Green synthesis of silver nanoparticles using leaf extract of *Lawsonia inermis* and *Psidium guajava* and evaluation of their antibacterial activity

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**Abstract**

The biosynthesis of silver nanoparticles has been proposed as a cost effective and environmental friendly alternative to chemical and physical methods. Plant mediated synthesis of nanoparticles is a green chemistry approach that interconnects nanotechnology and biotechnology. In the present study, synthesis of silver nanoparticles (AgNPs) has been performed using leaves extracts of two medicinal plants, *Lawsonia inermis* and *Psidium guajava* by reducing aqueous silver nitrate. Biologically synthesized silver nanoparticles have been widely used in the field of medicine. The silver nanoparticle formation was confirmed by the colour change of plant extracts. These silver nanoparticles were tested for antibacterial activity by using agar well diffusion method against the test organisms *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. The antibacterial activity of silver nanoparticles was analysed by measuring the zone of inhibition. The results of present study revealed that the silver nanoparticles synthesized by *Lawsonia inermis* and *Psidium guajava* showed significant antibacterial activity against selected bacterial strains. *Lawsonia inermis* and *Psidium guajava* silver nanoparticles showed highest activity against *Bacillus subtilis* (13mm) and *Escherichia coli* (11mm) respectively. Therefore they have an important advantage over commercial antibiotics and may prevent the risk for generation of antibiotic resistant bacterial strains.

**INTRODUCTION**

Nanotechnology is one of the most active research area in the modern material science. Depending upon their specific characteristics as like size, distribution and morphology, nanoparticles have distinct properties compared with the bulk form of the same material (Sriram and Pandidurai, 2010). Nanotechnology is now creating a growing sense of excitement in the field of life science especially in biomedical devices and biotechnology (Prabhu and Yamuna, 2010). Silver has long been recognized as a inhibitory effect on microbes present in medical and industrial process. The most important application of silver and silver nanoparticles is in medical industry such as topical ointments to prevent infection against burn and open wounds (Jain and Daima, 2009). Silver nanoparticles have been known for its inhibitory and bactericidal effects in the past decades. Antibacterial activity of silver containing materials may applied in medicine for reduction of infections on the burn treatment, prevention of bacterial colonization on catheters and...
elimination of microorganisms on textile, fabrics as well as disinfection in water treatment (Shameli and Mansor, 2012). In recent years, resistance to commercially available antimicrobial agents by pathogenic bacteria and fungi has been increasing an alarming rate and has become a serious problem in the field of medicine (Wright, 2005). Plant mediated synthesis of silver nanoparticles is a green chemistry approach that interlinks nanotechnology and plant biotechnology (Ahmed and Sharma, 2012; Dama et al., 1999; Poul et al., 1999, a, b.). The present study was carried out to synthesize the silver nanoparticles using Lawsonia inermis and Psidium guajava leaf extract and further synthesized silver nanoparticles were analysed for their antibacterial activity against pathogenic bacteria in laboratory condition Lawsonia inermis is a dwarf shrub, commonly known as Mehndi or Henna. It is renowned worldwide due to its cosmetic use for the reason of exclusive active principles in the leaves. It contains different variety of bioactive molecules (Shamkumar Deshmukh and Laxmikant Dama 2011; Tayyaba and Muhammad, 2014). Bio-inspired synthesis of nanoparticles provides advantages over chemical and physical methods because it is a cost effective and environment friendly and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. Biological synthesis of metal is a traditional method and the use of plant extracts has a new awareness for the control of disease, which are safe and devoid of phytotoxic effects. Currently, the increase of bacterial resistance to antibacterial agents poses a serious problem in the treatment of infectious diseases as well as in medical practices. In the present work, we reported a low cost convenient green synthesis approach to obtain large quantities of silver nanoparticles by reduction of silver ions using leaf extract of Lawsonia inermis and Psidium guajava. Hence the silver nanoparticles synthesized from the leaf extract of Lawsonia inermis and Psidium guajava may used against the bacterial pathogens.

