal ions, a distinct color change was noted only in the case of mercury (II) ions. In the case of mercury, the color of the AgNPs...
disappeared whereas no specific color changes were displayed in case of other metal ions. However, peak broadening and peak shift were observed for other metal ions. This showed the selectivity of AgNPs to mercury (II) ions.

Figure 6. Selectivity of AgNPs for mercury (Hg^{2+}) ions in (a) Water samples (b) Soil samples

![Graph showing absorbance of AgNPs with different metal ions](image1)

Figure 7. Change in absorbance of colloidal AgNPs in the presence of metal ions

![Bar chart showing absorbance of AgNPs with different metal ions](image2)

The mechanism involved is dependent on the electrochemical differences between the two metal ions, Ag^+ and Hg^{2+}. The standard reduction potential of Ag^+/Ag and Hg^{2+}/Hg is 0.80 V and 0.85 V respectively. This shows the occurrence of redox reaction between nanosilver and mercury (II) ions [28]. The elemental mercury then formed an amalgamation with the silver particles resulting in the disappearance of the SPR band of AgNPs. The other metal ions exhibited redox potentials lower than that of silver which resulted in very slight variations in the SPR band of AgNPs [29]. The synthesized AgNPs were found to be practical probes or
sensors in selective detection of mercury in environmental samples. The selectivity of AgNPs towards mercury (II) ions in water and soil samples is shown in Figure 6a and Figure 6b respectively. The synthesized AgNPs showed largest absorbance difference for Hg$^{2+}$ ions as seen in Figure 7a and 7b respectively for water and soil samples. The disappearance of the AgNP peak and the large absorbance difference seen for Hg$^{2+}$ ions makes the synthesized AgNPs a selective mercury nanosensor. This provides a positive indicator for qualitative detection of mercury ions leading further to quantitative applications.

3.6.2. Sensitivity of AgNPs towards mercury (II) ions in water and soil samples

![Figure 8. Sensitivity of AgNPs towards different concentrations of Hg$^{2+}$ ions in water samples](image)

The minimum detection limit of mercury (II) ions by AgNPs for visual and spectrophotometric detection of mercury was also determined by using various concentrations of Hg (II) ions in both water and soil samples. In the water samples, the color of the solution gradually disappeared and the absorbance of the SPR band gradually decreased with the increase in the concentration of Hg (II) ions in solution as seen in Figure 8a. The limit of detection or sensitivity of the present method visually is 200 μM of mercury (II) ions in water samples as seen in Figure 8b. The efficacy of AgNPs to quantitatively detect Hg$^{2+}$ ions was demonstrated by adding colloidal AgNPs to different concentrations of Hg$^{2+}$ ions and calculating the change in absorbance intensity monitored by UV-Visible spectrophotometry. A linear graph of change in absorbance intensity versus Hg$^{2+}$ ion concentration in micro molar (μM) in water samples was plotted to calculate the limit of detection spectrophotometrically as seen in Figure 10a. The linear regression coefficient ($R^2$) was 0.992 with a detection limit of 13.32 μM.
A similar result was obtained while assessing the limit of detection for mercury in soil samples. There was a change in SPR band with a change in concentration of mercury (II) ions as evident from Figure 9a. The limit of detection of mercury (II) in soil samples by the present method visually is 400 μM as seen from Figure 9b. A linear graph of change in absorbance intensity versus Hg^{2+} ion concentration in micro molar (μM) in soil samples was plotted to calculate the limit of detection spectrophotometrically as seen in Figure 10b. The linear regression coefficient (R^2) was 0.9604 with a detection limit of 223.3 μM.
Table 4. LOD values of Hg (II) ions in water and soil samples both visually and spectrophotometrically.

