

# Green Synthesis of Silver Nanoparticles using Various Plant Extracts and Evaluation of their Antimicrobial Activities

Kinjal Gohil<sup>1</sup>, Sureshkumar Dhakhda<sup>1\*</sup>, Vipul Patel<sup>2</sup>,  
Ajay Rathod<sup>3</sup> and Pradeep Kumar Singh<sup>1</sup>

<sup>1</sup>Rai School of Sciences, Rai University, Ahmedabad, Gujrat India.

<sup>2</sup>Department of Chemistry, School of Energy Technology,  
Pandit Deendayal Energy University, Gandhinagar, Gujarat, India.

<sup>3</sup>Department of Chemistry, KSKV Kachchh University, Bhuj, Gujarat, India.

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A reliable and green technique for the synthesis of silver nanoparticles has been developed using plant extracts and characterized by UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM). This research presented a simple and effective approach to synthesizing silver nanoparticles and tested their antibacterial activities. Plant extracts were prepared from *Azadirachta indica*, *Mangifera indica*, *Eucalyptus* and *Fenugreek*. In the UV-visible spectra peaks found around 350-450 nm indicated successful synthesis of silver nanoparticles. The size and shape of synthesized silver nanoparticles were studied by Scanning Electron Microscopic analysis, where the size of silver nanoparticles was found around 15 to 20 nanometers and a spherical shape was observed. X-ray diffraction spectra demonstrated characteristic Bragg peaks, and confirmed the cylindrical, face-centered composition of the silver nanoparticles. Good antibacterial activity was shown against Gram-negative bacteria and moderate activity was shown in antifungal study.

**Keywords:** Antibacterial; Antifungal Activity; Characterization;  
Green Synthesis; Silver Nanoparticles.

The nanotechnology field is currently expanding more in the manufacturing market, with a wide quest for innovative nanomaterials along with manufacturing technologies<sup>1</sup>. Living cells have long been recognized as the best examples of equipment that operates at the nano level and executes a multitude of operations that vary from energy production to distillation of specific materials with high efficiency<sup>2</sup>. Nanomedicine is an emerging interdisciplinary field that integrates nanoscale technologies with medical science,

leading to broad applications of nanomaterials in healthcare. Medicine is no longer only the domain of physicians; Nanoscale elements and gadgets serve as tools for detection, disease control and treatment for ease of pain and general wellness, preservation and advancement<sup>3</sup>. The field of nanotechnology can help us better comprehend live cells and molecular interactions. Several nanoparticle-mediated therapeutic techniques for various diseases and pharmaceutical uses have been clinically approved<sup>4</sup>. Oligodynamic

\*Corresponding author E-mail: sureshdhakhda@yahoo.com



silver possesses antibacterial activity that extends far beyond its virotoxicity with deadly effects spanning all microbiological domains<sup>5-6</sup>. The utilization of metallic nanoparticles in the delivery of medicines, pharmaceutical development, and innovative treatment methods has announced conflict against numerous awful illnesses<sup>61</sup>. They leverage the human system's inherent circulatory system and method of absorbing drugs by sick tissues<sup>6</sup>. Chemically generated methodology produces a large number of nanoparticles quickly, it necessitates the use of sealing substances to stabilize their dimensions. The compounds utilized during the manufacturing and stabilization of nanoparticles are hazardous and manufacture unwanted metabolites<sup>7</sup>. The rise of demand in natural methods for nanoparticle synthesis originates from the demand for ecologically friendly chemical methods for nanoparticle formation. Green synthesis methods are employed to minimize the production of byproducts, hence providing a more environmentally friendly and secure procedure<sup>8</sup>. As a result, there is a growing need for "Green Nanotechnology"<sup>9</sup>. Moreover, the use of plant products minimizes the expense of bacterial separation and media for cultivation, boosting the cost-effectiveness of microbial nanoparticle synthesis<sup>10</sup>. Nanobiotechnology is now a widely popular subject of research in the realm of substance science. Nanostructures have entirely different or improved features depending on their dimension, dispersion and forms<sup>11</sup>. Silver-based compound's antimicrobial action is used in healthcare to reduce sickness during healing from burns and arthroscopy and additionally to minimize germs colonization of implants, tubes, coronary transplants, dental implants, surgical metals, and the skin of people<sup>12</sup>. The nanoparticles of silver have applications in catalysis, biochemical detection, biosensing, photographic science and technology, including medicine because of their distinctive attributes<sup>13</sup>. The nanoparticles of silver offer significant possibilities for ecological applications including antimicrobial properties. Silver metal's antimicrobial abilities enable them to be suitable for usage in a variety of common items, such as fabrics, alimentary vessels, appliance parts, and healthcare equipment<sup>14</sup>. Silver is a potent antimicrobial agent with minimal toxicity<sup>15</sup>. The primary common use for silver and nanoparticles

