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Green Synthesis, Characterization and Antibacterial Activity of ZnO Nanoparticles

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Abstract

In the present study, mehendi extract (*Lawsonia inermis*) was used for phytosynthesis of ZnO nanoparticles using 0.1 M $Zn(NO_3)_2$ as precursor under alkaline condition using NaOH with vigorous stirring for 2 h. ZnO NPs obtained were characterized by UV-Vis spectroscopy, XRD, SEM and TEM that showed change in shape and size. Hexagonal particles were formed due to plant extract relative to the rod shaped particles in absence of plant extract. Further the antibacterial property of ZnO NP synthesized by green method was more effective than those synthesized in absence of plant extract. The antibacterial activity study of both the synthesized ZnO nanoparticles reveals that the nanoparticles synthesized using mehendi extract are more effective than the particle synthesized without mehendi extract. Thus, the use of leaf extract as capping agent would improve the antibacterial property of ZnO nanoparticle. However, bacteriocidal effect of these nanoparticles varies with respect to the organism tested.

Keywords

Antibacterial Activity, Green Synthesis, ZnO Nanoparticles, *Lawsonia inermis*

1. Introduction

Nanotechnology is the art and science of manipulating matter at the nanoscale to create new and unique materials and products with enormous potential to change society [1]. Nanoparticles can serve as "magic bullets", containing herbicides, chemicals, or genes, which target plant parts to release the content [2] [3].

Nano-zinc oxide has been widely used in industry for various applications like leather manufacturing, rubber industry etc. [4] [5]. ZnO nanoparticle is also drawing researcher's attention for its wide application in medicine, foods, agriculture, solar cell and cosmetics because of its high antibacterial, prospect for nano-nutrition, refractive index, high thermal conductivity, and UV protection property respectively [1] [6] [7] [8].

Green synthesis or phytosynthesis of nanoparticle is an eco-friendly approach which is in common practice [9] [10] [11] [12] [13]. The phytosynthetic approach is a simple alternative to chemical and physical methods due to low cost and less use of toxic chemicals. ZnO is selected for the current experiment because of its wide biological applications. In the present study, leaf extract of *Lawsonia inermis* (Mehendi) was used because of its medicinal properties [14]. Plants are rich in pytochemical sparticulary secondary metabolites such as tannins, terpenoids, flavonoids that have been demonstrated to have antibacterial properties [14] [15]. In the present study, an attempt has been made to compare the antibacterial property of ZnO nanoparticle synthesized in the presence and absence of leaf extract of *Lawsonia inermis* (Mehendi). It is hypothesized that use of leaf extract as capping agent would improve the antibacterial property of ZnO nanoparticle.

2. Materials and Methods

2.1. Plant Material

In our experiment the leaves of *Lawsonia inermis* were collected from Karimganj, Assam, India washed properly and was allowed to dry at room temperature. The plant was authenticated by the subject expert Dr. Partha Sarathi Das, Department of Botany and Biotechnology, Karimganj College Karimganj, Assam, India and confirmed by comparing the same with existing herbarium of the Department of Botany and Biotechnology, Karimganj College Karimganj, Assam, India. The dry leaf powder was obtained using grinder and used to prepare the plant extract by mixing 1 g of the leaf powder in 10ml of distilled water and heated in a water bath at 100˚C for 10 min. The solution was then filtered using Whatman No.1 filter paper and the filtrate was stored at 4˚C and used for ZnO NP synthesis. Different concentrations of the resulting filtrate (0%, 2.5%, 5%, 10%, 15%, 20%) were used as a reducing agent for ZnO NP synthesis and 5% plant extract was selected based UV-Vis spectroscopic studies.

2.2. ZnO NP-I & ZnO NP-II Synthesis

In this method we have dissolved 2.97 g (0.1 M) of $Zn(NO_3)_2$ salt in 90.7 ml of double distilled water which was then titrated drop wise along the wall of the conical flask with 9.3 ml of 2 M NaOH for 2 h under vigorous stirring at room temperature to obtain 100 ml final volume after titration. After 2 h of vigorous stirring the solution was filtered through a filter paper and the residue in the form of $Zn(OH)_2$ obtained was washed properly using double distilled water.

