Biosynthesis of Zinc oxide nanoparticles and their application for antimicrobial treatment of burn wound infections

Peng Wang¹,², Lei Jiang¹,² and Rongxia Han³

¹ Department of Aesthetic, Plastic and Burn Surgery, Yuhuangding Hospital, NO. 20, Yuhuangding East Road, Yantai 264000, People’s Republic of China
² Department of Plastic and Burn Surgery, Yantai Laiyang Central Hospital, NO. 111, Changshan Road, Laiyang 265200, People’s Republic of China
³ These authors have contributed equally to the work.

E-mail: rongxiahan@outlook.com

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Abstract

The current study demonstrated the green fabrication of Zinc oxide nanoparticles (ZnO NPs) using the leaf extract of Coleus amboinicus (C. amboinicus) by an environment-friendly method. The leaf extract of C. amboinicus acts as capping and reducing agent during the fabrication of ZnO NPs. The prepared ZnO NPs were characterized by utilizing analytical techniques such as photoluminescence spectroscopy (PL), Transmission Electron microscopy (TEM), Energy dispersive spectroscopy (EDS), Fourier transform infrared spectroscopy (FTIR), UV–visible spectroscopy (UV–vis) and x-ray diffraction spectroscopy (XRD). The XRD pattern confirmed the formation of polydisperse ZnO NPs having crystalline nature. The FTIR spectrum demonstrated the biomolecular capping on the surface of zinc oxide nanoparticles. The prepared ZnO NPs displayed excellent antimicrobial activities over several gram positive and gram-negative microbial pathogens. Further, wound healing studies in rats revealed that wound closure rate was greater in the ZnO NPs treated rats at all doses when related with the untreated group (negative control group), therefore, this indicates the effective wound healing ability of prepared ZnO NPs.

Introduction

Zinc oxide (ZnO) is recognized as one among the important and unique inorganic materials. Owing to its characteristic features and innovative applications in the broad fields of science and technology, zinc oxide has received enormous research interest. Zinc oxide also exhibits various important properties such as pyroelectric, semiconducting, optoelectronics, piezoelectric, and catalysis [1]. In addition, ZnO is an example of n-type semiconductor material having large band-gap energy of 3.37 eV with large exciton binding energy of 60 meV. Owing to their broad surface-to-volume ratio, zinc oxide nanoparticles (ZnO NPs) exhibit interesting chemical and physical properties.

ZnO NPs can be fabricated by various methods like metallorganic chemical vapor deposition [2], vapor phase deposition [3], zinc oxidation [4], vapor-liquid-solid [5], hydrothermal synthesis [6], and chemical vapor deposition [7]. The most common methods for fabricating ZnO NPs are vapor–liquid–solid and hydrothermal synthesis. Recently, biosynthesis of nanoparticles was performed utilizing micro-organisms, phyto extracts and natural bio-molecules owing to their non-hazardous, cheap, bio-degradable and eco-friendly nature. However, dispensability of ZnO NPs can be enhanced by surface capping agents, whereas this can easily be attained by green synthesis methods.

Numerous environment-friendly techniques have been utilized for the fabrication of ZnO NPs [8]. For instance, the leaf extract of Aloe barbadensis Miller has been already reported for the fabrication of water-dispersible ZnO NPs [9]. In the current study, the extract of C. amboinicus plant has been utilized for the
fabrication of ZnO NPs by using the principles of green chemistry. It is widely known that the extracts of plants play a major role in the fabrication of different NPs like graphene [10, 11], silver [12] etc.