of silver is within healthcare applications, including tropical lotions that stop illness in injuries and untreated cuts. Due to its attractive physiochemical properties, nanoparticles of silver serve a significant function in both science and medicine<sup>16</sup>. Silver compounds are known to have considerable suppressive and microbiological capabilities as well as a broad spectrum of antibacterial action for ages. It is also used to prevent and treat illnesses with a particular focus on infections<sup>17</sup>. Silver nanoparticles are known to have fungicidal effects as well as antiviral, anti-angiogenic and antiplatelet actions<sup>18</sup>. In the pursuit of advancing sustainable and cost-effective methodologies for nanoparticle synthesis, this research endeavors to explore the green synthesis of silver nanoparticles through the plant extracts in conjunction with silver nitrate<sup>19</sup>. The integration of various plant extracts as both reducing and stabilizing agents represents a departure from traditional chemical and physical techniques, offering a more environmentally friendly<sup>66</sup> and economically viable alternative<sup>20</sup>. By replacing chemical compounds and intricate synthetic procedures with biologically derived components, this study aims to contribute to the field of green synthesis<sup>21</sup>. Plant extracts not only provide a cheaper and more straightforward approach to the synthesis of silver nanoparticles but also align with the global shift towards eco-friendly practices<sup>22</sup>. The investigation encompasses the utilization of multiple plant extracts to discern their unique contributions to replace conventional chemical techniques<sup>23</sup>.

## MATERIAL AND METHODOLOGY

### Materials

All the plant leaves of *Azadirachta indica*, *Mangifera indica*, Eucalyptus and Fenugreek were collected from a residential area of Ahmedabad city (latitude 23°01'21.7833 North, longitude 72°34'24.963 East), India. Silver nitrate (99.9% Assay, Finar Chemicals Ltd.) was used as provided. Throughout all the experiments water was subjected to distillation and subsequently purified utilizing the Millipore Company's Milli-Q filtration device<sup>24</sup>. All the bacterial strains [*Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442)] and

fungal strains [*Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323)] purchased from Institute of Microbial Technology in Chandigarh, India. Ciprofloxacin and Norfloxacin standard drugs were used for screening antibacterial activity and Nystatin and Griseofulvin standard drugs were used for screening of antifungal activity.

### Experimental method

Experimental procedures were conducted under supervision at a steady temperature ranging from 25-30°C. The experimentation room was equipped with air conditioning, ensuring a stable and consistent temperature, with negligible fluctuations in humidity observed throughout the experiments<sup>25</sup>.

### Plant collection

The following plant's leaves were collected from a residential area of Ahmedabad city, India.

1. *Azadirachta indica*: Family: Meliaceae; Genus: *Azadirachta*; Species: *A. Indica*; Scientific name: *Azadirachta indica*; Common name: Neem<sup>26</sup>
2. *Mangifera indica*: Family: Anacardiaceae; Genus: *Mangifera*; Species: *M. Indica*; Scientific name: *Mangifera indica*; Common name: Mango<sup>27</sup>
3. Eucalyptus: Family: Myrtaceae; Subfamily: Myrtoideae; Genus: *Eucalyptus*; Species: *Eucalyptus obliqua*; Scientific name: *Eucalyptus teriticornis*; Common name: Nilgiri<sup>28</sup>
4. Fenugreek: Family: Fabaceae; Subfamily: Faboideae; Genus: *Trigonella*; Species: *T. foenum-graecum*; scientific name: *Trigonella foenum-graecum*; Common name: Methi<sup>29</sup>

### Preparation of plant extracts

Selected Plant leaves were collected from the residential area of Ahmedabad city, Gujarat



Fig. 1. Plant leaf extracts

(India). All the samples were thoroughly washed with tap water then distilled water and dried in the absence of sunlight<sup>30</sup>. 25 grams of minced leaf material was carefully taken in a 250 mL round bottom flask and 100 mL of demineralized water. This mixture was heated in a water bath for 1 hour at 100°C temperature<sup>31</sup>. After 1 hour mixture was cooled to room temperature and then filtered through Whatman filter paper number one. After filtration extracts were collected in plastic bottles shown in Figure 1.

### Preparation of 1 mM silver nitrate solution

Accurately weighed 0.169 grams of silver nitrate (99.9% purity) was dissolved in Milli-Q water and then diluted to a final volume of 100 mL<sup>32</sup>.

