Bio-synthesis and characterization of silver nanoparticles using *Terminalia chebula* leaf extract and evaluation of its antimicrobial potential

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**Abstract**

Due to increasing global conflicts, there is an emergent need to develop environmentally benign nanoparticles without the use of toxic chemicals. The bio-synthesis of silver nanoparticles (SNPs) using *Terminalia chebula* (TC) leaf extract becomes one of the more potential areas of research. The bio-reduction of metal ions is quite rapid and readily to be performed at room temperature. Present study describes a rapid and eco-friendly synthesis of TC-SNPs using TC leaf extract in a single pot process and is observed when the medium changes to brown color with the addition of silver ion. The obtained results confirmed that recorded UV spectra shows the characteristic surface plasmon resonance band for TC-SNPs in the range of 400–440 nm. Anti-bacterial activity of bio-synthesized TC-SNPs shows effective inhibition against human pathogens including, *Bacillus subtilis* (ATCC 6633) and *Escherichia coli* (ATCC 25922). Thus, the significant outcome of this study would help to formulate value added herbal based on nano-materials in biomedical and nanotechnology industries.

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1. Introduction

Indian tradition has a legacy in the use of medicinal plants to develop drugs from plants. Now-a-days herbal medicines are prescribed widely. Then the biologically compounds are incomprehensible because of their effectiveness and nominal side effects in clinical habitude and comparatively low cost [1]. Plants provide a predominant resource for a huge number of conventional medicines that have been in existence for hundreds of years in countries like India. Ayurveda, one of the ancient medicinal techniques in the world, bestows a considerable number of remedial useful compounds in the biomedical fields [2,3]. The combination of traditional and modern knowledge can produce better source of the active components for the treatment of diseases with fewer side effects [4]. Medicinally useful plants typically incorporate mixtures of many chemical combinations that may act in synergy to enhance the health [5]. It is believed that the drug of natural origin will play a vital role in health care particularly in the rural areas of India [6,7].

SNPs are widely used in industry and have an inhibitory effect on a number of microorganisms [8]. SNPs have been used in the fabrication of ointments and creams to inhibit the bacterial infection of burns and wounds [9]. Green synthesis is guiltless and eco-friendly method for originated NPs [10]. Nanoparticles (NPs) can be produced using a different types of physico-chemical methods to bring under control the problem of toxicity in synthesis since medicinal plants have a major role in the synthesis of NPs. Phytochemical compounds such as saponins, phenolic compounds, phytosterols and quinines present in plant [11] biomolecules have both preservative and reductive activity. In recent years, SNPs are fabricated using various plant extracts as a reducing agent [12–16]. Historically, silver is known to have a disinfecting effect and is found in applications ranging from traditional medicines to gastronomic objects. According to the earlier reports, SNPs were non-toxic and most effective against bacteria, virus and other micro-organisms at low concentrations without any side effects [17]. Moreover, various salts of Ag and their products are commercially produced as antimicrobial agents [18].

TC has been widely used as herb in Ayurveda, Unani and Homoeopathic medicine; also it is a center of attraction in modern medicine. This is due to the presence of a large number of different types of phytoconstituents. Especially, the leaves of TC were found to contain polyphenols (punicalin, punicalagin, terflavins B, C, and
2. Materials and methods

The fresh leaves of TC are collected from the Seshachala forest of Andhra Pradesh, South Indian states, India. Silver nitrate (AgNO₃), Muller Hinton Agar (MHA), Nutrient broth are purchased from HiMedia Laboratories Pvt. Ltd., Mumbai, India. The bacterial culture of Bacillus subtilis (ATCC 6633) and Escherichia coli (ATCC 25,922) are obtained from Microbial Type Culture Collection, Bangalore, India. Throughout the experiment double distilled water (ddH₂O) is used and all the reagents were used without any further purification.

2.1. Preparation of TC leaf extract and biosynthesis of Silver Nanoparticles (TC-SNPs)

The TC plant leaves are to be collected and dipped in ddH₂O to remove the surfaces adhered dust particles, later the leaves are separated from water and allowed for drying in dust free environment at room temperature for 48 h. These leaves are to be cut into small pieces for further study. 10 g of leaves are boiled in 100 mL of distilled water in 500 mL Erlenmeyer flask for 30 min at 65 °C to get 10% aqueous TC leaves extract. The extract has to be filtered with Whatmann grade no.1 filter paper to attain clear solution and is used to synthesize TC-SNPs. Collected extract is preserved at 4 °C for further experiments.