A natural recuperative reaction to tissue injury is wound healing, which is accomplished through a complex cascade of cellular and biochemical events that generate reconstitution, restoration, and resurfacing of the injured skin tensile strength [13, 14]. The process of wound healing involves with 4 phases, which includes hemostasis followed by inflammation, then proliferation, and finally maturation that typically causes the formation of scar [15, 16]. The fibrin substance is the major component of wound matrix into which the plasma proteins and cells migrate. Inflammatory cells like macrophages and neutrophils remove injured tissues during the initial 2 to 4 days of healing (inflammatory phase) and provides protection against infections and also releases mitogenic and chemotactic factors [17]. In the proliferative period, the fibroblasts present in the surrounding tissues start to proliferate on the fibrin component in order to produce collagen, which is an important event in this phase followed by simultaneous occurring of epithelialization and angiogenesis [18]. Then, the collagen molecules that are newly produced crosslink with the pre-existing protein and collagen molecules during the maturation period, resulting in the increase of scar tensile strength [19]. The phase of maturation starts by the second weekend of the healing and will last for an unspecified period [20]. The wound healing process has become more well established in recent years, but there has been a relentless quest for materials and techniques that may help to reduce the amount of scar tissue and eventually for remodelling the injury. It is noticed that considerable progress has been made in the preparation of engineered NPs for various biological applications [21].

On the other side, a substantial rise in the quantity of antibiotic resistances in opportunistic and medically important pathogenic microorganisms causes the scientific community to constantly develop novel drug targets and drugs. Owing to the incessant selection of antibiotic resistance traits, novel antibacterial agents have been progressed over the past few years and none of these agents has improved action towards multidrug resistant microorganisms and other pathogens. In addition, the antibacterial activities of the NPs and the advancement of new applications in this area are making them as a promising alternative against commercial antibiotics [22]. In the same way, the production of novel antiviral drugs, those maintain the viability of host cell and targets the virus is a difficult task, and also result in millions of consequences each year [23, 24]. For example, NPs have been investigated for their potential to reduce burn wounds [25] and infections in skin [26]. An immediate recovery with maximal function and minimal scarring is the utmost aim for wound healing [27].

The plant, Coleus amboinicus lour which is generally called as Indian Borage, is a tender fleshy perennial medical plant containing many phytochemicals like caryophyllene (bicyclic sesquiterpene), patchouline and carvacrol (monoterpenoid) as well as flavanoids like apigenin, genkwanin, quercetin, salvigenin, and luteolin that has major medicinal uses. This plant has been used especially in the diagnosis of hepatopathy, malarial fever, renal and cough, vesical calculi, hicouche, chronic asthma, anthelmintic, bronchitis, convulsions and colic [28]. The current study demonstrated the green fabrication of Zinc oxide nanoparticles (ZnO NPs) using the leaf extract of Coleus amboinicus (C. amboinicus) by an environment-friendly method. The prepared NPs have been studied for their antimicrobial and burn wound healing properties.

**Experimental section**

**Materials**

Bovine serum albumin (BSA), Dimethyl sulfoxide (DMSO), Zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O), Dimethyl-sulfoxide (DMSO), and KBr power were obtained from Sigma-Aldrich chemicals Ltd, Shanghai.

**C. amboinicus extract preparation**

The leaves of C. amboinicus plant were washed thoroughly using double distilled (DD) water in order to eliminate dust particles followed by drying under sunlight. About 20 g of dried leaves were finely powdered and then boiled with 100 ml DD water for about 40 min at a temperature of 90 °C. The resultant extract solution was allowed to cool at room temperature followed by filtering using cellulose nitrate (CN) membrane in order to achieve a clear solution of the extract. The extract obtained was stored in a refrigerator for further application.

**ZnO NPs synthesis**

About 50 ml of C. amboinicus leaf extract was boiled at a temperature of 60 °C–80 °C using stirrer heater. Then, about 5 g of zinc nitrate was taken and added to the above boiling solution until a deep yellow colour paste was obtained. Later, the obtained paste was shifted into a ceramic crucible followed by heating in a furnace for 2 h at a temperature of 400 °C, until it turned into a light white-coloured powder. This powder was utilized for future characterization and biological studies. Then, the obtained material was finely powdered with the help of a mortar and pestle, which allows for easy characterization in the future.
Antimicrobial activity