<table>
<thead>
<tr>
<th></th>
<th>Visual LOD</th>
<th>UV-visible spectrophotometric LOD</th>
</tr>
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<tbody>
<tr>
<td>Water samples</td>
<td>$2 \times 10^{-4}$ M</td>
<td>$1.332 \times 10^{-5}$ M</td>
</tr>
<tr>
<td>Soil samples</td>
<td>$4 \times 10^{-4}$ M</td>
<td>$22.33 \times 10^{-5}$ M</td>
</tr>
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</table>

The detection limit value of $1.332 \times 10^{-5}$ M of mercury ions in water samples by the synthesized AgNPs was found to be more efficient than the detection limit of Hg (II) ions by AgNPs synthesized from fresh water apple fruits which displayed a detection limit of $8.5 \times 10^{-5}$ M [30]. The detection limit of Hg (II) ions by the present method is close to the method used by Shiva Prasad et al., 2017 [31]. This clearly indicates the practical applicability of synthesized AgNPs for visual and spectrophotometric detection of mercury (II) ions present in environmental water and soil samples. The method makes use of a minimum quantity of 100 μL (0.01 mg/L) AgNPs for the detection of Hg (II) ions in both water and soil samples. The method can be scaled up considerably to improve the sensitivity and limit of detection of Hg (II) ions. A considerable increase in the concentration of AgNPs can further reduce the limit of detection of Hg (II) ions in both water and soil samples.

3. 7. Antimicrobial activity

The AgNPs synthesized from the aqueous root extract of *Soymida febrifuga* exhibited outstanding microbicidal activity when explored against four infectious microorganisms. Disc diffusion method was used for evaluating the antibacterial efficacy of synthesized AgNPs against two gram positive strains, *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative strains, *Pseudomonas putrida* and *Escherichia coli*.

The zones of inhibition were observed when the synthesized AgNPs were tested against the four pathogenic bacterial strains as seen from Figure 11. The zones of inhibition were limpid. The measured diameters of the zones of inhibition are represented in the Figure. 12 and Table 5.

The AgNPs synthesized from aqueous root extract of *Soymida febrifuga* evinced efficient microbicidal activity in comparison with the standard antibiotic, Ampicillin. The microbicidal activity of the AgNPs can be attributed to the small size of AgNPs and a large surface to volume ratio. The AgNPs manifested a dose-dependent antibacterial activity against all four disease-causing pathogenic microorganisms.

Dose-dependent antibacterial efficacy was also seen in AgNPs synthesized from *Urtica dioica* Linn. leaves [16]. In contrast to the standard antibiotic, Ampicillin, the antibacterial effectualness was more for AgNPs in the case of gram positive bacterial strains than gram negative bacterial strains.
Figure 11. Microbicidal potential of AgNPs synthesized from aqueous root extract of *Soymida febrifuga* (AMP) Ampicillin control (1) 5 μL AgNPs (2) 10 μL AgNPs (3) 15 μL AgNPs (4) 20 μL AgNPs

Figure 12. Bar diagram showing microbicidal activity of AgNPs
Table 5. Antibacterial activity of the synthesized AgNPs from aqueous root extract of *Soymida febrifuga* against some human pathogens

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Bacterial strains</th>
<th>Zone of inhibition in mm for 5 µL of AgNPs (0.54 μg/mL)</th>
<th>Zone of inhibition in mm for 10 µL of AgNPs (1.08 μg/mL)</th>
<th>Zone of inhibition in mm for 15 µL of AgNPs (1.62 μg/mL)</th>
<th>Zone of inhibition in mm for 20 µL of AgNPs (2.16 μg/mL)</th>
<th>Ampicillin (10 μg/10 μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Bacillus subtilis</em></td>
<td>9</td>
<td>11</td>
<td>16</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas putrida</em></td>
<td>15</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td><em>Staphylococcus aureus</em></td>
<td>15</td>
<td>17</td>
<td>20</td>
<td>22</td>
<td>7</td>
</tr>
</tbody>
</table>

The mechanism of the microbicidal efficacy of AgNPs has not been clearly elucidated till date. Nevertheless, sundry theories have been proposed by various researchers to describe the antimicrobial activity of AgNPs [32–34].