### Synthesis of Silver Nanoparticles

One millimolar silver nitrate ( $\text{AgNO}_3$ ) aqueous solution was filled in the 50 mL burette. Five milliliters of plant extracts were taken in different four sterilized glass beakers for each plant extract. It was placed on a Magnetic Stirrer. Silver nitrate was added dropwise into the plant extract. The color changed from yellow to dark brown after a few minutes, indicating the synthesis of silver nanoparticles shown in Figure 2. The beaker was always wrapped with aluminum foil. Synthesized silver nanoparticles were further utilized for characterization and antimicrobial testing<sup>33</sup>.

### Characterization Techniques

The samples were analyzed using Fourier-transform infrared spectroscopy (PERKIN-ELMER Spectrophotometer). The appropriate level of humidity remained the same during

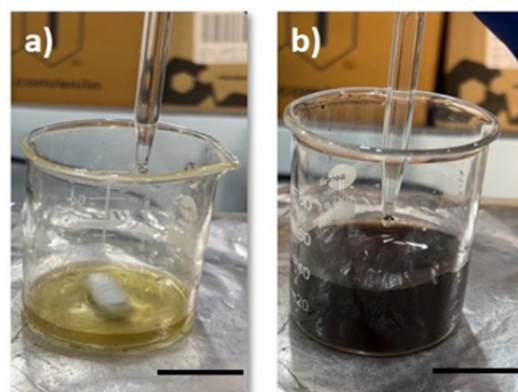


Fig. 2. a) Plant leaves extract before the addition of silver nitrate; b) Plant leaves extract after the addition of silver nitrate

the collection of FTIR spectra<sup>34</sup>. The Jasco ultraviolet (UV) spectrophotometer was used to do an Ultraviolet/visible spectroscopy examination. JEOL JSM-7900F FESEM was employed for obtaining sample micrographs<sup>35</sup>. To ensure representative compositional datasets reflecting Materials have been ground in small particles to obtain the mean volume content of the components before conducting XRD, Powder X-ray diffraction (PXRD) structures have been identified using a Bruker D8 DISCOVER X-ray diffractometer with Cu K $\alpha$  ( $\lambda = 1.5405 \text{ \AA}$ ) System within the  $2\theta$  scale of  $5^\circ$  to  $80^\circ$ , with an image acquisition pace is  $2^\circ$  per min<sup>36</sup>. By employing these methods, the shape, crystallinity, and stability of the synthesized nanoparticles were determined, showcasing their suitability for various applications<sup>37</sup>.

#### Antimicrobial effects

Well diffusion method was followed for the screening of antimicrobial activities<sup>38</sup>. Ciprofloxacin and Norfloxacin standard drugs were used for the antimicrobial study of Silver Nanoparticles samples synthesized using different plant extracts. AgNO<sub>3</sub> and Deionized water were used for the baseline condition<sup>39</sup>. The broth of Mueller Hinton was employed as a source of nutrients to cultivate and reduce an antibiotic solution to examine microbes<sup>40</sup>. The suspension amount for the experimental bacteria was adjusted to  $10^8$  CFU [Colony Forming Unit] per milliliter based on absorbance. Typical isolates were utilized

to test the antimicrobial and antifungal abilities of the strains<sup>63,41</sup>. Bacterial and fungal strains were purchased from the Institute of Microbial Technology, Chandigarh.

## RESULTS AND DISCUSSION

The nanoparticles of silver were formed utilizing a biosynthesis that utilized several leaf extracts as a provider of bio-reducing substances that are responsible for the breakdown of metallic ions into silver molecules. After about a full day of growth, the specimen was exposed to various characterization procedures<sup>42</sup>, with the following results:

#### UV/VIS spectrophotometric analysis

Synthesized silver nanoparticles were characterized by UV-visible spectroscopy. The ultraviolet-visible (UV-Vis) absorption spectra of the silver nanoparticles were investigated within the wavelength range of 300 to 800 nm. Figure 3 illustrates the absorption peaks of the silver nanoparticles synthesized, predominantly observed between 350 and 450 nm<sup>43</sup>. In the UV-visible spectroscopy of silver nanoparticles at different time durations are total of 4 different plant extracts are taken for the synthesis of silver nanoparticles. In Figure 4 among the four graphs, *Mangifera indica* leaves plant extract gives the highest absorption peak at 380 nm compared to *Azadirachta indica*, *Fenugreek*, and *Eucalyptus* leaves plant extract<sup>44</sup>.

