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Influence of Eucalyptus Leaves Extract on Antibacterial Activity of ZnO Nanoparticles

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In the current research work, zinc oxide nano particles are synthesized by simple precipitation method and studied its antibacterial activity. The antibacterial activity can be successfully enhanced for some kind of bacterium after the addition of eucalyptus leaves extract. The structural, morphological, and elemental properties of synthesized material and the effect of eucalyptus leaves extract on these properties are studied by using X-ray diffraction (XRD), Field Emission Scanning electron Microscopy (FESEM), and elemental analysis (EDAX). Enhanced crystalline size and porosity is observed after the addition of eucalyptus leaves extract while synthesizing zinc oxide (ZnO). In addition, eucalyptus leaves extract assists to avoid agglomeration of ZnO nano particles. Role of eucalyptus leaves extract in the antibacterial activity of synthesized ZnO nano particles are studied against gram positive bacterium (*Bacillus*) and gram negative bacterium (*Escherichia coli*). It reveals that after addition of eucalyptus leaves extract, antibacterial activity against gram positive bacteria (*Bacillus*) is enhanced. However, it does not show any antibacterial activity on a gram negative bacterium (*E. coli*). The eucalyptus leaves extract may be responsible for the resistance against *E. coli*.

1. Introduction

Earth atmosphere protects life on earth by creating water, absorbing ultraviolet solar radiation, warming surface by heat retention, etc. In last few decades, earth's atmosphere is affected due to hazardous gases and substances. Day by day these products are increasing due to rapid development, industrialization, and population growth.[1] Nano materials have gained most attention to overcome these issues.

Nano materials have various functions which are not observed in their bulk counterpart; therefore it acts as a bridge between bulk material and atomic structures.[1] Large surface area of nano material enhances the reactivity of nano materials with its surrounding. Based on the size, distribution, and morphology these materials are used for various applications such as electrochemistry, food technology, sensors, biomedicine, pharmaceutics, space industry, and opto-electronic device.[2,3] Highly ordered nano particles with desired shape and size are synthesized by using modern technology, which leads to develop new biocidal agents. Hence, these materials are also known as wonder of medical science.[3]

Nano particles are also synthesized by using plant extracts by the technique known as green synthesis. Compared to chemical methods, in green synthesis, expensive reducing agents are replaced by extract of natural products. Therefore, it is an interesting part in nano science and technology. In addition, it is simple, cost effective, gives good stability of nano particles, environmental friendly, free of chemical contamination, and there is no need to use high pressure.[4] During synthesis, it does not generate hazardous gases and substances. Also, nano particles produced by plants are more stable compared to that produced by organisms.[5]

Nowadays, bacterial contamination is major issue in human health, therefore there is urgent need to develop antibacterial bioactive materials. Biological molecules such as proteins, enzymes, sugars, etc. can be stabilized easily in nano form and easily allow interact with other bio-molecules. Consequently, it increases antimicrobial activity by improving interaction with micro-organism.[6] Various antimicrobial agents are used for this application and these are separated in two types, organic and inorganic. At high pressure and temperature, organic antimicrobial materials are less stable compared to inorganic antimicrobial material.[7] Therefore metal and metal oxides are more attractive; in addition, these materials sustain harsh conditions and are safe for human beings.[2]

Number of green synthesized materials such as Ag, Au, TiO_2, MgO, FeO_2, AlO, ZnO, etc., is tried by researcher for antimicrobial activity.[8] Among these nano particles, ZnO is more attractive because it shows high catalytic efficiency and strong adsorption ability. Therefore, it is useful for optical, piezoelectric, magnetic, and gas sensing application.[9] In addition ZnO nano particles show good absorption properties of UV-A and UV-B, therefore it is used for cosmetic application.[10] Different synthesis methods such as coprecipitation, solvothermal, sol–gel, hydrothermal, and microwave irradiation, etc., are tried by

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researcher.[6] But green synthesized ZnO nano particles have gained more importance because of its simplicity, eco friendliness and extensive antimicrobial activity.[10]

Eucalyptus is one of the most important plants belonging to the Myrtaceae family. It is tall and evergreen tree originated from Australia. Due to its medical advantages, it is cultivated in many countries. Extract of eucalyptus leaves is traditionally used as analgesic, anti-inflammatory, and antipyretic remedies for cold, flu, and sinus congestion.[11,12] In addition several studies show eucalyptus leaves are also useful for application as antibacterial, antifungal, antioxidative, and antiradical activities.[11,12]

The objective of current research work is to synthesize eucalyptus leaves extract with ZnO nano particles and to determine its antibacterial activity.