Anti-microbial activity assessment of the formed ZnO NPs over five bacterial pathogens among which three gram-negative strains namely Salmonella typhi (B-4420), Klebsiella pneumoniae (ATCC-27738), Escherichia coli (O157 : H7) and two gram-positive strains namely Staphylococcus aureus (6538 P) and Bacillus cereus (ATCC 7064) was performed utilising Muller-Hinton agar (MHA) medium by disk diffusion test (Kirby–Bauer test). About 10 mg per disc ciprofloxacin was utilized as a sterile blank disk and standard having diameter of 5 mm infused with known concentrations of DMSO (30%), plant extract (50 μl) and ZnO NPs (0.05, 0.01 and 0.1 M) was employed for the determination of antimicrobial activity.

Burn wound healing evaluation

In the present experiment, Sprague Dawley male rats of 7 to 9 weeks old and weighing 180 to 210 gm were utilized. The animal research was conducted in accordance with the protocols accepted by local Ethical committee of the Institute, and by following the instructions for care and use of laboratory animals. Prior to the experiment, the selected rats were sedated through intramuscular injection of 2% xylazine (10 mg per kg body weight (b.w)) and 10% ketamine (75 mg per kg b.w). After sedating the experimental animals, using electric hair clipper the back hair of the animals was shaved. On each rat dorsum, four circular burn wounds of diameter 10 mm were created using an aluminium bar which is boiled in water steaming at 100 °C for 30 s. The aluminium bar on the dorsum of rat was used without applying any pressure for a period of 10 s in every area. Before performing the treatments on the wounds, the necrotic area in the animal was punched after 2 days. Each of the experimental rats had four circular full-thickness skin burn wounds on the back. Until the 28th day, the experimental rats were placed in separate cages. The treatments were given daily and continued till 28th day. The burn wounds were separated into four groups in the following way:

1st Group: The burn wounds without treatment (Negative Control, n = 12).
2nd Group: The burn wounds were administrated topically using silver sulfadiazine of 1% (SSD group, n =12).
3rd Group: The burn wounds were administrated topically with a minimum-dose of ZnO NPs ointment: The mixture was prepared by the addition of 3 μl per wound of ZnO NPs to 50 mg per wound of silver sulfadiazine 1%
4th Group: The burn wounds were administrated topically with a maximum-dose of ZnO NPs ointment: The mixture was prepared by the addition of 24 μl per wound of ZnO NPs to 50 mg per wound of Silver sulfadiazine 1%

To investigate the healing rate, the percentage of wound closure was assessed by utilizing the below formula:

\[
\text{Wound Closure(\%) = } \frac{[(\text{area of the wound on day } 0) - (\text{area of the wound on indicated day})]}{\text{area of the wound on day } 0} * 100.
\]

Characterization

A D8-Advanced Bruker diffractometer was employed to perform the XRD patterns of the fabricated ZnO NPs across a 2θ range of 20°–70° using Cu Kα1 radiation (λ = 0.1546 nm). Perkin Elmer Lambda 950 UV-Vis spectrophotometer was employed to determine the optical absorption of ZnO NPs. The capping of biomolecules on the surface of the prepared ZnO NPs was analysed using a JASCO FTIR instrument. Furthermore, the morphology and size of the formed ZnO NPs was analysed by Transmission Electron Microscopy (JEOL TEM) instrument. Energy Dispersive Spectroscopy (BRUKER INDIA) instrument was used for chemical composition measurements. Photoluminescent spectrum of the formed ZnO NPs was studied using fluorescent spectroscopy (Hitachi) instrument.

Results and discussion

The optical absorbance spectrum of ZnO NPs displayed an absorption peak at a wavelength of 370 nm indicating the fabrication of ZnO NPs (figure 1). Additionally, the synthesis of ZnO NPs is confirmed visually by the change in colour of the solution into deep yellow. Interestingly, the reaction is finished in 3 h at a temperature of 60 °C–80 °C, which is relatively less and rapid than the earlier studies [29–31]. The absorbance at a wavelength of 383 nm indicated the formation of ZnO NPs and this absorbance may be because of the quantum confinement effects of the NPs. On the other hand, the optical absorbance spectrum of zinc nitrate and plant extract don’t exhibit any absorption peak in visible region indicating the successful formation of ZnO NPs.