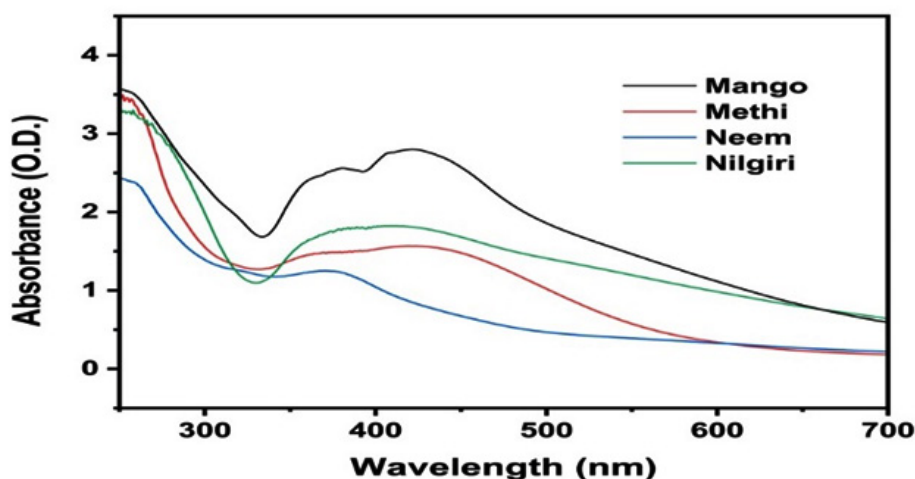


Fig. 3. UV-visible spectrum from silver nanoparticles from different plant extract

### FTIR analysis

The spectral band observed between 3490 and 3500  $\text{cm}^{-1}$  in the FT-IR spectra of silver nanoparticles corresponds to the stretching vibrations of O-H bonds in hydroxyl groups present in alcohols and phenols, or N-H bonds in amide functional groups. Notably, the peak observed around 1600-1650  $\text{cm}^{-1}$  signifies the presence of C-H stretching vibrations (Figure 5)<sup>70</sup>. The signal at 1041  $\text{cm}^{-1}$  represents glycoside or ether (C-O-C) bonds in the sample<sup>45</sup>. Ester groups present in the sample, such as -NH, -OH, C=O, and -C-O-C-, may be in charge of the bioreduction of  $\text{Ag}^{+1}$  to  $\text{Ag}^0$  nanoparticles<sup>46</sup>. The stretching frequency for silver nanoparticles was determined to be around 400-450  $\text{cm}^{-1}$ .

### X-ray diffraction (XRD) analysis

To acquire compositional data that accurately represents the overall composition of

the structures, initially, the samples were finely processed into powder before performing X-ray diffraction (XRD) analysis<sup>47</sup>. We then used a Bruker D8 DISCOVER X-ray diffractometer with Cu K $\alpha$  ( $\lambda = 1.5405 \text{ \AA}$ ) radiation to generate powder X-ray diffraction (PXRD) arrangements with a scan rate of 2 degrees per minute in the  $2\theta$  values ranging from 10 – 80<sup>48</sup>. In Figure 6 XRD patterns of the Neem, Mango, Methi and Nilgiri mediated Ag nanoparticles are shown. The X-ray diffraction (XRD) pattern of silver nanoparticles synthesized using different extracts of Neem, Mango, Methi and Nilgiri exhibits distinct peaks at  $2\theta$  values. These values correlate with the Bragg reflections of specific crystallographic planes, confirming the face-centered cubic (FCC) lattice systems of the silver particles. The peaks are observed at approximately 38, 44, 64, and 77 degrees, these relate to the levels of crystallography (111), (200),