To synthesize TC-SNPs, 0.01 M AgNO₃ (250 μL) is to be added to 5 mL leaf extract. After few seconds, TC-SNPs formed and monitored by brown color of colloidal suspension. Bio-reduction of Ag⁺ ions in aqueous extract is to be monitored initially as follows: 250 μL of 0.01 M AgNO₃ solution is added to different volumes of TC extract including 1:1, 1:2, 1:3, 1:4 & finally 1:5 mL (for example 1:4 ratio i.e., 1 part of 250 μL of 0.01 M AgNO₃ and 4 parts of 10% extract) and incubated for 30 min in a rotary shaker at 100 rpm at room temperature. TC-SNPs are separated by centrifugation at 12,000 rpm for 10 min to dispose some of unwanted bio-molecules; subsequently the pellet is redispersed in sterile ddH₂O. The purification of TC-SNPs by centrifugation and re-dispersion in sterile ddH₂O is constantly carried out to ensure the better elimination of free entities.

2.2. Characterization of nanoparticles

The bio-reduction of Ag⁺ ions in aqueous extract is to be monitored with UV–visible spectra and is recorded with UV–vis Spectrophotometer, LAB INDIA, UV–3092 from 200 to 800 nm at room temperature. The dried TC-SNPs are grinded with KBr pellets and are subjected to Fourier transform infrared spectroscopy (FT-IR) analysis using PerkinElmer Spectrum Two Fourier transform infrared spectrometer, UK, in the range of 400–4000 cm⁻¹. Mean diameter and size circulation of the nanoparticles are determined by dynamic light scattering (DLS) method using Brookhaven DLS Instrument, USA. Microscopy analysis of TC-SNPs was performed on a JEOL JEM-2010 high resolution transmission electron microscopy (HR-TEM) operating at an accelerating voltage of 200 kV. X-ray diffraction analysis of the bio-synthesized TC-SNPs cast onto glass slides are recorded using a Rigaku diffractometer (Cu-Kα radiation, λ=0.1546 nm) running at 40 kV and 40 mA were recorded XRD scans in the 20 range 20–80°. Field emission scanning electron microscopy (FESEM, Ikon analytical, FEI Quanta 200) experiments were performed to characterize the size and shape of bio-reduced TC-SNPs.

3. Result and discussion

UV–visible spectra of band peaks produced by TC-SNPs, with bio-synthesized leaf extract clearly shows that there is no significant peak showing a sign of pure extract (Fig. 1). When TC extract is subjected to AgNO₃, the reaction starts within seconds and the color change is observed visually. The solution color changes into brown indicating the formation of TC-SNPs [21]. Surface plasmon resonance (SPR) of Ag is appeared at 421 nm (Fig. 1A). In the present study, synthesized TC-SNPs with different extract ratio (V/V) such as 1:1, 1:2, 1:3, 1:4 mL and positive control (Gentamycin) (Fig. 5). The antibacterial effect of TC-SNPs has been determined on the basis of zone of inhibition in mm (Table 1).

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>Volume of tested material (10 μL)</th>
<th>Zone inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TC extract</td>
<td>13</td>
</tr>
<tr>
<td>2</td>
<td>1:1 TC-SNPs</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>1:2 TC-SNPs</td>
<td>14.5</td>
</tr>
<tr>
<td>4</td>
<td>1:3 TC-SNPs</td>
<td>14.5</td>
</tr>
<tr>
<td>5</td>
<td>1:4 TC-SNPs</td>
<td>14.5</td>
</tr>
<tr>
<td>6</td>
<td>Gentamycin</td>
<td>18</td>
</tr>
</tbody>
</table>

