PHOTO–IRRADIATION BASED BIOSYNTHESIS OF SILVER NANOPARTICLES BY USING AN EVER GREEN SHRUB AND ITS ANTIBACTERIAL STUDY

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The photo-irradiated based biosynthesis of nanoparticles is an eco friendly approach for research interest in nanotechnology. Silver nanoparticles are frequently formed by chemical processes which are toxic and flammable in nature, hence this research study is reported on an environmentally friendly photo-irradiation based process of antibacterial silver nanoparticles by using gui hua (Osmanthus Fragrans) leaves, an ever green shrub with strong fragrance. Gui hua a bio-organism present in leaves extracts, that is eco friendly and contains flavonoids which act as reducing agent by forming stable and controlled shape silver nanoparticles. Formation and characterization of photo- irradiated biosynthesised silver nanoparticles were confirmed by UV-vis spectroscopy, X-Rays Diffraction, Energy-Dispersive X-ray spectroscopy (EDX) and Transmission Electron Microscope (TEM) period. The antibacterial activity of synthesised silver nanoparticles in the presence of bacterial strains E.coli and S. aureus are studied by well diffusion method.

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1. Introduction

Nanoparticles generation with an eco friendly and photo-irradiation is a cost-effective, non toxic and heat step reduced method, which can serve in the application for antibacterial textiles [1-3]. Nanoparticles can be achieved by a number of chemical, physical and biological methods [4,5]. The usual method of creating nanoparticles utilizes harmful chemical are toxic to the human body and can be considered as environmentally unsafe. The biological method includes enzymes, microorganisms, plants and plant extracts which are eco friendly approach compare to chemical and physical process for low cost, energy efficient and nontoxic production of silver nanoparticles [6-7].

Biosynthesis have received much attention as a feasible alternative for the development of metallic nanoparticles having antibacterial property where plants extract was used for the synthesis of nanoparticles without any chemical ingredients[8-10]. Plants [11], algae [12], bacteria [13] and fungi [14-15] are used for the synthesis of nanoparticles but absence of research

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report was found on photo-irradiated biosynthesis of silver nanoparticles by using gui hua (Osmanthus Fragrans) leaves extracts. The formation of silver nanoparticles having antibacterial activity by photo-irradiation approach could be advantageous over heat evaporation, electrochemical reduction, photochemical reduction and chemical reduction methods.

Gui hua a Chinese name for the Osmanthus Fragrans, it is small, an ever green and deep rooted shrub with succulent- looking firm leaves. It is one of the ten most famous flowers in China and Taiwan, traditionally the symbol of love and romance. The word Osmanthus is derived from Greek Osma, meaning "fragrant", and Anthos, meaning "flower"[16]. According to the Chinese medical science the plant protects against coughs. It is also used to enhance the flavour of other medicines or to disguise obnoxious flavours in herbal medicines. Besides the medical usage, the osmanthus fragrans can be found in ingredients for cosmetics and insect repellent agent for clothes [17].

2. Materials and methods

2.1. Materials

The gui hua (Osmanthus Fragrans) leaves were collected from the trees allocated in Jiangnan University campus, Wuxi, China. Silver nitrate (AgNO₃) was purchased from Sigma Aldrich Chemicals (USA), all the glassware are washed and sterilized, and distilled water was used throughout the experiment process.

2.2.1 Flavonoids test

To assure the presence of flavonoids in guihua leaves extract about 0.2 ml of extract solution were mixed with CH₃OH while heating, a chip of metal added to the mixture and followed by addition of few drops of HCL, appeared a reddish orange colour indicated the presence of flavonoids[18].