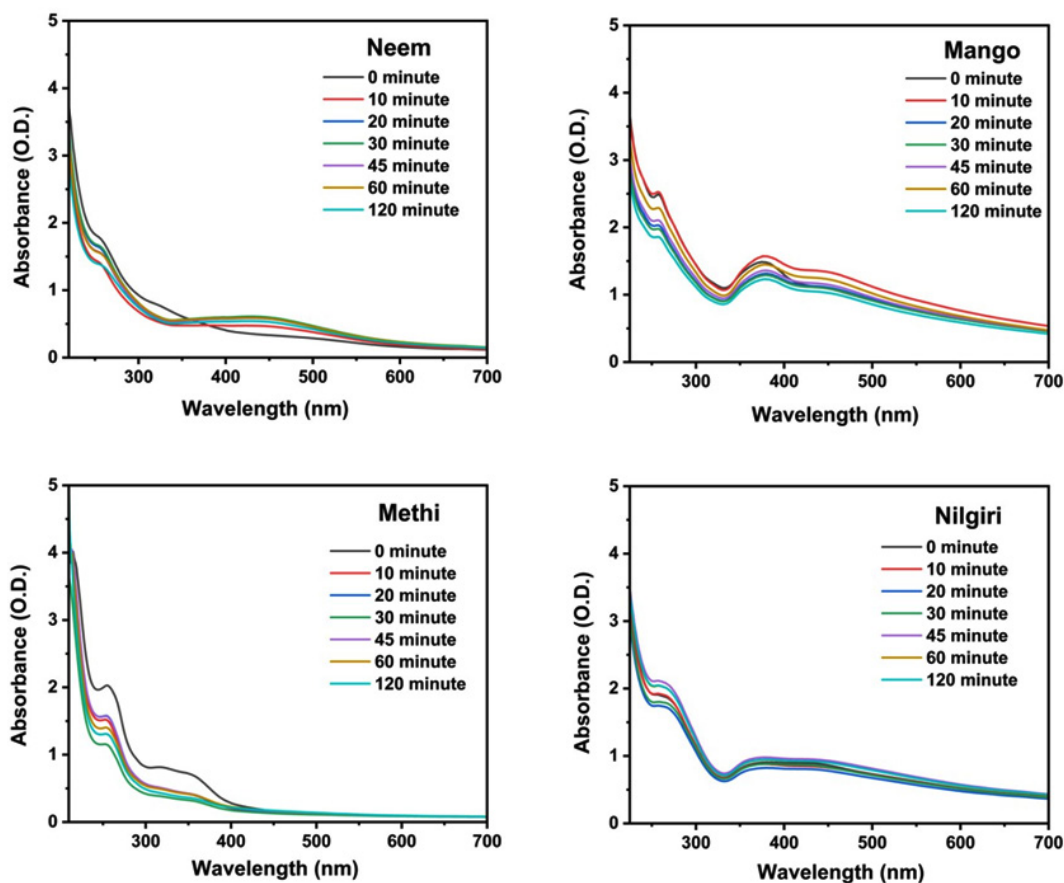


Fig. 4. UV-visible spectroscopy of Silver Nanoparticles at different time durations

(220), and (311)<sup>49</sup>. Peak intensities vary among the different samples, as evidenced by the above figure 6. These findings indicate the successful synthesis of Silver Nanoparticles in the prepared sample. The XRD patterns of the prepared samples agree with the previously reported XRD patterns<sup>50</sup>.

**Scanning electron microscopic (SEM) analysis**

The size and shape of prepared AgNPs have been identified using Field emission scanning electron microscopy (FESEM). Using ImageJ software particle size for every sample is measured. Figure 7 depicts the morphological properties of

silver nanoparticles synthesized with various plant extracts, displaying diversity in micrometer-scale parameters. As shown in Figure 7 a) was observed that the morphology of silver nanoparticles, synthesized through neem mediation, exhibited a predominantly spherical shape, interspersed with cuboidal and rectangular forms<sup>51</sup>. The particles displayed a polydisperse distribution and tended to aggregate into intricate irregular structures, lacking well-defined morphological boundaries. As shown in Figure 7 b), SEM images unveil the presence of flake-like structures in the synthesized

