Synthesis, Characterization And Assessment of Anti Microbial Behaviour of Goat Faecal Mediated Silver Nanoparticles - Fed on Tirumala Hills

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ABSTRACT
To synthesize, characterize, and to assess the anti-microbial activity of silver nanoparticles (AgNPs) induced by goat fecal matter. The AgNPs were processed by the microwave heating method and the characterization was accomplished by employing various spectroscopic approaches such as UV-Visible, FTIR spectroscopy, XRD, Particle size, and Zeta potential analysis. The \( \lambda_{max} \) for both extracts were found at 426& 438nm. The wideband corresponded to O-H stretching vibrations at 3384.0 cm\(^{-1}\), 3273.9 cm\(^{-1}\), and 3366.2 cm\(^{-1}\), bands at 2918.5 cm\(^{-1}\), 2922.5 cm\(^{-1}\), 2853.2 cm\(^{-1}\), and 2850.2 cm\(^{-1}\) corresponded to the N–H and C–H stretching. The bands at 1638.1 cm\(^{-1}\), 1651.9 and 1686.5 cm\(^{-1}\) corresponded to the C=C stretch. Bands of 1460.3 cm\(^{-1}\), 1450.4 cm\(^{-1}\), 1409.2 cm\(^{-1}\), and 1376.3 cm\(^{-1}\) corresponded to C=N, C–C bond stretching vibrations. The stretch of C-O indicates bands at 1159.7 cm\(^{-1}\), 1033.2 cm\(^{-1}\), and 1032.8 cm\(^{-1}\). The synthesized AgNPs demonstrated good anti-microbial activity on gram +Ve (S.aureus) and gram -Ve (E.coli) bacteria. Bio/Green synthesized AgNPs have shown improved biological performance, this tends to minimize production cost, pollution-free, less chemical usage, and stable generation of nanoparticles.

INTRODUCTION
An investigator’s primary goal is often to meet certain enormous thresholds and to implement new ideas in research and innovation. Nanotechnology is evolving as a booming domain with its implementation in scientific and technological aspects producing current products on a nanometric scale (Albrecht et al., 2006). Nanotechnology is a fascinating platform, particularly in the biotechnology sector (Natarajan et al., 2010). Due to its potential applications and simple production, nanopar-
ticle research is slowly growing today (Gopinath et al., 2012). Numerous forms of nanoparticles have appeared, including copper, magnesium, titanium, zinc, gold, and silver (Schabes-Retchkiman et al., 2006), but the most powerful antimicrobial agents are known to be silver nanoparticles (AgNPs) as they destroy bacteria and other microbes (Gong et al., 2007). AgNPs play a crucial task mostly in the field of nano and medical technology compare with other nanoparticles. Silver is one of the most commercialized nanomaterials, with an approximate production of 500 tones of silver nanoparticles, which is forecast to expand in the upcoming years (Larue et al., 2014).

Green/biosynthesis helps in the reduction of production cost and to maintain an eco-friendly environment. It does not require high temperature, pressure, energy, and toxic chemicals. By design, various technologies have been built for the manufacturing of different nano-length materials. This has contributed to the growth of the comparatively recent and relatively unknown field of research focused on nanomaterial biosynthesis. Production using bio-organisms is consistent with the ideas of green technology. “Biological/Green synthesis” of nanoparticles utilizes eco-friendly, non-toxic, and stable reagents (Li et al., 2007).

MATERIALS AND METHODS

Chemicals and reagents
Goat fecal matter, AR grade of ethanol, and Merck grade silver nitrate were used.

Experimental research
Sample collection
Goat fecal matter has been retrieved from the confined region of ArepalliRangampet, Chandragiri (M), Chittoor (Dist), India, rinsed thoroughly with distilled water, dried at ambient temperature in the shade, and packed for further use in an airtight envelope.

Preparation of Extracts
Preparation of water extract
In a mortar, 20 g of dried and powdered goat feces was transferred, smashed with a pestle, and placed into a jar and Percolated with 150 ml of 95 % ethanol, capped with aluminium foil and kept for 72 hours, allowed to filter. The filtrate was employed as a reducing medium for the production of AgNPs.