The residue was then dried at 70˚C in a hot air oven for 48 h to obtain fine ZnO NP powder. In the ZnO NP-II synthesis the above solution for ZnO NP-I was titrated with 5% plant extract along with NaOH drop wise and then filtered, washed and dried using the aforesaid method for ZnO NP-I.

2.3. Characterization of ZnO Nanoparticle

The above synthesized ZnO NP was characterized using UV-Vis spectroscopy, SEM, TEM and XRD. The morphology was investigated using field emission scanning electron microscopy. For FESEM alcoholic dispersion of synthesized ZnO NP was put on a properly cleaned glass slide followed by spin coating. Uv-Vis spectroscopy of the sample was done using Biospectrometer (Eppendorf) in which 10 mg of ZnO NP was resuspended in 15 ml distilled water and sonicated for 10 min after which the sample was scanned from wavelength 300 - 700 nm.

X-Ray diffraction of the sample powder was carried out in a PANalytical X-PERT PRO applying a monochromator to select the Kα1 component of the employed copper radiation (Wavelength of 1.54056 Å). The data have been collected in the range 20 - 80 with a step size of 5˚. The XRD data has been depicted in **Figure 3**, the grain size of the synthesized sample have been collected using Scherrer formula [16]:

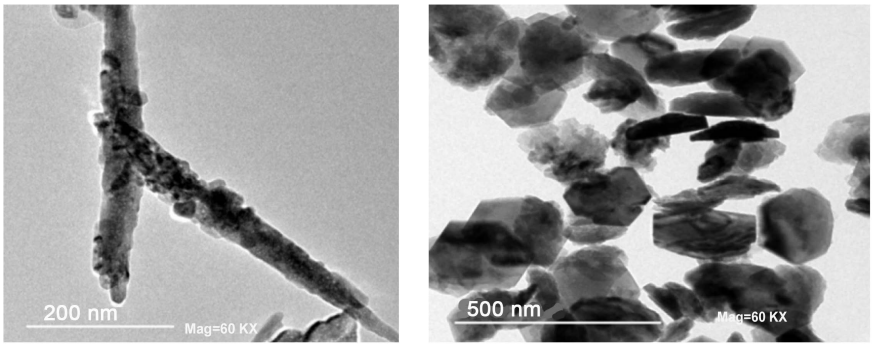
$$D_{hkl} = \mathrm{K}\lambda / \beta_p \cos\theta$$

where D_{hkl} is the average grain size, K the shape factor (here, 0.9), λ is the x-ray wavelength, β_p is the full width at half maximum (FWHM) intensity (here, 101 and 002 peak of the ZnO nanoparticle ZnO NP-I and ZnO NP-II spectrum fitted with Gaussian for precision measurement and θ is the Bragg's angle.

For TEM analysis, powder samples were dispersed in triple distilled water using sonication, and a drop of the dispersion was placed onto a carbon coated copper grid and dried at room temperature. Further selected area electron diffraction (SAED) patterns were recorded to determine the growth orientation of the synthesized ZnO and was put into a uniform carbon coated copper TEM grid and dried in vacuum.

2.4. Antibacterial Activity of ZnO Nanoparticles (ZnO NP-1 & ZnO NP-II)

The antimicrobial activity of both the synthesized ZnO nanoparticles was performed and their antimicrobial efficacy was compared against four bacterial strains (*Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*). The lyophilized cultured of the four bacterial strains were revived in conical flasks containing 20 ml of nutrient broth with 5 ml of inoculum and incubated at 37˚C. From this revived bacterial culture 100 ul of inoculum were added to 20 ml of LB broth media followed by the addition of synthesized ZnO nanoparticle dispersion of concentration of 0 µg/ml, 100 µg/ml, 200 µg/ml and 500 µg/ml with respect to the total volume of 20 ml (**Figure 1**). The inoculated



(a) (b)

Figure 1. Transmission electron micrograph of synthesized ZnO nanoparticles in absence (ZnO NP-I) ((a) and (c)) and presence (ZnO NP-II) ((b) and (d)) of mehendi (*Lawsonia inermis)* extract by using Zinc nitrate as precursor.

culture were incubated at 37˚C with gentle stirring. The growth of bacterial strains was observed in liquid medium by measuring absorbance at 600 nm against control using UV-VIS spectrophotometer [17].