The nano size of the AgNPs enhances the surface area of the particles making the AgNPs more susceptible to penetration into the bacterial cell. With the increase in the concentration of AgNPs, there is a substantial increase in the surface area for attachment to the bacterial cell wall resulting in inhibition of the bacterial growth and reproduction. The *Soymida febrifuga* aqueous root extract primed AgNPs displayed more effective bactericidal property against gram positive bacterial strains in comparison to the gram negative bacterial strains. This can be attributed to the structural organization of the bacterial cell wall. The gram positive bacteria have a plasma membrane as their cell wall and do not possess the bacterial outer membrane.

In contrast, the gram negative bacteria have an extra outer membrane made up of lipopolysaccharides and proteins as a part of their cell wall. The structural differences in the cell wall composition of the gram positive and gram negative bacterial strains are the result of differential antibacterial activity shown by AgNPs. The AgNPs interact quickly with the naked peptides on the cell wall of gram positive strains and penetrate readily through the cell wall to inhibit the bacterial growth [35]. However, as the outer wall of the gram negative bacterial strain is constructed with a thick lipopolysaccharide layer, it provides an effective barrier and prevents the swift entry of AgNPs through the membrane [36]. Another fact which explains the effective microbicidal activity of AgNPs towards gram positive strains in comparison to gram negative bacterial strains is the fact that the cell walls of gram positive bacteria bind large quantities of metals than the gram negative bacteria [37]. Similar results were reported by Niska et al., 2016, Umadevi et al., 2011 and Abalkhil et al., 2017.

A concentration as low as 5µL (0.54 μg/mL) of colloidal AgNPs synthesized from aqueous root extract of *Soymida febrifuga* has exhibited effective microbicidal activity against gram positive and gram negative bacterial strains. The antibacterial activity reported by Dhand...
et al. showed effective detrimental effect at a low concentration of 0.2675 mg/L of AgNPs [38]. A concentration of AgNPs as low as 0.54 μg/L synthesized from *Soymida febrifuga* aqueous fruit extract exhibited effective antibacterial activity [39]. The antibacterial activity reported from same concentrations of AgNPs synthesized from aqueous root extract of *Soymida febrifuga* exhibited greater zones of inhibition when compared to the AgNPs synthesized from aqueous fruit extract of *Soymida febrifuga*. However, the antibacterial activity was less for *Escherichia coli* [39]. The AgNPs thus produced are cost-efficient, eco-friendly, non-toxic and effective microbicidal agents which can be used for the treatment of human pathogenic bacteria and biofilms as well.

### 4. CONCLUSIONS

An uncomplicated, lucrative and environmentally benign method of AgNP synthesis maneuvered by aqueous root extract of *Soymida febrifuga* was explained in the present study. For the first time, *Soymida febrifuga* aqueous root extract has been used for the synthesis of AgNPs. The functional biomolecules, flavonoids present in the aqueous root extract of *Soymida febrifuga* participated in the reduction and stabilization of AgNPs. The prepared AgNPs were found to be spherical in shape with an average particle size of 21.81 nm. The AgNPs were well scattered. The synthesized AgNPs were checked for their practical application as a colorimetric nanosensor or probe in the detection of mercury (II) ions in environmental water and soil samples. The biosynthesized AgNPs in a very small volume of 100 μL (0.01 mg/L) showed selective and sensitive detection of mercury (II) ions. The present method can be employed for detection of toxic mercury (II) ions present in environmental samples. This can help in the detection and further prevention of mercury pollution in the environment. The AgNPs with concentration as low as 0.54 μg/mL have shown excellent microbicidal properties against gram positive and gram negative bacterial strains. The current study is environment centric and sustainable method which makes use of a minimum quantity of plant material (0.1% of plant extract) and precursor salt (1 mM AgNO₃) for the synthesis of stable AgNPs. The method can be adopted for the colorimetric sensing of mercury (II) ions in environmental samples all over the world. This can help reduce mercury pollution in the environment which is utmost hazardous to living organisms.

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References