The PL spectrum of the fabricated ZnO NPs utilizing C. ambonicus leaf extract is depicted in figure 2. The spectrum of PL displayed the presence of a peak at a wavelength of 396 nm which is characteristic to UV emission. On the other side, the peak existed at a wavelength of 418 nm could be because of the Zinc vacancy.
The emission found at 450 nm, corresponding to the oxygen vacancy due to the transition among the shallow donors to valence band and the emission found at 466 nm is because of zinc and oxygen vacancy or interstitials. Also, the peak found at 481 nm emission peak is due to the transition between the interstitial oxygen and oxygen vacancy and the wavelength peak found at 492 nm corresponds to green emissions because of oxygen vacancy.

The XRD pattern displayed the existence of ZnO NP peaks found at 77.03°, 72.5°, 69.0°, 67.9°, 66.3°, 62.8°, 56.5°, 47.5°, 36.2°, 34.4° and 31.7° with distinctive planes correlated to (202), (004), (201), (112), (200), (103), (110), (102), (101), (002) and (100) correspondingly. The obtained results also confirm the wurtzite structure of prepared ZnO NPs (figure 3). Moreover, no other impurities in the crystalline form and an unusual shift in the peaks of diffraction were seen, therefore, this indicates the purity of the synthesized product. On the other side, the growth of anisotropic ZnO NPs is proved by the existence of comparatively high peak intensity (101), which indicates the crystallites preferred orientation. Additionally, the XRD patterns displayed the values of planes that are in close accordance with JCPDS NO: 89-7102 [32, 33]. Further, the crystalline size for prepared ZnO NPs can be calculated using the Debye–Scherrer equation as follows and is found to be 15 nm.

![Figure 1. UV-Visible optical absorbance spectrum of ZnO NPs.](image1)

![Figure 2. PL spectrum of the biofabricated ZnO NPs.](image2)
\[
D = \left(\frac{K\lambda}{\beta \cos \theta}\right) \text{Å}
\]

D = average crystallite size; \(\beta\) = corrected line broadening of ZnO NPs; \(\lambda\) = wavelength of x-ray; \(K\) = shape factor (0.9) and \(\theta\) = Bragg angle.

Figures 4(A), (B) displayed the Transmission Electron Microscopy (TEM) images of fabricated ZnO NPs at various magnifications. From the figure, it is observed that the formed ZnO NPs are polydisperse with a particle size ranging from 15–20 nm. Furthermore, EDS spectrum of ZnO NPs displayed the existence of peaks corresponding to oxygen and zinc elements (figure 4(C)). Additionally, the presence of extra peaks corresponding to carbon and chlorine arises due to the biomolecular capping.

Figure 5 depicted the FTIR spectra of the formed ZnO NPs, prepared from aqueous extract of C. amboinicus plant leaf using a biosynthetic method. Figure 5 shows bands of absorption present at 710, 849 and 768 cm\(^{-1}\) which corresponds to vibrations from out of plane bending of aromatic C–H in typical mono substituted benzene ring, 1, 4 di-substituted benzene ring and 1, 2, 3 tri substituted ring of benzene. The band present at 1029 cm\(^{-1}\) is distinctive to the stretching vibrations of C–N bond present in amine functional group [34]. On the other side, the absorption bands noticed in the region of 1411–1617 cm\(^{-1}\) correlates to the aromatic ring vibrations. The second weak IR absorption bands found at 2900–2750 cm\(^{-1}\) region, confirms the existence of...
ZnO NPs

Table 1. Antimicrobial activity of Zno NPs, Zinc nitrate solution, plant extract, ciprofloxacin drug and DMSO.