2.5. Statistical Analysis

Each experiment was repeated thrice and data presented are mean ± SE (n = 3) and least significance difference (LSD) test was used for comparison between pairs of treatments. The data analysis was carried out using a statistical package, SPSS v. 10 (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Synthesis and Characterization of ZnO NP-I and ZnO NP-II

ZnO nanoparticles were synthesized by using leaf extract of *Lawsonia inermis* extract as detailed above. The size and morphology of the nanoparticles was characterized by using various techniques. The UV-Vis spectra for ZnO NP-I and ZnO NP-II are shown in **Figure 2(a)** and **Figure 2(b)** respectively which clearly shows a blue shift resulting from reduction in size from NP-I to NP-II.

The SEM morphology images of the ZnO NPs as synthesized without plant extract (NP-I) and with plant extract (NP-II) are shown in **Figure 2(c)** and **Figure 2(d)** respectively. The SEM images confirm formation of ZnO nano particles with hexagonal structures having sizes nearly 100nm for ZnO NP-I and 75 nm for NP-II.

The TEM micro graphs also confirm the synthesized particles to be in the same range as predicted XRD and SEM. It also shows formation of hexagonal structures with average particle size decreased from 100 nm for ZnO NP I to 75 nm in ZnO NP II. The results are in good agreement with that of XRD and SEM analysis.

Structural analysis of the sample was made by X-ray Diffraction studies with Cu K_α radiation (λ = 1.5418 Å) as X-ray source at 40 kV and 30 mA in the scanning angle (2θ) from 20˚ to 80˚. The resulted XRD pattern was analysed using