Anti-bacterial activity of TC-SNPs was examined against pathogenic bacteria including B. subtilis (ATCC 6633) and E. coli (ATCC 25,922) using agar well-diffusion method [20] at different compositions such as 1:1, 1:2, 1:3, 1:4 mL and positive control (Gentamycin) (Fig. 5). The antibacterial effect of TC-SNPs has been determined on the basis of zone of inhibition in mm (Table 1).
bound to Ag\(^+\) or not during TC-SNPs synthesis, pristine TC extract and TC-SNPs samples were subjected to FT-IR studies. Fig. 2 (TC) shows FTIR spectrum of pristine TC extract, distinctive peaks observed for –OH, pH-NH\(_2\), R-NH\(_2\), >C=O, C–H and C=C groups at around 3378, 1118, 1380, 1040, 1721, 91694, 2848 and 1604 cm\(^{-1}\), respectively. Fig. 2 (TC-SNPs) shows FTIR spectrum of TC-SNPs shows peaks for –OH, >C=O, N–H bending, C–H, C–N stretching, and C–O symmetrical stretching, 3435, 1162, 1721, 1634, 598, 1401 cm\(^{-1}\), respectively. Also, disappeared 1606, 1526 and 1316 cm\(^{-1}\) peaks and also gets new peak at 607 cm\(^{-1}\). Relative shifts of TC-SNPs are observed at 1694–1646, 1192–1181 and 1116–1032 cm\(^{-1}\). This clearly shows that oxidized poly phenols have capped the surface of the TC-SNPs.

FESEM micrograph sown in Fig. 3(a), reveals that TC-SNPs are sphere-shaped and uniformly dispersed with an average size of 60 nm. This is further confirmed by DLS experiments. EDX spectrum displayed in Fig. 3 shows strong signal related to the presence (of Ag 60\%) of TC-SNPs and also some of the weak signals for C, O, and Al elements are also obtained Fig. 3(b). DLS analysis shows that the size of TC-SNPs is approximately ~18 nm (Fig. 4A). TEM analysis clearly shows the size of the individual TC-SNPs ranged from 10 to 30 nm (Fig. 4B) which correlates with DLS. XRD of bio-synthesized TC-SNPs is confirmed by the characteristics peak observed in (Fig. 4C). In the XRD pattern, four prominent peaks are observed at 2\(\theta\)= 39\(^\circ\), 46\(^\circ\), 65\(^\circ\) and 78\(^\circ\), which corresponds to (111), (200), (220) and (311) Bragg’s reflections, respectively of the face-centered cubic (fcc) structure of TC-SNPs.

The average size of TC-SNPs is 27.26 nm is obtained by calculation of the crystalline size of TC-SNPs by using “Scherrer equation” and Bragg angle from XRD data as follows [22]:

\[
D_p = \frac{0.94\lambda}{\beta \cos \theta}
\]

where, \(D_p\)=Average Crystallite size, \(\beta\)=Line broadening in radians, \(\theta\)=Bragg angle, \(\lambda\)=X-ray wavelength.

TC leaf extract and TC-SNPs shows anti-bacterial activity against both \(B.\ subtilis\) and \(E.\ coli\), and the results located in Table 1. Pristine TC leaf extract has less inhibition (Fig. 5) whereas zone of inhibition increased with TC-SNPs which were reduced by various
ratios of TC leaf extracts (1:1–1:4). This clearly indicates that the anti-bacterial activity is increased when associated with TC leaf extract due to TC-SNPs [23–25]. The exact mechanism for the anti-bacterial activity of SNPs is not known till now. However, theoretically many studies report that the SNPs could bind to the bacterial membrane, invade the cell and cause appetite of proton motive force which leads to the distraction of bacterial cell by forming pores on the bacterial cell wall.

4. Conclusion

The present study reports the facile approach of bio-synthesis of TC-SNPs from AgNO₃ using the aqueous extract of TC. The adopted method is well suited with green chemistry principles as the plant extract serves as a dual functional molecule acting as a reductant and also as a stabilizing agent for the synthesis of TC-SNPs. The UV-visible spectra has confirmed the reduction of Ag⁺.

Fig. 4. (A) Particle size determination by Dynamic light scattering (B) TEM image and (C) XRD analysis of TC-SNPs.

Fig. 5. Anti-bacterial activity of TC leaf extract and TC-SNPs against Escherichia coli and Bacillus subtilis pathogenic strains.
ions at 421 nm on 24 h of incubation time. XRD analysis confirms the crystalline face centered cubic (fcc) structure of TC-SNPs. From FTIR and XRD analysis it is observed that TC leaf extract act as a stabilizer for the synthesis of TC-SNPs. The stability of the TC-SNPs is also recorded as stable in Zeta potential estimation by DLS. Further, the bio-synthesized TC-SNPs show significant anti-bacterial action on tested pathogenic microorganisms. As a result it is observed that a fine tuning of process variables may give the end product with typical physical characteristics.

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