Preparation of ethanolic extract
In a mortar, 50 g of dried and powdered goat feces was transferred, smashed with a pestle, and placed into a jar and Percolated with 150 ml of 95 % ethanol, capped with aluminium foil and kept for 72 hours, allowed to filter. The filtrate was employed as a reducing medium for the production of AgNPs.

Analysis of Phytochemical’s
A phytochemical investigation of amino acids, alkaloids (Trease and Evans, 1989), carbohydrates, glycosides, flavonoids, phenols, tannins, triterpenoids, proteins, and saponins was performed on both filtrates (Amrita and Praveen, 2015).

Preparation of 0.1 M silver nitrate solution
Weighed nearly 17g of AR grade silver nitrate and delivered to a volumetric flask of 1000ml, dissolved in 100ml of distilled water, and rendered up to 1000ml of final volume with distilled water.

Silver nanoparticles synthesis
10 ml of both the water and ethanol extracts of goat feces were introduced to 2 different jars having 100 ml of 0.1 M silver nitrate solution (AgNO3). Then the fusion was preserved in a micro-oven and illuminated at 180W for about 2 min. The light yellow color has been changed to a distinctive golden yellow color distinctive of the silver nanoparticles (Ahmad et al., 2011).

Silver nanoparticles characterization
Nanoparticles microscopic images
Under an Olympus-BX51 digital microscope, the shape and size of the developed nanoparticles of both ethanolic and water extract of goat feces were noted.

Ultraviolet-Visible spectrophotometry
The spectrum of absorption for both extracts was examined by scanning between 200-800 nm wavelength ranges using the Agilent UV-Visible Spectrophotometer (Menon et al., 2017).

Fourier transforms infrared spectrophotometry
Separately, the specimens of the two extracts were mounted on the ATR diamond crystal and analyzed with the software tool Agilent Resolution Pro in the 400-4000 cm⁻¹ absorption range. Each sample spectrum was produced using 32 single numeric average scans with a 4 cm⁻¹ resolution power (Swati and Kumar, 2016).

Assessment of particle size and zeta potential
The particle size & Polydispersity-index (PI) of the shaped NPs were calculated at a scattered angle of 90°C and the measurement was done at 25°C. The zeta potential of the preparation was also determined with the aid of the electrophoretic mobility method using a transparent disposable zeta cell.
X-ray powder diffraction
The crystallinity of produced nanoparticles was tested by using X’ pert Pro diffractometer (\(\lambda\)-Cu = 1.5148 A0, Cu-K\(\alpha\) radiation, Phillips) operating at a temperature of 30 mA and 40 Kv reported in 2\(\theta\) the scale from 200 and 600 scanning rate 10min-1) (Anju et al., 2018).

Assessment of anti-microbial activity
The in-vitro anti-microbial activity of formed AgNPs was assessed on both Streptococci (gram+) E. coli (gram-) bacteria through the agar contact method, employing streptomycin as a basic standard. In this process, the nutrient agar medium was sterilized at 121\(^\circ\)C for half an hour. In each sterilized Petri dish, 20 ml of the above-prepared medium was poured and allowed for solidification at ambient temperature. The inoculation was done with 24hr broth cultured test bacteria. Using borer wells of 6 mm diameter of wells, a sterile L-shaped rod was punched with 100 \(\mu\)l or 0.1 ml of fresh subcultured species. 50 \(\mu\)l samples were added in every well. Petri dishes were incubated over 24 hours at 37\(^\circ\)C, as well as the inhibition zone (ZOI) was recorded in mm and contrasted to the standard (Antony et al., 2016).

RESULTS
The phytochemical examination was conducted on both water and ethanolic extracts of goat feces. The positive results for carbohydrates, amino acids, flavonoids, proteins, and saponins, suggesting the existence of reducing agents, are shown in Table 1. Figures 1 and 2, A yellow colored spherical shape gave us conformation about the formed AgNPs.