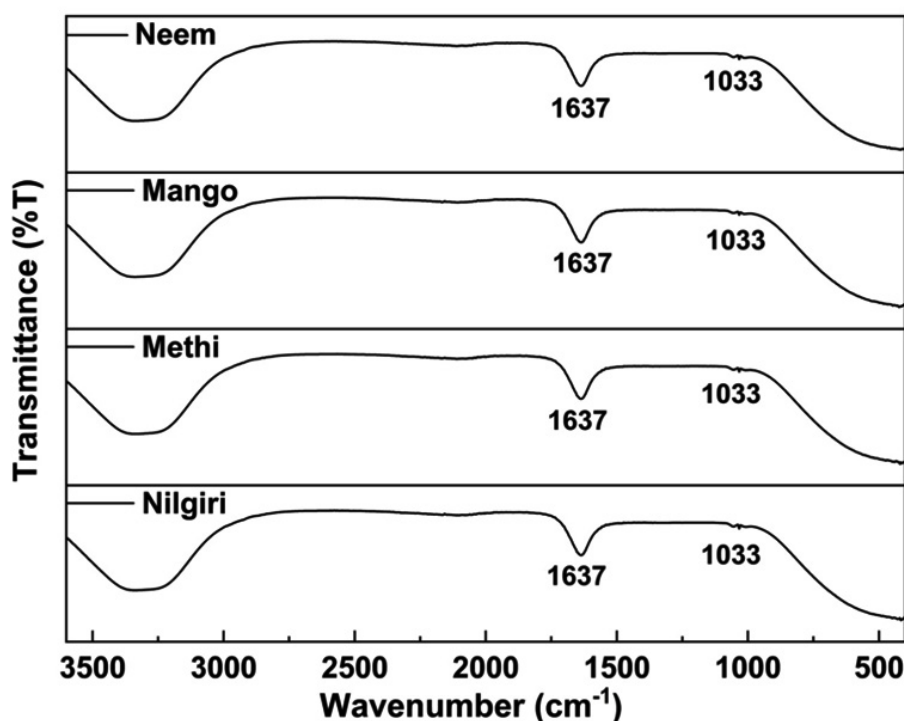


Fig. 5. FT-IR of synthesized silver nanoparticles

Table 1. Antibacterial Activity

Sr No.	Code No.	Minimal Inhibition Concentration [ $\mu\text{g/mL}$ ]			
		<i>E. Coli</i> (Gram-negative) MTCC 443	<i>P. Aeruginosa</i> (Gram-negative) MTCC 1688	<i>S. Aureus</i> (Gram-positive) MTCC 96	<i>S. Pyogenus</i> (Gram-positive) MTCC 442
1	A	50	100	100	500
2	B	62.5	50	100	50
3	C	50	100	100	125
4	D	100	100	50	100
5	Ciprofloxacin (Std.)	25	25	50	50
6	Norfloxacin (Std.)	10	10	10	10

silver nanoparticles through mango mediation their size ranged from 42 to 201 nm. The spherical shape-like morphology is evident from the SEM image as shown in Figure 7 c) having particle sizes ranging from ~12 to 46 nm. As shown in Figure 7 d), synthesized silver nanoparticles through Nilgiri mediation, the SEM image illustrates the presence of spherical agglomerated particles having particle sizes ranging from ~68 to 106 nm. Therefore, SEM analysis confirms the presence of silver nanoparticles<sup>52</sup>.

#### Antimicrobial Activity

The antibacterial activity of silver nanoparticles was studied against pathogenic bacterial strains of gram-negative and gram-positive using the well diffusion method. For screening of antibacterial and antifungal activities: *Escherichia coli* (MTCC 443), *Pseudomonas*

*aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 282) and *Aspergillus clavatus* (MTCC 1323) strains were used. Ciprofloxacin and Norfloxacin standard drugs were used for screening antibacterial activity and Nystatin and Griseofulvin standard drugs were used for screening of antifungal activity. The Institute of Microbial Technology in Chandigarh, India provided all microorganisms<sup>53-54</sup>. The antimicrobial capability was evaluated using the Broth Dilution Technique. It is a non-automated on-site microbial resistance study. It is done using containers for testing shown in Figure 8.

