Synthesis of zinc oxide nanoparticles using leaf extract of *Ajuga parviflora* Benth. In Wall and their characterization

Anuj Kandwal, Pranjali Purohit, Arun K Khajuria, Goutam Kumar, MC Purohit and Niranjan Kumar Mandal

Abstract

*Ajuga parviflora* is one of the potent medicinal herb of Himalaya belongs to Lamiaceae family used in number of different folk medicine time immemorial. In this work, we design to synthesize zinc oxide nanoparticles from zinc nitrate via green route using the leaf extracts of *Ajuga parviflora*. Zinc oxide nanoparticles were characterized by XRD, EDX, TEM and FTIR analysis. Zinc oxide nanoparticles showed UV-visible spectral bands between 310-330 nm. EDX analysis of nanoparticles confirms the presence of zinc and oxygen. TEM analysis revealed that nanoparticles are spherical in shape with the average particle size less than 20 nm. X-Ray Diffraction (XRD) structural analysis affirms the synthesis of crystalline zinc oxide nanoparticles. FTIR spectra confirms the presence of phytochemicals of plant extract which played a key role in the synthesis of zinc oxide nanoparticles.

Keywords: Zinc oxide nanoparticles, *Ajuga parviflora*, TEM, XRD, crystalline

Introduction

*Ajuga parviflora* is an important medicinal herb of Himalaya belongs to Lamiaceae family commonly used to control hypertension, hepatitis and diabetes [1, 2]. It exhibited a variety of biological activities, such as antibacterial, antifungal, antioxidant, insecticidal [3-5]. *Ajuga parviflora* is a short-lived perennial herb with its stem branched from base, pink purple to blue, short flowers and leaves, sessile, hairy, ovate and tinged with purple on lower surface [6, 7]. Nano science is a new and advanced inter-disciplinary field under discussion which is based on the elementary properties of Nano-size materials [8, 9]. Metallic nanoparticles can be synthesized by various methods including physical, chemical and biological. However, green synthesis of metallic nanoparticles is more stable, non-hazardous, ecofriendly, efficient and cost effective than the long established chemical and physical methods [10, 11]. Therefore, researchers are largely focusing on the green routes of synthesis of metallic nanoparticles. Zinc oxide nanoparticles have drawn the attention of many researchers owing to their several applications ranging from material science to biological science. Phytochemicals present in the extracts of various parts plants (roots, leaf, fruits, seeds and bark) acts as capping and stabilizing agent of zinc oxide nanoparticles [15-17]. Various researchers have reported synthesis of zinc oxide nanoparticles using different plant materials [18-22]. Due to large surface area to volume ratio and high catalytic activity of zinc oxide nanoparticles, they are used in several catalytic processes. Moreover, antimicrobial activities of zinc oxide nanoparticles have also been reported [23-25]. After reviewing data, it has been found that no report has been texted regarding the green synthesis of ZnO nanoparticles by using aqueous leaf extract of *Ajuga parviflora*. The objective of present study is to synthesize zinc oxide nanoparticles through the leaf extracts of *Ajuga parviflora* and its characterization by FTIR, XRD, EDX and TEM analysis.

Material and Methods

Collection of plant materials

Fresh leaves of *Ajuga parviflora* were collected from Kandolia, Pauri Garhwal, Uttarakhand and authenticated from Herbarium Jammu University and its accession no. HBJU 16003 was collected.
Plant Extract preparation
Fresh leaves of *Ajuga parviflora* were thoroughly washed under tap water for 15 min followed by washing with double distilled autoclaved water. Then, only healthy leaves were shade dried until the constant weight of leaves were achieved. Then, these dry leaves were grinded into fine powder using mortar and pestle and 5 g of finely powdered *Ajuga parviflora* was taken with 100 ml double distilled water and heated at 65 °C for 25 minutes. Then, the extract was allowed to cool at room temperature and filtered using Whatman Filter paper No. 1.

Green synthesis of Zinc oxide Nano particles
50 ml of *Ajuga parviflora* leaf extract was taken in a 250 ml of Erlenmeyer conical flask. It was heated on magnetic stirrer at 60 °C for 15 minutes. Then, 50 ml of 100 mM zinc nitrate hexa hydrate solution was poured drop by drop to it with continuing stirring. Few drops of 1 M NaOH solution was also added to it in order to maintain pH ranging 9-12. The change in colour of solution from brown to yellow was considered as a visual marker for the synthesis of nanoparticles. Then, the solution was centrifuged for 10 minutes at 7500 rpm and washed with distilled water followed by acetone to remove the impurities. The obtained material was dried at 40 °C for 24 hours in oven followed by mashing in air tight bottles for further characterization and anti-microbial activities.