Characterization of synthesized silver nanoparticles

UV-Visible Spectrophotometry
Synthesized nanoparticles were exposed to the study of the UV-Visible Spectrophotometer which
### Table 1: Phytochemical components of water and ethanolic extract of goat feces

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical constituents</th>
<th>Water extract</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4.</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5.</td>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>Phenols &amp; Tannins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Triterpenoids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8.</td>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9.</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

### Table 2: FT-IR interpretation in water extract of AgNPs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Functional group</th>
<th>Peak wave no (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O–H</td>
<td>3273.9</td>
</tr>
<tr>
<td>2.</td>
<td>N–H</td>
<td>2918.5</td>
</tr>
<tr>
<td>3.</td>
<td>C–H</td>
<td>2850.2</td>
</tr>
<tr>
<td>4.</td>
<td>C=C</td>
<td>1638.1</td>
</tr>
<tr>
<td>5.</td>
<td>Nitrates from AgNO(_3)</td>
<td>1539.4</td>
</tr>
<tr>
<td>6.</td>
<td>C–N</td>
<td>1460.3</td>
</tr>
<tr>
<td>7.</td>
<td>C–C</td>
<td>1409.2</td>
</tr>
<tr>
<td>8.</td>
<td>C–O</td>
<td>1032.8</td>
</tr>
</tbody>
</table>

### Table 3: FT-IR interpretation in Ethanolic extract of AgNPs

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Functional group</th>
<th>Peak wavedo (cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>O–H</td>
<td>3384.0</td>
</tr>
<tr>
<td>2.</td>
<td>O–H</td>
<td>3366.2</td>
</tr>
<tr>
<td>3.</td>
<td>N–H</td>
<td>2922.5</td>
</tr>
<tr>
<td>4.</td>
<td>C–H</td>
<td>2850.2</td>
</tr>
<tr>
<td>5.</td>
<td>C=C</td>
<td>1686.5</td>
</tr>
<tr>
<td>6.</td>
<td>C=C</td>
<td>1651.9</td>
</tr>
<tr>
<td>7.</td>
<td>C–N</td>
<td>1450.3</td>
</tr>
<tr>
<td>8.</td>
<td>C–N</td>
<td>1376.3</td>
</tr>
<tr>
<td>9.</td>
<td>C–O</td>
<td>1159.7</td>
</tr>
<tr>
<td>10.</td>
<td>C–O</td>
<td>1033.2</td>
</tr>
</tbody>
</table>

### Table 4: AgNPs- X-ray powder diffraction in water extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Obtained peak intensity</th>
<th>Total peaks intensity</th>
<th>%Degree of crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>879</td>
<td>15225.4</td>
<td>5.77%</td>
</tr>
</tbody>
</table>

### Table 5: AgNPs- X-ray powder diffraction in Ethanol extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Obtained peak intensity</th>
<th>Total peaks intensity</th>
<th>%Degree of crystallinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>16456</td>
<td>29692.21</td>
<td>55.42%</td>
</tr>
</tbody>
</table>
Figure 7: AgNPs particle size in Water extract

Table 6: Zone of inhibition against *Staphylococcus aureus* and *E.coli* from both extracts

<table>
<thead>
<tr>
<th>S. No</th>
<th>Components</th>
<th>Zone of inhibition (ZOI) in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>1</td>
<td>Control</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Water extract</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>Ethanolic extract</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>Standard</td>
<td>15</td>
</tr>
</tbody>
</table>

gave us an idea of the development of nanoparticles in the very first stage itself. The reduction of silver (Ag) ions into the shape of nanoparticles were tracked by calculating the Ultraviolet-Visible spectra of two extracts by scanning about 200 to 800 nm. Peaks at 426nm and 438nm suggest the development of nanoparticles made by silver nitrate.