2.2.2 Preparation of the extract

To prepare the extract solution, leaves were randomly collected from the gui hua trees, washed twice with distilled water to remove dust and particulate matters present on them and placed 24 hours to dry at room temperature. After the initial washing and drying process leaves were cut into tiny size and boiled for ten minutes at 100 °C in Kirby flask, then filtrated the extracted solution twice by Whatman filter paper No.1 and let it be cool.
2.3. Synthesis of Silver Nanoparticles

Take 5ml of extract solution of leaves in a conical flask, and pour it in the beaker containing 5ml silver nitrate 3mM aqueous solution. After adding cover the flask with paper or cloth to avoid the light and temperature effect on the solution. Remove the covers as the flask is placed in bright sunlight, as the sun rays hit the solution within 2-3 minutes a colour change observed with continuous stirring. UV-vis spectra showed strong SPR band at 430nm and colour change to golden brownish is a clear indication of the formation of nanoparticles in solution. It is important here to discuss that the same procedure was adapted in the absence of extract solution no colour change was observed, that is why we strongly believe that potential phenolic flavonoids present in the extract solution of gui hua plays significant role in bioreduction of silver ions to synthesize nanoparticles.

2.4. UV-visible spectral analysis

The nanoparticles were characterised at primary stage by UV-vis spectroscopy, which is proved to be a very functional technique for the analysis of nanoparticles. Hence UV-vis spectra of colloidal silver solution were recorded as a function of wavelength on UV-4802 series double beam UV/Vis spectrophotometer (UNICO) from 300-700 nm operated on a resolution of 1 nm, using a quartz cuvette with water as a reference.

2.5. Energy-dispersive X-ray Spectroscope (EDX)

EDX spectroscopy was carried out to confirm the presence of silver atoms in photo-biosynthesised AgNPs with EDX detector (Linkisi 5000), equipped on Transmission Electron Microscope (TEM, JEOL/JEM2100 Japan).

2.6. Transmission Electron Microscopy (TEM)

The size and morphology of the synthesised nanoparticles were examined by using a Transmission Electron Microscope (TEM, JEOL/JEM-2100 Japan). The sample analysis was prepared by drop-coating the silver nanoparticles solutions onto carbon coated copper TEM grids.
The samples dropped on grids were allowed to dry at room temperature, the shape and size of synthesised nanoparticles was determined from the images and micrograph.

### 2.7. X-rays Diffraction (XRD)

To investigate the crystalline nature and structure of photo irradiated-biosynthesized silver nanoparticles X-rays diffraction was conducted, the sample was analysed on a Bruker D8 Advance X-ray diffractometer (Germany) using Cu-Kα irradiation ($\lambda=1.5406$ Å) and a scanning rate of 3- 90°, 4°±1/min.

### 2.8. Fourier Transform Infra Red Spectroscopy (FTIR)

FTIR measurements were carried out in order to obtain the information about the functional groups present around the AgNps for their stabilization and understand the transformation due to the reduction process; the sample was washed and dried at room temperature. FT-IR spectra was performed on Bruker vertex 70 spectrometer. Dried powdered extract were palleted with potassium bromide (KBr, 1:10). The spectra were recorded in the wavelength ranging from 500-4000 cm⁻¹ and analysed by subtracting the spectrum of pure KBr.

### 2.9. Antibacterial assay

Photo irradiated-biosynthesized nanoparticles were tested against pathogenic bacteria *Escherichia coli* (gram-negative) and *Staphylococcus aureus* (gram positive) by Well diffusion method. To gain the most effective output, different concentrations were used.

### 3. Results and discussions

It was noticed that AgNPs synthesised by photoreduction method had narrower size distribution as opposed to the broad size distribution obtained by a thermal reduction method [19,20]. Compared to the other prescribed processes, photoreduction is the more safe method to generate nanoparticles [20].

#### 3.1. UV-visible spectroscopy

UV-visible spectroscopy is very useful technique to analyse the particles formation and stability of the metal nanoparticles in aqueous solution [21]. Plasmon Resonance band is the most important feature of optical absorbance spectra among nanoparticles which is because of electron oscillation that gathers collectively around the surface of metal particles, the brownish colour change of silver nanoparticles became visible due to excitation of surface plasmon vibrations by absorbance at 430nm range of the curve.
3.2. TEM Analysis

The size and morphology of photo-irradiated biosynthesized silver nanoparticles were typically being measured by TEM shown in figure 3.