2. Results and Discussion

The crystal phase composition of synthesized pure ZnO and ZnO +eucalyptus leaves nano particles is investigated by using X-ray diffraction (XRD) pattern as shown in **Figure 1**a–c. All patterns match well with JCPDS card no. 36-1451 of ZnO. The reflection (100), (002), (101), (102), (110), and (103) corresponding to ZnO are present in XRD patterns. The average crystallite size (D) was calculated using Scherrer formula. The observed average crystallite size is 35.53, 39.7, and 41.5 nm for ZnO and ZnO + 5 mL eucalyptus leaves extract and ZnO + 10 mL eucalyptus leaves extract, respectively. It clearly indicates eucalyptus leaves extract promotes to enhance crystalline size of material.

The morphology of the ZnO and ZnO + 10 mL eucalyptus leaves extract nanoparticles are also examined by Field Emission Scanning electron Microscopy (FESEM). **Figure 2**a,b shows the micrograph of (a) ZnO and (b) ZnO + 10 mL eucalyptus leaves extract nanoparticles, which demonstrates the agglomerated ZnO nano particles. However, after addition of 10 mL eucalyptus leaves extract, porous ZnO nano particles are observed. It can be concluded that addition of 10 mL eucalyptus leaves extract restrict agglomeration of ZnO nano particles. Furthermore, representative EDAX patterns of pure ZnO and ZnO+10 mL eucalyptus leaves extract nanoparticles were recorded to investigate the elemental composition. The EDAX pattern of pure ZnO

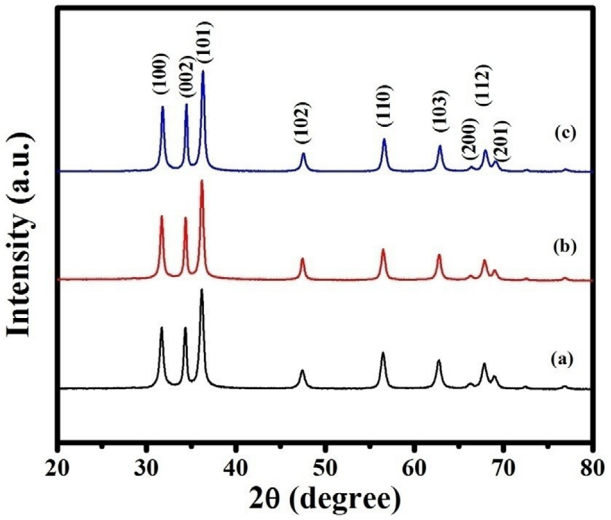

Figure 1. XRD patterns of (a) sample 1 (Pure ZnO) (b) sample 2 (ZnO + 5 mL eucalyptus leaves extract), and (c) sample 3 (ZnO + 10 mL eucalyptus leaves extract).

nanoparticles (the inset of Figure 2a) clearly shows the exclusive presence of Zn and O element, additional peaks are not observed it confirms the absence of other impurities in the sample. Similarly, ZnO+10 mL eucalyptus leaves extract (the inset of Figure 2b) shows the presence of Zn and O. This result is in good agreement with the literature report confirming the successful formation of ZnO nano particles.

A study of antibacterial activity was performed against gram positive bacterium (Bacillus) and gram negative bacterium (*E coli*) and it is shown in **Figure 3**a-b. Thickness for inhibition zone was calculated for different samples and it is listed in **Table 1**. These results reveal that, without eucalyptus leaves extract, ZnO nano particles show antibacterial activity for both gram positive bacteria (bacillus) and gram negative bacteria (*E coli*). However, after addition of eucalyptus leaves extract, increased antibacterial activity against gram positive bacteria (bacillus) is observed; however, antibacterial activity against gram negative bacterium

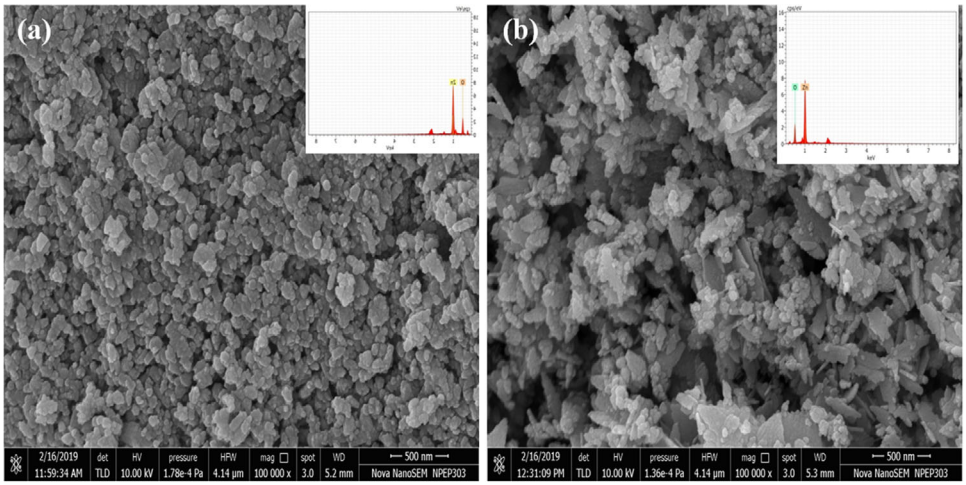

Figure 2. FESEM image of (a) ZnO (b) 10 mL ZnO + eucalyptus leaves extract.