**Fourier transform-infrared spectroscopy (FTIR)**

To assess the potential biological molecules accountable for the bio-reduction of Ag+, synthesized nanoparticles were exposed to FT-IR spectral analysis in the range of 400-4000 cm⁻¹ and their interpretation values were given in Tables 2 and 3. The broad band’s at 3384.0 cm⁻¹, 3273.9 cm⁻¹, and 3366.2 cm⁻¹ in the spectra region corresponded to O–H stretching vibrations indicates the presence of alcohol and phenol group of flavonoids, bands at 2918.5 cm⁻¹, 2922.5 cm⁻¹, 2853.2 cm⁻¹, and 2850.2 cm⁻¹ corresponded to the N–H and C–H stretching of aromatic compounds. The bands in the aromatic ring corresponded to the C=C stretch at 1638.1 cm⁻¹, 1651.9 cm⁻¹, and 1686.5 cm⁻¹. The intense band reveals the presence of AgNO₃ nitrate at 1539.4 cm⁻¹. Bands at 1460.3 cm⁻¹, 1450.4 cm⁻¹, 1409.2 cm⁻¹, and 1376.3 cm⁻¹ correlate with stretching vibrations of C–N, C–C bonds suggesting the existence of proteins. The C–O stretch in the amino acid was shown by bands at 1159.7 cm⁻¹, 1033.2 cm⁻¹, and 1032.8 cm⁻¹.
Assessment of the particle size and zeta potential
It was calculated that the particle size of the produced AgNPs was 15 and 46.5 nm in both water and ethanolic extracts. The PI value was observed to be 0.719 and 1.725 which suggests a very wide range distribution. The zeta-potential of produced AgNPs was found to be -4.7 and -7.7 mV, exhibiting excellent stability and surface properties of formed nanoparticles.

X-ray powder diffraction pattern
The degree of crystallinity of the biosynthesized AgNPs in the water extract was examined using X-ray diffraction patterns depicted five sharp peaks and well-described diffraction lines at $2\theta = 21.725^\circ$, $27.826^\circ$, $29.687^\circ$, $32.251^\circ$, and $35.525^\circ$ which indexed the planes of 86, 92, 98, 100, and 110. Similarly, four sharp and well-described diffraction lines are depicted in the ethanolic extract at $2\theta = 35.555^\circ$, $54.418^\circ$, $56.210^\circ$, and $75.052^\circ$ which indexed the planes of 100, 108, 110, and 311, that indicates the formed AgNPs have cubic crystal facial structure. The degree of crystallinity of formed nanoparticles in both extracts was found to be 5.77% & 55.42% shown in Tables 4 and 5.

Antimicrobial activity
The formed AgNPs have shown their antibacterial effects on both bacteria such as $S. aureus$ (+Ve) and $E. coli$ (-Ve)). Table 6 reveals the radial diameters of the zone of inhibition for the formed AgNPs, which is 9 mm (aqueous extract) and 11 mm (ethanolic extract) for $S. aureus$ and 8 mm (aqueous extract) as well as 12 mm (ethanolic extract) for $E. coli$. Such antibacterial activity can be traced because of the existence of amines and carboxyl groups on their cellular membrane and the frequency of enhanced Ag ion empathy to those groups. Because of their incredibly large surface area, nanoparticles have demonstrated powerful antibacterial activity which may provide closer interaction with microorganisms.