MATERIAL AND METHODS
Preparation of leaf extract
Fresh leaves of Lawsonia inermis and Psidium guajava were collected locally, leaves were brought to laboratory and thoroughly washed with distilled water to remove all the dust and unwanted particles, cut into small pieces and dried at room temperature. About 100gm of finely incised leaves powder were extracted in methanol, after 48hours, the extracts were filtered by using Whatman filter paper No. 1 and filtrate were used for synthesis of silver nanoparticles (Shanmuga and Vasantha, 2015).

Preparation of Silver Nanoparticles
The procedure for the preparation of the silver nanoparticles has been adopted from Savithramma and Lingarao, 2011 with slight modifications. 1mM solution of silver nitrate was prepared and used for synthesis of silver nanoparticles. 5ml methanolic extract of Lawsonia inermis and Psidium guajava were mixed separately into two flasks containing 95 ml of freshly prepared silver nitrate solution and incubated in the dark condition overnight at room temperature.

Characterization of silver nanoparticles
The samples were observed under UV-Visible spectrophotometer for its maximum absorbance and wavelength to confirm the reduction of Silver nitrate was showed in Figure 1. (Lalitha and Subbaiya, 2013). Equal amount of overnight incubated sample and freshly prepared 1mM silver nitrate (1ml each) were taken into quartz cuvette and UV-Visible spectrum analysis was carried out compared with 1ml of 1mM silver nitrate used as blank to detect the reduction of silver ions (Arulmooorthy and Vasudevan, 2015). The concentration of silver nanoparticles produced was measured by using UV double beam spectrophotometer, between ranges of 200 to 700 nm.

Separation of synthesized silver nanoparticles
The overnight incubated sample was centrifuged at 8000rpm for 10 min, after centrifugation pellet was air dried and used for further testing.

FTIR analysis of silver nanoparticles
Fourier transform infrared spectra for the green synthesized silver nanoparticles of Lawsonia inermis and Psidium guajava was obtained in the range between 4000 to 500cm\(^{-1}\).

Antibacterial activity of silver nanoparticles
Mueller Hinton agar is chosen among the culture media because it gives satisfactory growth for most non fastidious organisms like Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli and it shows good bacteria culture reproducibility. Antibacterial activity of synthesized silver nanoparticles and methanolic extracts of both plants were tested by agar well diffusion method against E. coli, Pseudomonas aerogenosa, Bacillus subtilis and Proteus vulgaris. The culture was inoculated by spread plate method. Amoxicillin was used as standard control and DMSO was used as negative control. The plates were then incubated for 24 hours at 37\(^\circ\)C.
RESULTS AND DISCUSSION
The results are shown in Figure 1 to 7.

**Green synthesis of silver nanoparticles**
It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles. Silver nanoparticles synthetic route has been used for silver nanoparticles synthesis (Subramani and Jerome, 2014). *Lawsonia inermis* and *Psidium guajava* extracts were used to produce silver nanoparticles in the present study, silver ions were reduced to silver nanoparticles when plant extract was mixed with AgNO₃ solution, reduction was followed by change in greenish to yellowish in colour were showed in Figure 2.

![Figure 1. (a) 1mM silver nitrate with leaves extract, (b) Lawsonia inermis and Psidium guajava silver nanoparticles after centrifugation.](image)

**UV-Visible spectral Analysis**
An UV-Visible Spectroscopy is one of the important techniques to study the formation of metal nanoparticles (Saware, 2014). 1ml Sample of *Lawsonia inermis* and *Psidium guajava* were collected and monitored for the bioreduction of silver ions in distilled water, UV-visible spectral analysis between 200 to 600 nm showed maximum absorbance at 440 nm for *Lawsonia inermis* and 480nm for *Psidium guajava* (Figure 2 and 3).

![Figure 2. UV-Visible absorbtion spectrum of Lawsonia inermis silver nanoparticles.](image)
FT-IR analysis was used for the characterization of the *Lawsonia inermis* extract for the resulting nanoparticles (Figure 5). Absorbance bands in the region of 4000-500 cm\(^{-1}\) are 3346, 2922, 2852, 1716, 1619, 1446, 1375, 1165, 1031 and 553 cm\(^{-1}\). These absorbance bands are known to be associated with the stretching vibrations for O-H stretch, C-H asymmetric stretching, C=O stretch, C=C aromatic ring (s) stretch, C-H (s) stretch, C-N stretch, C-H stretch having the functional groups hydroxyl, alkanes, ketones, amines and alkyl halides.