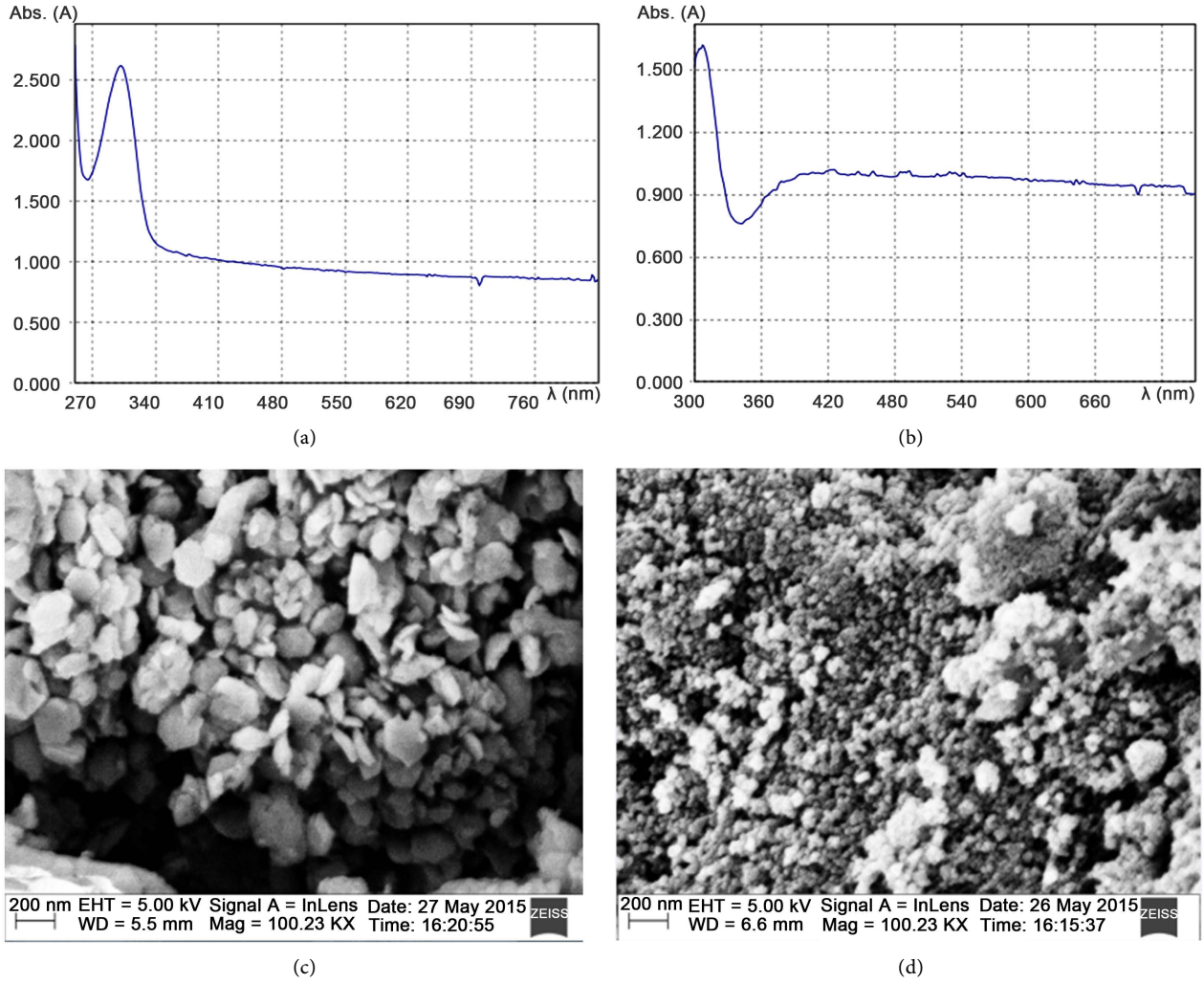


Figure 2. UV-visible spectra ((a) and (b)) and scanning electron micrograph ((c) and (d)) of synthesized ZnO nanoparticles in absence (ZnO NP-I) and presence (ZnO NP-II) of mehendi (*Lawsonia inermis)* extract by using Zinc nitrate as precausor.

X'Pert High Score software by search match tool, it was found to match with the ICDD Reference Pattern of Zinc Oxide, 01-070-8072.

As depicted in **Figure 3(a)** and **Figure 3(b)**, typical XRD pattern of ZnO samples having polycrystalline nature with major peaks along the planes (100), (002), (101), (102), (110), (103), and (112). The highest intensity peak is found along the plane (101).Other peaks with lower intensities were observed along (200), (201), (004), (202) etc. The observed XRD pattern is analyzed, fitted and refined using X'Pert High Score software by search match tool, it was found to match with the ICDD Reference Pattern of Zinc Oxide, 01-070-8072. The ZnO NPs exhibit hexagonal phase (space group $p6_{3mc}$) with wurtzite structure a = 0.3329 nm, c = 0.5185 nm.

3.2. Antibacterial Activities of ZnO Nanoparticles against *E. coli*

In case of *E. coli* the growth of bacterial populations has decreased significantly at 100 μg/ml and 200 μg/ml ZnO nanoparticle relative to the control. Absorbency