- Macrodilution Method in Tubes
- Microdilution format using plastic trays

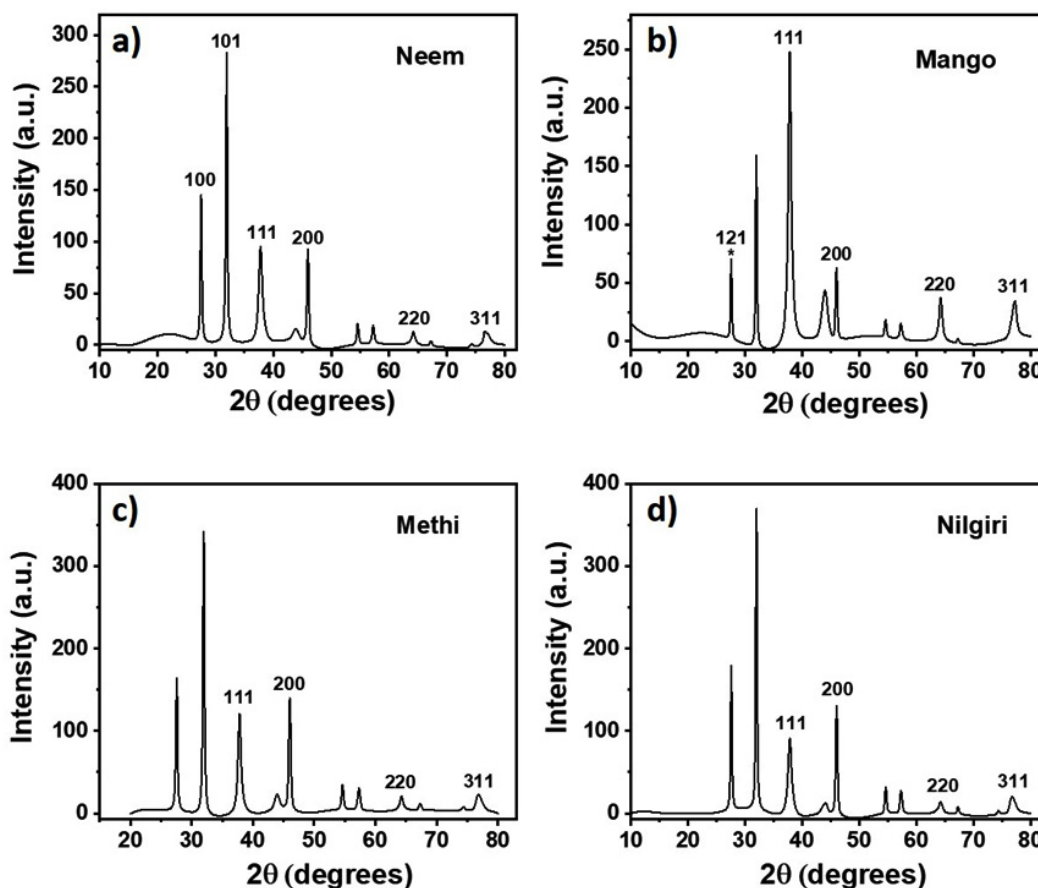


Fig. 6. a) *Azadirachta indica*, b) *Mangifera indica*, c) Fenugreek and d) Eucalyptus are the PXRD patterns of the silver nanoparticles synthesized by various plant extracts

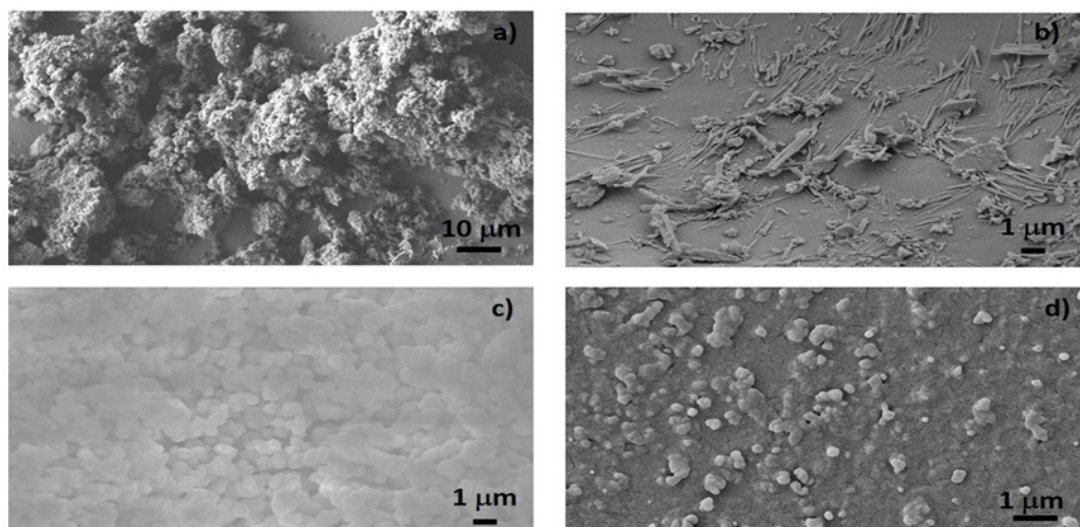
Table 1 denoted as A (*Azadirachta indica*), B (*Mangifera indica*), C (*Eucalyptus globulus*) and D (*Fenugreek*). Antimicrobial and antifungal activities were assessed, with Table 1 indicating that sample B, synthesized from Mango (*Mangifera indica*) leaves extract, exhibited the highest antimicrobial activity<sup>68</sup>, while Table 2 reveals that sample C, synthesized from Nilgiri (Eucalyptus) leaves extract, observed the highest antifungal activity<sup>67</sup>.

The inherent advantages of the biosynthesis technique extend beyond economic considerations, encompassing its profound eco-friendliness<sup>55</sup>. The diminished reliance on chemical compounds not only mitigates environmental impact but also aligns with the imperative of

sustainable practices, contributing to the well-being of future generations. The consequential reduction in chemical usage is particularly significant, given the ubiquitous applications of silver nanoparticles in pharmaceuticals, cosmetics, dyes, and other industries<sup>56</sup>. The affordability of the biosynthesis process translates directly into cost-effective end products, such as medicines and cosmetics, making them more accessible to a broader demographic<sup>57</sup>. The collective adoption of this biosynthesis technique over chemical and physical methods holds transformative potential, not only benefiting human health but also mitigating the ecological footprint associated with traditional synthesis approaches. Thus, advocating for the widespread implementation of biosynthesis emerges as a