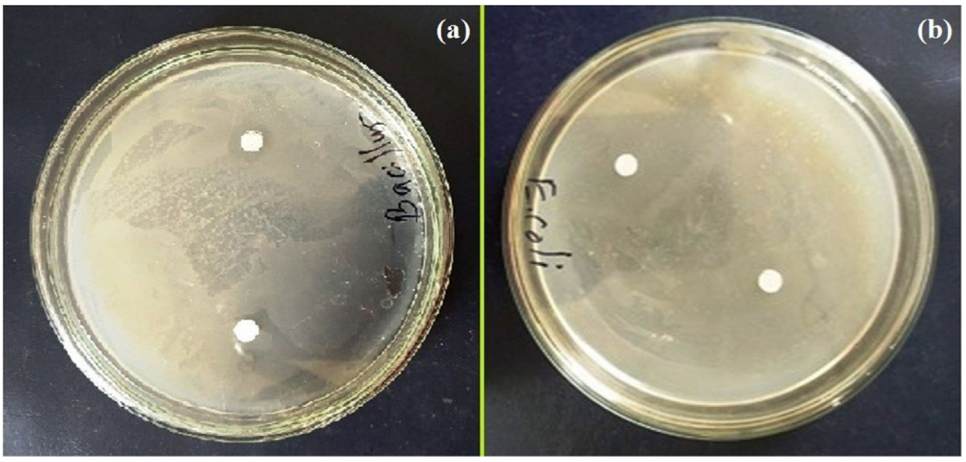

Figure 3. Antibacterial activity (a) against gram positive bacteria (bacillus) and (b) gram negative bacteria (*E coli*).

Table 1. Quantitative analysis of antibacterial activity against Bacillus and *E. coli*.

S.N.	Sample	Thickness of zone of inhibition [mm] against *Bacillus*	Thickness of zone of inhibition [mm] against *E. coli*
1	ZnO nanoparticles	0.5	0.7
2	Eucalyptus leaves extract	0.6	0.0
3	5 mL extract + 0.5 g ZnO nano particles	1.4	0.0
4	10 mL extract + 0.5 g ZnO nano particles	0.6	0.0

(*E coli*) has disappeared. This result reveals that eucalyptus extract does not help in resisting *E. coli*.

These results are observed because eucalyptus leaves extract contain chemical composition of different chemicals such as *E. maideni; E. astrengens; E.cinerea; E. leucoxylon; E. lehmani; E. sideroxylon; E. bicos-tata*. This composition may vary with the leaves age, geographical origin, harvest date, extraction method, etc. After addition of eucalyptus leaves extract, these molecules become stable on the surface of ZnO nano particles and support to stabilize ZnO nano particles. Eucalyptus leaves extract itself shows antibacterial activity against gram-positive bacteria (*Bacillus*), which results enhanced antibacterial activity against gram positive bacteria (*Bacillus*). However, chemical composition present on the surface of ZnO nano particles may resist antibacterial activity against gram negative bacteria (*E. coli*). Therefore, a ZnO nano particle with eucalyptus leaves extract does not show any antibacterial activity against gram negative bacteria (*E. coli*).

3. Conclusion

The present study for antibacterial activity of ZnO nano particles using powdered extract of eucalyptus leaves provides environmental friendly, cost effective, and simple technique to synthesis metal oxides. The ZnO nano particles are synthesized in a range of 35 to 42 nm. The crystalline size increases with the addition of eucalyptus leaves extract. In addition, eucalyptus leaves extract restrict agglomeration of ZnO nano particles. Its antibacterial activity is studied against gram positive bacterium (*Bacillus*) and gram negative bacterium (*E. coli*). After addition of eucalyptus leaves extract, enhanced antibacterial activity against gram positive bacteria (*Bacillus*) is observed. After addition of eucalyptus leaves extract however, the molecules present in eucalyptus leaves extract become stable on the surface of ZnO nano particles and support to stabilize ZnO nano particles. Eucalyptus leaves extract itself shows antibacterial activity only against gram positive bacteria (*Bacillus*); therefore enhanced antibacterial activity against gram positive bacteria (bacillus) is observed, however, the antibacterial activity against gram negative bacterium (*E. coli*) is diminished.