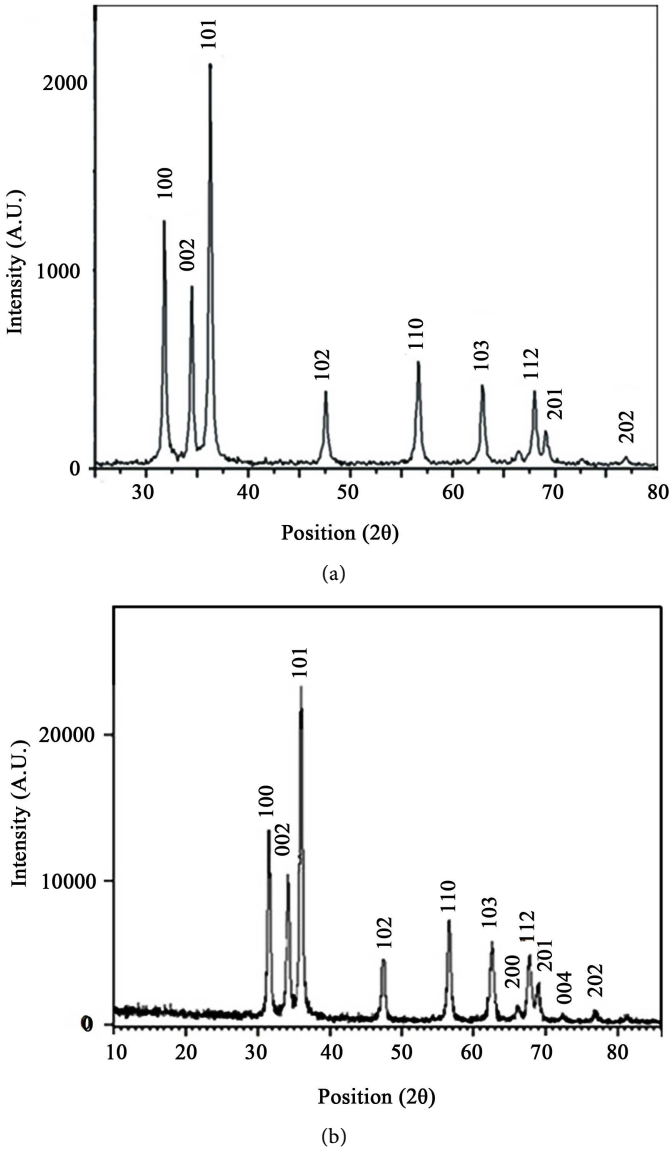


Figure 3. XRD pattern of ZnO nanoparticles synthesized in absence (ZnO NP-I) (a) and presence (ZnO NP-II) (b) of mehendi (*Lawsonia inermis*) extract by using Zinc nitrate as precausor.

readings taken on 1st, 2nd, 3rd, 4th, 5th, 6th and 24th hours suggest that 200 μg/ml of both ZnO NP-I and ZnO NP-II nanoparticles show optimum antibacterial activity against *E. coli*. However when the antibacterial efficacy of ZnO NP-I and ZnO NP-II is compared readings suggest that ZnO NP-II has better antibacterial activity than ZnO NP-I compared to the control (**Figure 4**). Similarly ZnO NP-II nanoparticle at 500 μg/ml also showed enhanced anti-bacterial effect than ZnO NP-I on 1st-6th and 24th hour. The antibacterial activity of ZnO NP-I at 100 μg/ml is however slightly higher than that of ZnO NP-II compared to control. This enhanced anti-*E. coli* effect of ZnO NP-II is due to the synergistic effect of increased bioactive surface area of ZnO nanoplates with hexagonal shape synthesized using *Lawsonia inermis* extract and *Lawsonia inermis* extract

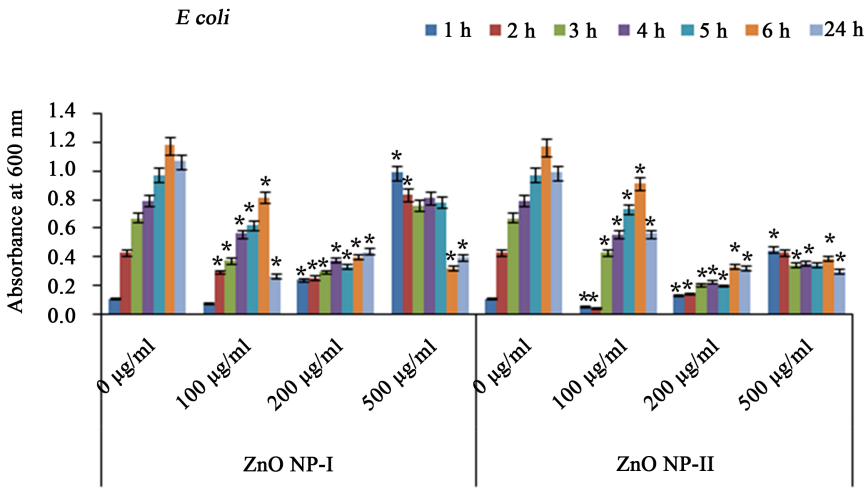


Figure 4. Antibacterial efficiency of ZnO NP-I and ZnO NP-I I nanoparticles at 0, 100, 200 and 500 µg/mL tested on *E. coli* after 1, 2, 3, 4, 5, 6 & 24 h incubation. Data presented are mean ± SE (n = 3). Bars superscript with "*" indicates significant mean difference from control (0 mg/ml) at $p < 0.05$ in multiple comparison by LSD test.