| Sample                       | Salmonella typhi | Klebsiella pneumoniae | Escherichia coli | Staphylococcus aureus | Bacillus cereus |
|------------------------------|------------------|-----------------------|------------------|-----------------------|-----------------|
| DMSO (30%)                   | —                | —                     | —                | —                     | —               |
| ZnNO₃ (0.3 M)                 | 1.02 ± 0.18      | 1.31 ± 0.23           | 1.01 ± 0.09      | 2.35 ± 0.27           | 2.65 ± 0.17     |
| Plant extract                | 0.98 ± 0.30      | 1.01 ± 0.09           | 0.4 ± 0.16       | 1.6 ± 0.19            | 1.02 ± 0.18     |
| ZnO NPs (0.01 M)             | 1.01 ± 0.09      | 3.65 ± 0.21           | 4.66 ± 0.14      | 2.01 ± 0.21           | 3.34 ± 0.28     |
| ZnO NPs (0.05 M)             | 6.6 ± 0.19       | 3.31 ± 0.30           | 7.68 ± 0.45      | 5.65 ± 0.31           | 5.4 ± 0.49      |
| ZnO NPs (0.1 M)              | 10.35 ± 0.322    | 7.28 ± 0.31           | 9.10 ± 0.42      | 4.52 ± 0.28           | 8.80 ± 0.45     |
| Ciprofloxacin (10 μg/disc)   | 11.99 ± 0.48     | 11.02 ± 0.28          | 13.5 ± 0.21      | 9.99 ± 0.61           | 10.2 ± 0.61     |

Bacillus cereus displayed mild effect over all the tested bacterial strains. DMSO utilized as a solvent did not display any anti-bacterial effect, whereas the aqueous extract of Coleus amboinicus displayed mild effect over all the tested bacterial strains. The findings displayed that gram-positive bacteria are more resistant to the treatment of ZnO NPs when compared to the gram-negative bacteria. This may be due to the existence of a thick layer in the latter group cell walls (peptidoglycan). One of the researcher investigated the effect of ZnO NPs on mesophilic and halophilic microbial species and demonstrated that gram-positive bacteria, B. subtilis is less sensitive to ZnO NPs then the gram-negative bacteria, Enterobacteria [36]. They also reported that the existence of peptidoglycan thick layer in their cell wall is responsible for the resistance in gram-positive bacteria. Another researcher also identified an excellent antibacterial ability against gram-negative bacteria utilizing the cumin seeds-fabricated ZnO NPs, and reported that the existence of a peptidoglycan thick layer in their cell wall is responsible for the slight resistance of the gram-positive bacteria [37], which is in accordance with the results obtained in the current study. It has also been

![Figure 5. FTIR spectrum of prepared ZnO NPs.](Image)

Aromatic aldehydes [35]. In the same way, weak absorption band found at 2049 cm⁻¹ is because of the vibrational stretching of C≡C bond. The broad and intense band noticed at 3237–3565 cm⁻¹ is related to stretching vibrations of OH bond. The absorption band at 450–540 cm⁻¹ showed the existence of ZnO NPs. On the other side, the broad absorption bands in the spectrum of FTIR observed at 2900, 3237, 1614 cm⁻¹ showed the existence of amine, hydroxyl and aldehyde functional groups on the synthesized ZnO NPs surface [34, 35]. Furthermore, the vibrations which corresponds to the aromatic ring also proved that phyto extract biomolecules could enhance the ZnO NPs stabilization via capping in the aqueous medium. The above results proved the fabrication of ZnO NPs which is stabilized by the biomolecules of Coleus amboinicus phyto extract.

