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# ANTIMICROBIAL ACTIVITY OF ZnO NANOPARTICLES: SYNTHESIS USING *ARTOCARPUS GOMEZIANUS* FRUIT MEDIATED FACILE GREEN COMBUSTION METHOD

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## ABSTRACT

Zinc Oxide nanoparticles have been proven to be competent antibacterial and antifungal activity for medical applications because of their strong redox ability, nontoxicity, long-term stability and low cost. Spherical ZnO nanoparticles were synthesized by eco-friendly green combustion method using citrate containing *Artocarpus gomezianus* fruit extract as a fuel. Prepared ZnO nanoparticles exhibit significant antibactericidal activity against *Bacillus cereus, Escherichia coli, Serratia marcescens* and antifungal activity against *Malassezia furfur* using the zone of inhibition method. The study successfully demonstrates synthesis of spherical ZnO nanoparticles by simple eco-friendly green combustion method exhibit antimicrobial activities. Further, this method can effectively need for the preparations of ZnO for the medical applications in controlling pathogenic bacteria with better dispersion and consequently, better efficiency in food and textiles industry.

**KEYWORDS:** ***Green synthesis; ZnO nanoparticles; Antibacterial activity; Antifungal activity.***

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# INTRODUCTION

ZnO nanoparticles exhibit completely new or improved properties of the bulk materials and these novel properties are derived due to the variation in specific characteristics such as size and morphology of the particles.[1] ZnO nanoparticles have a wide variety of applications in solar cells, catalysts, gas sensors, luminescent devices etc.[2-4] Green combustion synthesis is wet-chemical method, which has been proved to be an excellent technique for preparing several grams due to its short processing time, low processing temperature and low cost as well as good ability to achieve high purity in making single or multiphase complex oxide powders. Chemical synthesized prepared nanoparticles of various methods such as, sol-gel process, micelle, precipitation, hydrothermal and pyrolysis etc.[5-8] In general, chemicals used for the nanoparticles synthesis and stabilization are toxic and led to non-eco-friendly by-products are very expensive and use of hazardous chemicals mostly which cause danger to the environment and human beings.[9] In the present study spherical ZnO nanoparticles were prepared by green combustion method with different volume of citrate containing *Artocarpus gomezianus* fruit source as fuel. The green chemistry approach for the synthesis of ZnO NPs using plants avoids the generation of toxic byproducts. Hence, green combustion synthesis is eco-friendly alternate wet-chemical method. Which has been proved to be an excellent technique for preparing several grams due to its short processing time, low processing temperature and cost effective as well as good ability to achieve high purity in making single or multiphase complex oxides.[10-11] The obtained spherical ZnO nanoparticles are used to study antibacterial and antifungal activities. To the best of our knowledge this is the first attempt made to study ZnO Nps synthesized from *Artocarpus gomezianus* fruit extract as antibacterial and antifungal activity.

# MATERIALS AND METHODS

### *Chemicals*

All the chemicals used were of analytical grade and were used without any further purification. Zinc nitrate was purchased from Merck. All glassware used in the laboratory experiments were cleaned with a fresh solution of $HNO_3$/HCl (3:1, v/v), washed thoroughly with doubly distilled water and dried before use. Double distilled water was used in all experiments.

### *Preparation of ZnO NPs*

The citrate containing *Artocarpus gomezianus* fruit source was collected from Mangalore, Karnataka. The collected fresh, healthy fruits were washed thoroughly with double distilled water and finely cut into small pieces. Then finely cut pieces were dried at room temperature for 10 days under dust free condition and crushed into the fine powder. 10 g of fine powder were boiled in 100 mL doubled distilled water to prepare 10% crude solution, filtered and stored in refrig rator for further usage. In a typical synthesis, 5 mL of 10% crude solution was added to 3g of $Zn(NO_3)_2.6H_2O$ already dissolved in 10 mL of double distilled water. This reaction mixture was mixed well using magnetic stirrer for about 5-10 min. and then placed in a preheated muffle furnace maintained at $400 \pm 10^0$ C. The reaction mixture boils froths and thermally dehydrates forming foam. The entire process was completed in a few minutes. Further foam was cooled and collected at room temperature. Same procedure followed for 10mL and 15 mL of 10% crude solution.

### *Antibacterial activity and Antifungal activity*

To perform MIC (minimum inhibitory concentration) for the given sample. The nanoparticles obtained from 5 mL, 10mL and 15 mL of 10% crude sample were dissolved in DMSO (Di Methyl SulphOxide) to give a concentration of 10 mg/mL and were marked as stock respectively. 0.5 ml of the stock was serially diluted to obtain working concentrations of 0.5 mg/mL, 0.05 mg/mL, 0.005 mg/mL and 0.0005 mg/mL. Sterile Muller Hinton agar was poured into autoclaved petriplates. Four wells were bored. 100 μL of 24 h test cultures (*Escherichia coli, Serratia marcescens, Bacillus cereus*) was spread plate onto the well bored media. To the four wells, 100 μL of different working concentrations of the sample were loaded. The plates were incubated along with a control containing DMSO and antibiotic (ampicillin) for 17 h period at 37˚C. Following the incubation period, the zone of inhibition of each well was measured using scale. The diameters of the inhibition zones were measured in mm and the values are recorded. Sterile Potato dextrose agar media was poured into autoclaved petriplates. Four wells were bored. 100μl of spore suspension (*Malassezia furfur*) were spread plate onto the well bored media. To the four wells, 100μL of different concentrations from the working solution of the samples were loaded. The plate was incubated along with a control containing DMSO and antifungal (fluconazole) at room temperature for a period of 96 h. After the incubation period, the zone of inhibition of each well was measured and the values are noted. Triplicates are maintained in each compound and the average values are calculated for the ultimate antibacterial activity and antifungal activity.