ingredients that ligated to ZnO nanoparticles (Zno NP-II). Guo *et al.* [18] also reported the increased antibacterial activity of Ta-doped ZnO nanoparticles having pure hexagonal wurtzite structure of polycrystalline ZnO nanoparticles that showed enhanced antibacterial activity due to increased bioactive surface of hexagonal Ta-doped plates compared to control non Ta-doped ZnO nanoparticles. Liu *et al*., demonstrated the antibacterial effect of ZnO nanoparticles against *Escherichia coli* and found that the effect increases with increase in ZnO nanoparticle concentration [19]. Previous report suggest that acetate and ethanol extracts *L. inermis* expressed tremendous antibacterial activity against E.coli (gram negative bacteria) confirming the antibacterial potential of *L. inermis* [20] [21]. The combined antibacterial effect of individual ZnO nanoparticle and *L.inermis* resulted in enhanced antibacterial effect of ZnO NP-II nanocomposites. Recently Moghaddam *et al*., [22] reported that appreciable antibacterial activity of green synthesized ZnO nanoparticles was achieved against *E. coli* at 100 µg/ml concentration.

3.3. Antibacterial Activity of ZnO Nanoparticles against *P. aeruginosa*

The antibacterial activity of ZnO NP-I and ZnO NP-II against *P. aeruginosa* is appreciable at 100 µg/ml, 200 µg/ml and 500 µg/ml concentration compared to control with 100 µg/ml and 200 µg/ml showing very good antibacterial effect relative to control on 1st, 2nd, 3rd, 4th, 5th and 6th hour (**Figure 5**). Lee *et al*., [23] reported the antibacterial effect of ZnO nanoparticles bio-fabricated with *C. religiosum* (L.) against virulent *Pseudomonas aeruginosa* that inhibited *Pseudomonas aeruginosa* induced biofilm formation. Similarly Mahendra *et al*., [24] demonstrated the antibacterial effect of ZnO nanoparticles coated with *C. religiosum* against *P. aeruginosa* with MIC of 312.5 µg/ml and 20.33 mm zone of

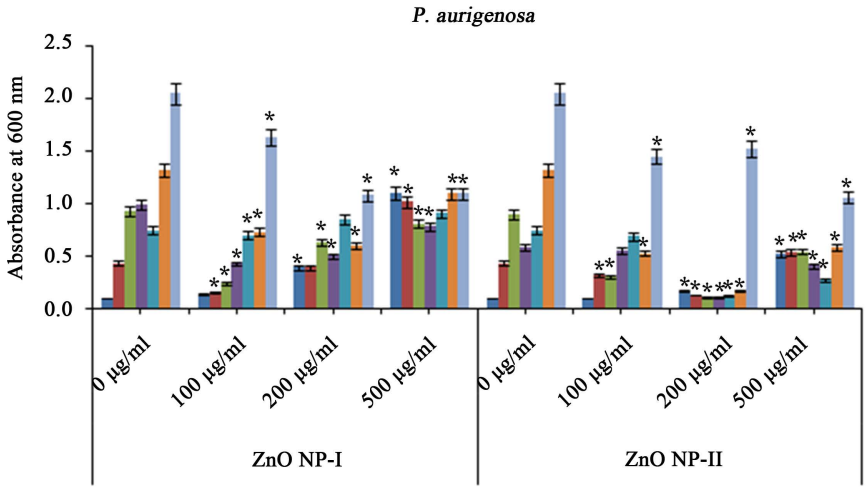


Figure 5. Antibacterial efficiency of ZnO NP-I and ZnO NP-I I nanoparticles at 0, 100, 200 and 500 µg/mL tested on *P. aurigenosa* after 1, 2, 3, 4, 5, 6 & 24 h incubation. Bars superscript with "*" indicates significant mean difference from control (0 mg/ml) at $p < 0.05$ in multiple comparison by LSD test.