4. Experimental Section

Preparation of Zno Nanoparticles: ZnO nanoparticles were synthesized by direct precipitation method. Zinc nitrate ($Zn(NO_3)_2.6H_2O$ (LOBA CHEMIE PVT LTD 98%) and KOH (Himedia 99%) are used as precursors. Aqueous solution of zinc nitrate (0.2M) and KOH (0.4 M) were prepared with deionized water. Followed by this, under vigorous stirring, KOH solution was slowly added to zinc nitrate solution at room temperature. It results in the formation of white suspension. The resultant suspension was washed number of times with double distilled water and absolute alcohol. Then, obtained white suspension was subjected to centrifugation and solid product was separated. Collected pest was dehydrated at 60°C under the IR lamp for 2 h. Then for homogenation, solid product was grinded in agate mortar.

Collection of Eucalyptus Leaves: The fresh leaves of eucalyptus were collected early in the morning and were washed thoroughly two—three times with running tap water and sterilized with double-distilled water. The leaves sample was allowed to dry at room temperature (≈32°C).

Extraction of Eucalyptus Leaves: A total 20 g of eucalyptus leaves powder in dried form was added to 100 mL of double distilled water and magnetically stirred for 2 h at 60°C. Light yellow colored solution was obtained. After cooling to room temperature, obtained solution was filtered through Whatman No. 1 paper. For further study, pre calculated amount of eucalyptus leaves extract (0, 5, and 10 mL) was mixed with already prepared 2 g zinc oxide nanoparticles. The mixture was continuously stirred for homogeneous mixing. During the process of constant heating and stirring,

light yellow colored gel was formed after removal of excess water. This gel was kept under IR lamp for 2 h, it was decomposed and whitish yellow colored powder was formed. After grinding and homogenation of whitish yellow colored powder in agate mortar, it was preserved for further studies. The resultant products were named as sample 1 (ZnO + 0 mL eucalyptus leaves extract), sample 2 (ZnO + 5 mL eucalyptus leaves extract), and sample 3 (ZnO + 10 mL eucalyptus leaves extract).

The crystalline properties of powder were studied by using X-ray diffractometer (Philips PW-3710) with Cu (Kα) as radiation source. The morphological properties and elemental properties were analyzed using field emission scanning electron microscope (FE-SEM, Hitachi S-4200).

Antimicrobial Studies: A bacterial strain of interest was grown in pure culture. Using a sterile swab, a suspension of the pure culture was spread evenly over the face of a sterile agar plate. The antimicrobial agent was applied to the center of the agar plate (in a fashion such that the antimicrobial does not spread out from the center). A hole can be bored in the center of an agar for a liquid substance. The agar plate is incubated for 18–24 h (or longer if necessary), at a temperature suitable for the test microorganism.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Data openly available in a public repository that issues datasets with DOIs.

Keywords

antibacterial activity, eucalyptus leaves extract, morphology, nanoparticles, X-ray diffractions

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[1] I. Hussain, N. B. Singh, A. Singh, H. Singh, S. C. Singh, *Biotechnol. Lett.* **2016**, *38*, 545.
[2] S. Ahmed, A. S. A. Chaudhry, S. Ikram, *J. Photochem. Photobiol., B* **2017**, *166*, 272.
[3] S. Gunalan, R. Sivaraj, V. Rajendran, *Prog. Nat. Sci.: Mater. Int.* **2012**, *22*, 693.
[4] K. Elumalai, S. Velmurugan, S. Ravi, V. Kathiravan, S. Ashokkumar, *Spectrochim. Acta, Part A* **2015**, *143*, 158.
[5] C. Jayaseelan, R. Ramkumar, A. A. Rahuman, P. Perumal, *Ind. Crops Prod.* **2013**, *45*, 423.
[6] A. Raja, S. Ashokkumar, R. Pavithra Marthandam, J. Jayachandiran, C. P. Kathiwada, R. G. R. K. Kaviyarasu, M. Swaminathan, *J. Photochem. Photobiol., B* **2018**, *181*, 53.
[7] K. Elumalai, S. Velmurugan, *Appl. Surf. Sci.* **2015**, *345*, 329.
[8] S. R. Senthilkumar, T. Sivakumar, *Int. J. Pharm. Sci. Res.* **2014**, *6*, 461.
[9] R. Sivaraj, P. K. S. M. Rahman, P. Rajiv, H. A. Salam, R. Venckatesh, *Spectrochim. Acta, Part A* **2014**, *133*, 178.
[10] M. Ramesha, M. Anbuvannan, G. Viruthagiri, *Spectrochim. Acta, Part A* **2015**, *136*, 864.
[11] K. Sebei, F. Sakouhi, W. Herchi, M. L. Khouja, S. Boukhchina, *Biol. Res.* **2015**, *48*, 7.
[12] O. O. Ayepola, B. A. Adeniyi, *J. Appl. Sci. Res.* **2008**, *4*, 1410.