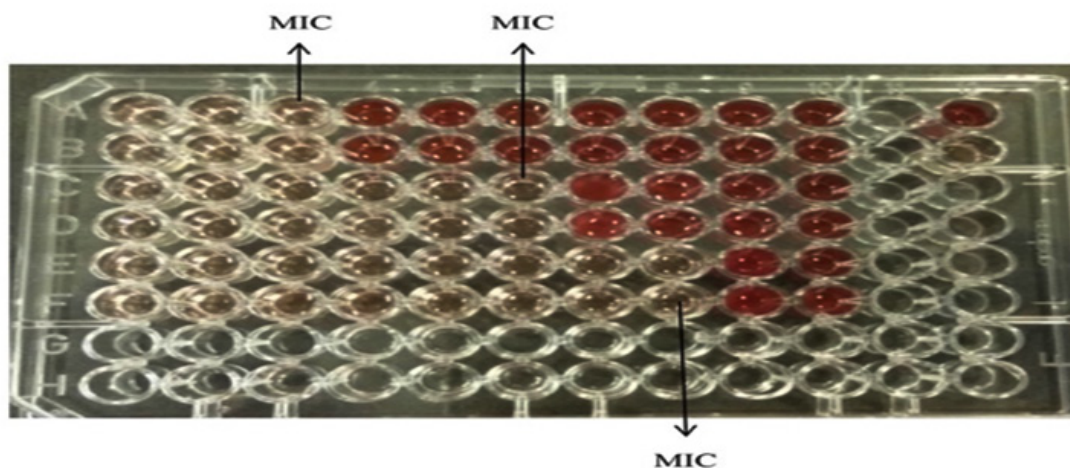
**Table 2.** Antifungal activity table

Sr. No.	Code. No.	Minimal Fungicidal Concentration [ $\mu\text{g/mL}$ ]		
		<i>C. Albicans</i> MTCC 227	<i>A. Niger</i> MTCC 282	<i>A. Clavatus</i> MTCC 1323
1	A	500	1000	1000
2	B	>1000	500	500
3	C	250	500	500
4	D	500	500	1000
5	Nystatin (Std. Drug)	100	100	100
6	Griseofulvin (Std. Drug)	500	100	100



**Fig. 7.** a) *Azadirachta indica*, b) Fenugreek, c) *Mangifera indica* and d) Eucalyptus are the SEM images of the silver nanoparticles in micrometer parameters synthesized by different plant extracts.





**Fig. 8.** Broth Microdilution Format using plastic trays which is a standard method of Microdilution

crucial strategy for fostering a more sustainable and harmonious coexistence between human activities and the environment<sup>58</sup>.

### CONCLUSION

Reported research work has shown the successful synthesis of silver nanoparticles by green methods from various plant extracts aiming to replace conventional chemical compounds and techniques with eco-friendly biological methods. The utilization of natural, low-cost biological reducing agents in green nanotechnology has been demonstrated, a green synthetic route for silver nanoparticles<sup>59</sup>. Synthesized silver nanoparticles were characterized by UV-visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD) and Scanning electron microscopy (SEM). The characterization data confirms that the silver nanoparticles have a size of less than 100 nm. The application involved the evaluation of synthesized silver nanoparticles for their potential as highly effective antibacterial and antifungal agents<sup>65</sup>. The microbiological analysis results indicate strong action against specific gram-negative bacterial strains and moderate activity against selected fungus strains. The demonstrated effectiveness of this green synthesis approach highlights its significance in promoting environmentally conscious practices.

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#### Conflict of Interest

All authors declare no conflict of interest.

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#### Authors' Contribution

Kinjal Gohil is a research scholar; she has performed all experiments, collected all experimental data, and drafted a manuscript. Sureshkumar Dhakhda is a corresponding author as well as a research guide; he has provided complete research guidance to research scholars. He has coordinated with the research scholar to generate all experimental data, including methodologies for sampling plants, the synthesis of silver nanoparticles, and the characterization and study of antimicrobial activities. Also provide guidance to scholars on the writing of the paper, correction of the drafted paper, and revision of the manuscript as per the reviewer's comments. Vipul Patel has helped the scholar to generate X-ray diffraction (XRD) spectra and interpret the XRD spectra of synthesized silver nanoparticles. Ajay Rathod has helped to generate and interpret the scanning electron microscopic data of synthesized silver

nanoparticles. Pradeep Kumar Singh has helped to research scholar for the study of antimicrobial activities.

#### Data Availability Statement

Not applicable because we have already shared all data in manuscript.

#### Ethics Approval Statement

This study did not involve the use of humans or animals.

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