![TEM Image of synthesized Silver Nanoparticles](image)

The morphology is apparently spherical; size of formed AgNPs are observed in range of 20 ± 3 nanometers and polydispersed.

3.3. EDAX analysis of AgNPs

EDAX pattern shows the crystalline and elemental composition of silver nanoparticles photosynthesized from the extracted solution of *Osmanthus Fragrans* leaves. The strong signal in
the silver region was observed at 3 keV for silver nanoparticles due to the surface plasmon resonance.

In graph (Figure 5) obtained from EDAX analysis shows the existence of silver elements. It indicates the reduction of silver ions into elements of silver. The “Cu” presented in fig. 5 because of copper grid base used for analysis[22].

### 3.4. XRD analysis of photo-biosynthesized AgNPs

To verify the results of UV-vis spectral analysis and crystalline nature of photo-biosynthesised silver nanoparticles, samples of colloidal suspension of AgNPs was examined by X-Ray Diffraction. The particle size of silver nanoparticles was also calculated from the XRD pattern according to the line width of the plane, refraction peak using following Scherer’s equation. i.e.

\[
D = \frac{K\lambda}{\beta \cos \theta}
\]

- D = the crystallite size of AgNPs particles
  - \(\lambda\) = the wavelength of x-ray source (0.1541 nm) used in XRD
  - \(\beta\) = the full width at half maximum of the diffraction peak.
  - K = the Scherer constant with a value (0.9 to 1).
  - \(\theta\) = the Bragg angle.
XRD pattern of examined AgNps is shown (In Figure 6). Peaks at 36°, 39°, 48° and 56° can be assigned to reflections from 111, 200, 220 and 311 plane of face-centred-cubic (fcc) phase of the crystalline nature of silver nanoparticles with size 21 nm[23]. Thus the XRD data reveals the crystalline nature of synthesized nanoparticles.

3.5. Fourier Transform Infra Red (FT-IR) analysis

FT-IR spectroscopy was carried out in order to identify information about chemical groups around the silver nanoparticles for their stability and understand transformation phenomena of functional group due to reduction process[5], major peaks are observed in figure.7 are at 3440, 2890, 1760, 1630, 1380, 1120, 823, 670cm⁻¹.
The broad peak at 3440 cm\(^{-1}\) is a possible OH stretch of hydroxyl group, which suggests the presence of phenolic compound in the extract solution\[6\], peaks at 2890 cm\(^{-1}\) can be assigned to \(-\text{CH}\) and \(-\text{CH}_{2}\) stretching bonds, peak at 1760 cm\(^{-1}\) can be attributed to (C=O), 1630 cm\(^{-1}\) peak to carbonyl carboxylic stretching band of peptide linkage (stretching of amides), peak present at 1380 cm\(^{-1}\) can be a possible O-H band of carboxylates, 1120 cm\(^{-1}\) can attribute to C-N, peak 823 cm\(^{-1}\) can refer to NH-band where as peak present at 670 cm\(^{-1}\) can possibly belongs to C-H (alkynes).

Gui hua are known to be rich in various phytochemical like flavonoids, phenolic acid and lactones \[25\], and found in various plant parts like leaves, root, fruits, bark, stem and flowers \[26\]. Accordingly the high content source of flavonoids and phenolic compounds in gui hua leaves extract supports the potential production of Ag\(^{+}\) to Ag\(^{0}\) (In Figure 8b). The flavonoids play a role in the reduction of silver ions to silver nanoparticles \[27,28\]. Comparing photo irradiated-biosynthesised silver nanoparticles via gui hua leaves extract makes it different from earlier synthesised silver nanoparticles by easy access to raw material as it is ever green shrub, cost effective and less time consumption \[29\]. After synthesis of silver nanoparticles application on textile fibres (i.e. cotton, silk and wool) is our next objective to accomplish antibacterial property on the fabrics.
Fig. 8a. Schematic diagram of Photo irradiated-Biosynthesis process of AgNps.