Figure 4. FT-IR analysis of silver nanoparticles synthesized using *Lawsonia inermis*.

FT-IR absorbance bands of *Psidium guajava* silver nanoparticles (Figure 5) in the region of 4000-500 cm\(^{-1}\) are 2923, 1689, 1605, 1446, 1169, 1031, 827, 560, 538 and 523 responsible for the functional group alkanes, ketones, aromatics and aliphatics groups and alkyl halides which showed C-H, C=O, C-C and C-Br stretching.

Figure 3. UV-Visible absorption spectrum of *Psidium guajava* silver nanoparticles.

FT-IR analysis of silver nanoparticles synthesized using *Lawsonia inermis* and *Psidium guajava*
Antibacterial activity of silver nanoparticles

The antibacterial activities of synthesized silver nanoparticles were screened against pathogenic bacterial strains *E. coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis* and *Proteus vulgaris* by agar well diffusion method. Zone of inhibition of *Lawsonia inermis* against *E. coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis* and *Proteus vulgaris* was 12mm, 11mm, 13mm and 9mm respectively, amoxicillin showed 20mm zone of inhibition in average (Table 1). Zone of inhibition of *Psidium guajava* against *E. coli*, *Pseudomonas aerogenosa*, *Bacillus subtilis* and *Proteus vulgaris* was 11mm, 8mm, 9mm and 10mm respectively (Table 1). Silver nanoparticles synthesized by *Lawsonia inermis* showed maximum antibacterial activity against *Bacillus subtilis* (Figure 6) whereas *Psidium guajava* showed maximum activity against *E. coli* (Figure 6). The Silver nanoparticles synthesized via green route are toxic to human pathogenic bacterial strains hence it has a great potential in biomedical applications. The present study showed a simple, rapid and economical route to synthesize silver nanoparticles using medicinal plants.

Figure 6. Antibacterial activity of silver nanoparticles of *Lawsonia inermis* and *Psidium guajava* against (a) *E. coli*, (b) *Pseudomonas aerogenosa*, (c) *Bacillus subtilis*, (d) *Proteus vulgaris*.
Table 1: Average zone of inhibition of silver nanoparticles against some bacterial strains.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Silver <em>L. inermis</em> Zone of inhibition (mm)</th>
<th>Silver <em>P. guajava</em> Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>12 ±0.13</td>
<td>11 ±0.14</td>
</tr>
<tr>
<td><em>Pseudomonas aerogenosa</em></td>
<td>11 ±0.12</td>
<td>8 ±0.09</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>13 ±0.19</td>
<td>9 ±0.11</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>9 ±0.11</td>
<td>10 ±0.12</td>
</tr>
</tbody>
</table>

Figure 7. Zone of inhibition of silver nanoparticles synthesized using *Lawsonia inermis* and *Psidium guajava* against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Proteus vulgaris*.

According to Kavitha and Baker (2013), green synthesis has advances over chemical and physical method as it is cost operative, atmosphere friendly and easily scrabbled up for large scale synthesis and in this method there is no need to use high energy, temperature and toxic chemicals. Green synthesis offer better influence, control over crystal growth and their steadiness. Green synthesized nanoparticles are cheap and economical having many applications in science, the results of present study showed synthesized silver nanoparticles have significant bactericidal activity against some bacterial pathogens, which is helpful to minimize the antibiotic dose in the field of medicine.

CONCLUSION
The biosynthesis and characterization of silver nanoparticles from selected medicinal plants have been performed successfully. These green synthesized silver nanoparticles have significant antibacterial activity against some human pathogenic strains hence this is a new platform to overcome on the hurdles of multidrug resistance of bacteria. The reduction of aqueous silver ions by the leaves extract of the *Lawsonia inermis* and *Psidium guajava* plant has been performed. In the present study we found that *Lawsonia inermis* and *Psidium guajava* can be a good source for synthesis of silver nanoparticles. Applications of these ecofriendly nanoparticles in bactericidal, wound healing and other medical and electronic fields makes this method potentially helpful for the large scale synthesis of other nanomaterials.

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