inhibition which supports the present findings. At 24th hour the high absorbancy of the bacterial culture may be due to the formation of cell debris and toxic substances that increased the turbidity of the bacterial culture media. ZnO NP-II synthesized using *Lawsonia inermis* extract has enhanced antibacterial efficiency than ZnO NP-I which is due to the synergistic antibacterial effect of both heaxagonal wurtzite structure ZnO NP-II nanoparticles (as suggested by TEM images) that resulted in enhanced surface bioactivity and *Lawsonia inermis* derived bioactive compounds that ligated to the ZnO NP-II nanoparticles. Similar to our findings Guo *et al*., [18] also reported the enhanced antibacterial activity of Ta-doped ZnO nanoparticles that showed pure hexagonal wurtzite structure of polycrystalline ZnO nanoparticles that showed enhanced antibacterial activity due to increased bioactive surface of hexagonal Ta-doped plates compared to control non Ta-doped ZnO nanoparticles. Habbal *et al*., [14] also reported the high in-vitro anti-*P. aeruginosa* activity of L. inermis which support our present findings. Similarly Al-Rubiay *et al*., [25] and Jeyaseelan *et al*., [20] demonstrated the antibacterial effect of alcoholic extract of *Lawsonia inermis* with MIC values of 0.5 - 0.57 µg/ml. Naseem *et al*., [26] demonstrated the antibacterial activity of iron nanoparticle synthesized using *Lawsonia inermis* and *Gardenia jasminoides* leaves extract confirming the antibacterial potential of metal nanoparticles and *L.inermis* nanocomposites. Literature survey suggests that few reports are available on the antibacterial study of ZnO nanoparticles and *L. inermis* nanocomposites to directly support our study.

3.4. Antibacterial Activity of Synthesized ZnO Nanoparticles against *S. aureus*

The present study of antibacterial activity of Zno NP-I and ZnO NP-II against *Staphylococcus aureus* suggest that both ZnO NP-I and ZnO NP-II have a little

retarded antibacterial effect against *S. aureus* compared to other three bacteria in the present study (Figure 5). However ZnO NP-II at 100 µg/ml 200 µg/ml and 500 µg/ml concentrations shows a little improved anti-*Staphylococcus aureus* activity on 1st, 2nd, 3rd, 4th, 5th and 6th hour than ZnO NP-I compared to control which is due to the synergistic antibacterial mechanisms of ZnO nanoparticle and *Lawsonia inermis* bioactive compounds that ligated to the ZnO nanoparticle surface and differences in the shape of ZnO NP-I which are nanorods while the ZnO NP-II being hexagonal plates may have easily penetrated the membrane barrier mediating bacterial membrane damage and cell leakage leading to cell death. In context to this Badoni *et al*., [27] reported that ZnO nanoparticles bio-fabricated with *R. parviflora* extract showed very good anti-*Staphylococcus aureus* activity at 200 µg/ml concentration (Badoni *et al*., [27]. The anti-*Staphylococcus aureus* activity of ZnO nanoparticle and *Lawsonia inermis* nanocomposites (ZnO NP-II) has increased with rise in concentration. Karvani *et al*., also reported a similar increase in anti-*Staphylococcus aureus* activity of ZnO nanoparticles with increasing ZnO nanoparticle concentrations [28].

3.5. Antibacterial Activity of ZnO Nanoparticles against *B. subtilis*

Our study suggests that both ZnO NP-I and ZnO NP-II showed significant anti-*Bacillus subtilis* effect with 100 µg/ml and 200 µg/ml showing optimum antibacterial activity on 1st, 2nd, 3rd, 4th, 5th and 6th hour (Figure 6 and Figure 7). In this case also ZnO NP-II has enhanced anti-*Bacillus subtilis* efficacy than ZnO NP-I again suggesting the combined antibacterial effect of individual ZnO nanoparticle having increased surface bioactivity than ZnO NP-I and *Lawsonia inermis* derived bioactive ingredient that got ligated on ZnO nanoparticle. Hsueh