**Anti-microbial activity**

The anti-microbial activity results of antibiotics, DMSO, zinc nitrate solution, extract and formulated ZnO NPs determined utilizing Kirby–Bauer method over 5 strains of pathogenic micro-organisms are displayed in table 1. During the analysis of ZnO NP anti-microbial activities, we have seen that the 0.1 M ZnO NPs inhibitory activity over Bacillus cereus and Salmonella typhi showed no considerable change when related to the standard ciprofloxacin, and an obvious difference was noticed over Klebsiella pneumoniae, Escherichia coli and Staphylococcus aureus (table 1). The low concentrations of ZnO NPs exhibited moderate inhibitory activity over both gram-positive and gram-negative tested bacteria. The solution of zinc nitrate showed moderate to mild effect, whereas the aqueous extract of Coleus amboinicus displayed mild effect over all the tested bacterial strains. DMSO utilized as a solvent did not display any anti-bacterial effect (table 1). The findings displayed that gram-positive bacteria are more resistant to the treatment of ZnO NPs when compared to the gram-negative bacteria. This may be due to the existence of a thick layer in the latter group cell walls (peptidoglycan). One of the researcher investigated the effect of ZnO NPs on mesophilic and halophilic microbial species and demonstrated that gram-positive bacteria, B. subtilis is less sensitive to ZnO NPs then the gram-negative bacteria, Enterobacteria [36]. They also reported that the existence of peptidoglycan thick layer in their cell wall is responsible for the resistance in gram-positive bacteria. Another researcher also identified an excellent antibacterial ability against gram-negative bacteria utilizing the cumin seeds-fabricated ZnO NPs, and reported that the existence of a peptidoglycan thick layer in their cell wall is responsible for the slight resistance of the gram-positive bacteria [37], which is in accordance with the results obtained in the current study. It has also been
demonstrated that the formation of reactive oxygen species (ROS) within the cell and the zinc ion binding to the bacterial cell membrane results in the cell disruption [37].

**Wound healing activity**

The effectiveness of ZnO NPs in the healing of burned wounds was examined in rat model. Figure 6 demonstrated that the ZnO NPs were more efficient in the healing of burned wounds when compared to the untreated group and the SSD groups ($p < 0.001$). On the day seven, fourteen and twenty-eight, every burned wound was checked for inflammation, hyperemia and redness. No inflammation symptoms were noticed in groups treated with ZnO NPs when compared with the negative control and the SSD groups on the day seven and fourteen after the surgery. In contrast, inflammation and redness was observed clearly at wound site in untreated group, on the day seven and fourteen. In comparison with untreated group and SSD groups, fast wound contraction was clearly noticed in the rats treated with ZnO NPs (Wound closure rate with different treatments were shown in Table 2). After the 28 days of wound formation, control and the SSD groups showed mild redness, whereas no indication of inflammation, hyperaemia and redness was observed in the group administrated with ZnO NPs. These results are in agreement with previous reports where wound tissues were totally improved after 7th day of healing with complete reepithelialisation [38]. Moreover, the plant

![Figure 6. Images showing the burn wound healing activity of ZnO NPs at high and low doses compared with silver sulfadiazine and negative control treatment at 0, 7, 14, and 28 days of post wounding.](image)

| Sample          | 28th day | 14th day | 7th day     |
|-----------------|----------|----------|-------------|
| High ZnO NPs    | 100      | 100      | 94.27 ± 0.37|
| Low ZnO NPs     | 100      | 99.94 ± 0.06 | 72.00 ± 2.42 |
| SSD             | 99.10 ± 0.25 | 98.94 ± 0.34 | 60.05 ± 3.78 |
| Negative control| 98.56 ± 0.58 | 80.32 ± 2.52 | 57.05 ± 2.41 |
bimolecules that are capped on the surface of ZnO NPs reduce the side effects promoting the burn wound healing procedure and hence the prepared NPs are suggested as good dressing material for wound healing.

Conclusions

A simple, biosynthetic and low cost approach for the synthesis of ZnO NPs utilizing the leaf extract of C. amboinicus is reported in this study. The FT-IR analysis displayed the phyto extract biomolecules decoration on the ZnO NPs surface. XRD and TEM analysis revealed the crystalline polydispersed ZnO NPs formation. Wound healing studies in rats revealed that the wound closure rate was greater in the ZnO NPs treated rats at all doses when related with the untreated group (negative control group), therefore, this indicates the effective wound healing ability of prepared ZnO NPs.

ORCID iDs

Rongxia Han @ https://orcid.org/0000-0003-1380-5548

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