Characterization of green ZnO nanoparticles
Formation of zinc oxide nanoparticles was preliminary examined by using Elite-double beam -UV-visible spectrophotometer. Then, zinc oxide nanoparticles was subjected to X-ray diffraction (XRD) analysis (CuKα radiation, λmax = 1.54 Å) and its spectra was reported in the range of 2θ from 0° to 75°. FTIR analysis ranging from 4000-500 cm⁻¹ was to recognize the phytochemicals present in plant extract responsible for capping and stabilizing nanoparticles. The average size of ZnO nanoparticles was calculated by using Debye Scherrer’s equation:

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

Where D is average crystallite size, K is the Scherrer's constant, \( \lambda \) is the X-ray wavelength, \( \beta \) is the full width at half maxima (FWHM) and \( \theta \) is the Bragg's diffraction angle.

EDX analysis
EDX was carried out for the composition of nanoparticles. Fig. 2 shows the presence of ZnO with sharp signals of Zn and O at around 1 keV and 0.5 keV respectively. Peak of C may be attributed due to the bioactive components present in plant extract.

![Fig. 1: UV-visible spectrum of ZnO nanoparticles](image-url)
XRD analysis
XRD spectra showed the crystalline nature of ZnO nanoparticles and the average crystallite size was calculated using Debye-Scherrer’s equation \[ d = \frac{K\lambda}{B\cos \theta} \] \[ (26-28) \]. The average calculated size of ZnO nanoparticles is less than 13 nm. XRD spectra of synthesized nanoparticles showed sharp peaks at \( 2\theta \) = 32.00°, 34.62°, 36.47°, 47.72°, 56.79°, 63.01° and 68.25° which are indexed to (100), (002), (101), (102), (110), (103) and (112) respectively (Table 1) of hexagonal closed packed nano crystals (Fig. 3).

TEM analysis
TEM analysis was used to study the surface morphology ZnO nano structures (Fig. 4). These results confirmed that ZnO nanoparticles were spherical in shape with their average size less than 20 nm.

Table 1: Peak List for Average Size Calculation of Nanoparticles

<table>
<thead>
<tr>
<th>2\theta (°)</th>
<th>hkl</th>
<th>FWHM left</th>
<th>( d )-spacing (Å)</th>
<th>Rel. int. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>32.00</td>
<td>100</td>
<td>0.70</td>
<td>2.79</td>
<td>58.91</td>
</tr>
<tr>
<td>34.62</td>
<td>001</td>
<td>0.35</td>
<td>2.58</td>
<td>75.54</td>
</tr>
<tr>
<td>36.47</td>
<td>101</td>
<td>0.56</td>
<td>2.46</td>
<td>100</td>
</tr>
<tr>
<td>47.72</td>
<td>102</td>
<td>1.04</td>
<td>1.90</td>
<td>15.60</td>
</tr>
<tr>
<td>56.79</td>
<td>110</td>
<td>0.65</td>
<td>1.62</td>
<td>35.42</td>
</tr>
<tr>
<td>63.01</td>
<td>103</td>
<td>0.71</td>
<td>1.47</td>
<td>28.76</td>
</tr>
<tr>
<td>68.25</td>
<td>112</td>
<td>1.47</td>
<td>1.37</td>
<td>21.33</td>
</tr>
</tbody>
</table>
FTIR analysis
FTIR peak in Fig. 5 at 3411.7 cm\(^{-1}\) corresponds to stretching vibrations of hydroxyl group. Further, peaks at 1571.6 cm\(^{-1}\), 1492.8 cm\(^{-1}\) and 1412.9 cm\(^{-1}\) may be attributed to C=O frequency of extensively conjugated systems, C=C aromatic stretching and C-O stretching of ArOH respectively. Further, peak at 462.7 cm\(^{-1}\) shows the presence of ZnO crystals.

Conclusion
It can be concluded that Ajuga parviflora leaf extract mediated synthesis of zinc oxide nanoparticles is non-toxic, rapid, non-hazardous and cost effective. Spherically shaped, crystalline zinc oxide nanoparticles with their average size less than 20 nm are synthesized. Phytochemicals presents in leaf extract effectively acts as capping and stabilizing agents of zinc oxide nanoparticles. Thus, it is an alternative, promising and better route for the synthesis of zinc oxide nanoparticles than the conventional physical and chemical methods.

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Conflict of Interest
The authors declare that there is no conflict of interests regarding the publication of this article.

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