Whereas, a = Leaves Extracted Solution (LES), b = Silver Nitrate (AgNO₃), c = LES + AgNO₃ and d = Silver Nanoparticles (AgNPs).

Fig. 8b. Probable pathway of Photo irradiated-Biosynthesis of AgNps.
3.6. Antibacterial activity

Since ancient time silver is used as an antimicrobial weapon for wound dressings and other medical purposes. By attracting a wide range of applications in biomedical through its antibacterial studies AgNPs are well known universally[30]. Antibacterial activity of photo-biosynthesised silver nanoparticles was carried out against *E. coli* (gram negative) and *S.aureus* (gram positive) with different concentrations as shown in Figure 9(a) and 9(b), which produces inhibition zone of different ranges were shown in Table1. The agar well diffusion method was used to determine the antimicrobial behaviour of gram positive (*S. Aureus*) and gram negative (*E. coli*) bacterial strains. Media were equipped in soy broth yeast extract (TSAYE) & LB agar. Plates were solidified at room temperature for 30 minutes; proceeding to inoculation with10 μL of each bacteria containing approximately $10^6$-7 CFU/ml. Punched wells (5mm) were served as reservoirs of extract and antibiotics. AgNPs samples were inoculated in the wells and plates were incubated at 37 ± 2 °C for 24 hours. A zone of inhibition against each strain was determined in millimetres (mm). The experiments were repeated three times to confirm the results.

![Fig. 9 (a) Escherichia Coli](image1)

![Fig. 9 (b) Staphylococcus Aureus](image2)

Table 1: Bactericidal activity of AgNPs in terms of zone inhibition around the well

<table>
<thead>
<tr>
<th>S No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>AgNPs(µg/mL)</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>E.coli (mm)</td>
<td>2 ±1</td>
<td>NA</td>
<td>9 ±1</td>
<td>13 ±1</td>
<td>25 ±1</td>
</tr>
<tr>
<td>S.aureus (mm)</td>
<td>NA</td>
<td>3 ±1</td>
<td>11±1</td>
<td>14 ±1</td>
<td>23 ±1</td>
</tr>
<tr>
<td>Extract. Solution(µg/mL)</td>
<td>10</td>
<td>NA</td>
<td>-NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Water(as zero)</td>
<td>NA</td>
<td>10</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA= Not Apply
Silver nanoparticles having size ranges from 10-100nm are strong bactericidal potential against gram positive and gram negative [31]. The well diffusion assay is used to test the sensitivity of the bacterial strains towards antibacterial; the clear zone around wells reflects the overall bacterial sensitivity. AgNps are loaded into wells on the nutrient agar plates spread with bacterial strains. A good zone of inhibition is observed for both gram positive and gram negative the bacterial strains tested [32]. A zone of inhibition increased with increasing the concentration of silver nanoparticles (Figure 10).

### 4. Conclusion

In this research study, an eco friendly and green approach was carried out by photo irradiation-biosynthesis of antibacterial silver nanoparticles; using an ever green shrub gui hua (*Osmanthus Fragrans*) leaves extracts. Silver ions in an aqueous solution were exposed to leaves extracts in bright sunlight, used as a constructive agent for the synthesis of silver nanoparticles. The antibacterial analysis of AgNps was carried out by the Well diffusion method against *E.coli* and *S. aureus* bacterial strains. Hence such methods for synthesis of nanostructure materials at inferior cost and with natural energy will promote functionalised fabrication on industrial stage and it will be quite practical for using silver nanoparticles on application in medical textiles.

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References