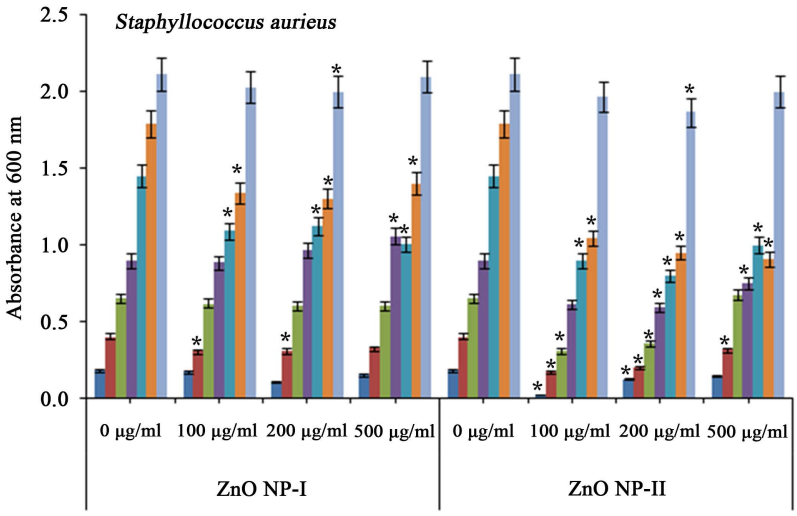


Figure 6. Antibacterial efficiency of ZnO NP-I and ZnO NP-I I nanoparticles at 0, 100, 200 and 500 µg/mL tested on *Staphyllococcus aurieus* after 1, 2, 3, 4, 5, 6 & 24 h incubation. Bars superscript with "*" indicates significant mean difference from control (0 mg/ml) at $p < 0.05$ in multiple comparison by LSD test.

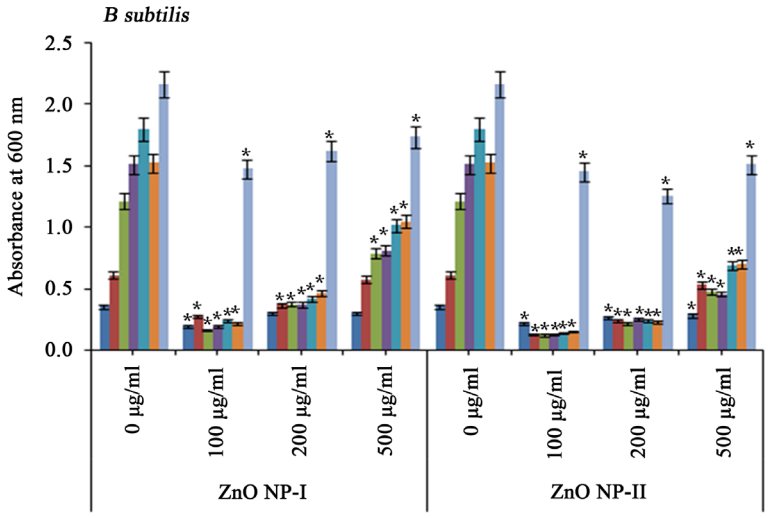


Figure 7. Antibacterial efficiency of ZnO NP-I and ZnO NP-I I nanoparticles at 0, 100, 200 and 500 µg/mL tested on *B subtilis* after 1, 2, 3, 4, 5, 6 & 24 h incubation. Bars superscript with "*" indicates significant mean difference from control (0 mg/ml) at $p < 0.05$ in multiple comparison by LSD test.

et al., [29] and Mastanaiah *et al.* [30] demonstrated the anti-*Bacillus subtilis* activity of ZnO nanoparticle and *Lawsonia inermis* respectively suggesting the antibacterial potential of ZnO nanoparticle and *Lawsonia inermis.*

4. Conclusion

ZnO NPs have potential antibacterial property that may be attributed to its various structural and functional properties. The antibacterial activity study of both the synthesized ZnO nanoparticles reveals that the nanoparticles synthesized using mehendi extractare are more effective than the particle synthesized without mehendi extract. Thus, the use of leaf extract as capping agent would improve the antibacterial property of ZnO nanoparticle. However, bacteriocidal effect of these nanoparticles varies with respect to the organism tested. Detail understanding of the molecular mechanism of the synthesized nanoparticles may help to modulate their antibacterial potential for the benefit of mankind.

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