DISCUSSION
This research reflects an effort to synthesize AgNPs with goat fecal matter. After the chemical reac-
tion, the solution mixture’s color turned to pale yellow which suggested AgNO₃ reduction. Lee et al., in 2013 found similar results using cow milk (Lee et al., 2013). The formation of AgNPs within the sample mixture has also been verified by the UV-Vis spectrophotometer; it is a crucial tool for assessing the structure and stability of metal nanoparticles in both aqueous and ethanolic solutions (Gnanajo-bitha et al., 2012). Figures 3 and 4 showed the UV-Visible absorption band at 426 nm and 438 nm corresponded to the absorbance of silver nanoparticles. Figures 5 and 6 of the FT-IR spectra revealed the absorption bands at 3384.0 cm⁻¹, 3273.9 cm⁻¹, and 3366.2 cm⁻¹ in the spectral region correlates with O–H stretching vibrations, suggesting the alcohol and Phenolic group of flavonoids and polyphenols and N-H stretching vibrations in 1⁰ and 2⁰ amines of amino acids, proteins, and peptides (Shivayo-geeswar E Neelagund and Mahesh Midatharaha-liChikkanna, 2018). Bands at 2918.5 cm⁻¹, 2922.5 cm⁻¹, 2853.2 cm⁻¹, and 2850.2 cm⁻¹ corresponded to the N–H and C–H stretching of aromatic compounds (Munajad and Subroto, 2018). The bands in the aromatic ring corresponded to the C=C stretch at 1638.1 cm⁻¹, 1651.9 cm⁻¹, and 1686.5 cm⁻¹. The solid band at 1539.4 cm⁻¹ reveals the presence of AgNO₃ nitrate, bands at 1460.3 cm⁻¹, 1450.4 cm⁻¹, 1409.2 cm⁻¹, and 1376.3 cm⁻¹ coincide to C-N, C-C bonds, suggesting the existence of proteins (Lalitha et al., 2013). Bands at 1159.7 cm⁻¹, 1033.2 cm⁻¹, and 1032.8 cm⁻¹ suggest the stretching vibrations of C-O in the amino acid (Prakash et al., 2013), similar outcomes have been reported by some researchers (Pawlak and Mucha, 2003). Such functional groups have a significant role in the stabilization of AgNP’s as stated in several studies (Niraimathi et al., 2013). The average particle size (mean size) of AgNPs was 15 nm and 46.5 nm in both water and ethanolic extracts with a Polydispersity index (PDI) of 0.719 and 1.725 shown in Figures 7 and 8.

This result implies that the particles are quite stable and show a wide range of distribution (Awad et al., 2019). Figures 9 and 10 show the zeta potential of formed nanoparticles in both extracts that is -4.7 and -7.7 mV exhibiting excellent stability and surface properties of formed nanoparticles. Figures 11 and 12 display the X-ray diffraction pattern.
Figure 10: Zeta potential of Ethanol extract of AgNPs

Figure 11: Graph that illustrates X-ray powder diffraction of AgNPs in Water extract

Figure 12: Graph that illustrates X-ray powder diffraction of AgNPs in Ethanol extract

The number of reflections for AgNPs in aqueous extract appears at $2\theta = 21.725^\circ(86)$, $27.826^\circ(92)$, $29.687^\circ(98)$, $32.251^\circ(100)$, and $35.525^\circ(110)$. For ethanol extract appears at $2\theta = 35.555^\circ(100)$, $54.418^\circ(108)$, $56.210^\circ(110)$, and $75.052^\circ(311)$ respectively (Pung et al., 2012), which elucidates the centered cubic face structure of silver nanoparticles (Sathyavathi et al., 2010). The ZOI for the formed AgNPs was 9 mm (in aqueous extract), and 11 mm (in ethanol extract) for S. aureus and 8 mm (in aqueous extract), and 12 mm (in ethanol extract).
extract) for *E. coli*. The nanoparticles produced showed strong gram-positive antimicrobial activity compared to gram-negative bacteria (Gnanadesigan *et al.*, 2011). The results obtained showed strong antibacterial activity of AgNPs against all species, relative to the other studies.

**CONCLUSION**

Through this paper, we explored the green/biosynthesis of AgNPs employing goat feces as a reducing agent, which concludes with consistent outcomes demonstrated by their characteristics. This research was accomplished by utilizing the fecal material of goats feeding everyday on the hills of Tirumala. The nanoparticles produced are of good particle size and have shown good microbial resistance behavior. The green/biosynthesis also helps one to produce innovative thoughts about the synthesis of metal nanoparticles which are cheaper, eco-friendly, reduction of chemical usage, and stable. Such experiments could be subject to drug targeting/delivery and chemotherapeutic operations in the future.

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**Conflicts of Interest**

The writers announce that this dissertation does not have potential conflicts of interest.

**Author’s Contribution**

A. Anil and R Praveena contributed to the literature search, data acquisition, C. Sravan Kumar and Mohammed Idress contributed to the preparation and characterization of nanoparticles, C. Chitra Shekar contributed to manuscript preparation and editing, Ashok Thulluru and C. Naresh Babu contributed in review. The complete manuscript was read by all authors and accepted.

**REFERENCES**