# RESULT AND DISCUSSION

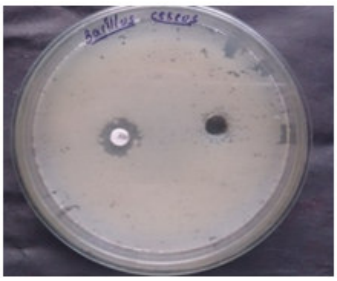
Antibacterial activity of ZnO was studied against *Escherichia coli, Serratia marcescens, Bacillus cereus,* bacterial strains using agar well diffusion method. The pathogenic bacterial strains of *Bacillus cereus with various concentration* 0.0005 to 0.5 (mg/100μL) of 1,2,3,4 samples respectively showed the zone of inhibition shown in Figure 1 and the data was shown in Table 1.

**Table 1**
***Zone of inhibition of spherical ZnO nanoparticles against Bacillus cereus***

| Sample | Sl.No. | Concentration (mg/100μL) | Zone of inhibition (mm) |
|---|---|---|---|
| 5 mL | 1 | 0.5 | 11.5 ± 0.66 |
| | 2 | 0.05 | ne |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 10 mL | 1 | 0.5 | 12.5 ± 0.33 |
| | 2 | 0.05 | 12.0 ± 0.51 |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 15 mL | 1 | 0.5 | 12.0 ± 0.57 |
| | 2 | 0.05 | ne |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |

***Values are mean inhibition zone (mm) ± S.D of three replicates***
***Note: 'ne' indicates no effect***

***Control***



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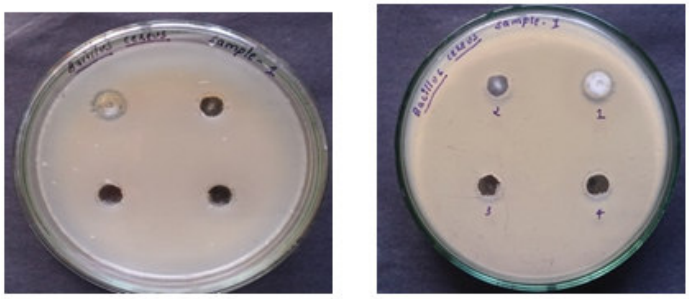

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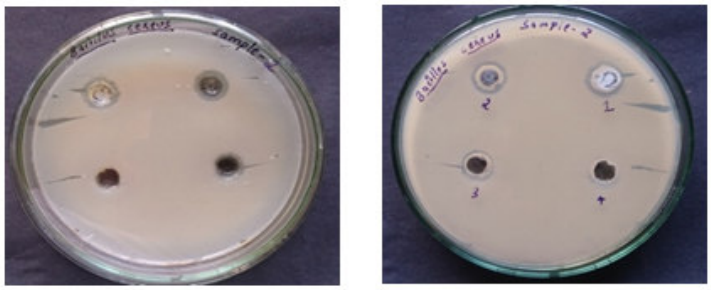

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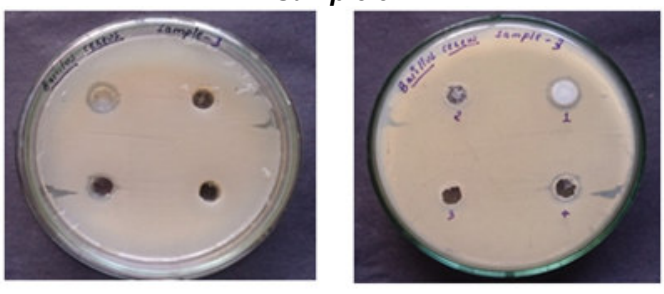


**Figure 1**
***Photographs showing antibacterial activity in zone of inhibition method with Bacillus cereus 0.0005 to 0.5 (mg/100μL) of 1,2,3,4 samples respectively.***

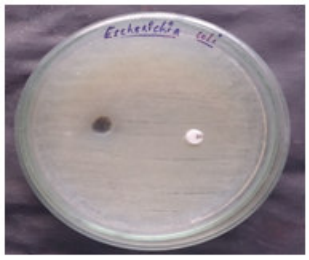
The pathogenic bacterial strains of *Escherichia coli with various concentration* 0.0005 to 0.5 (mg/100μL) of 1,2,3,4 samples respectively showed the zone of inhibition shown in Figure 2 and the data was shown in Table 2.

**Table 2**
***Zone of inhibition of spherical ZnO nanoparticles against Escherichia coli.***

| Sample | Sl.No. | Concentration (mg/100μL) | Zone of inhibition (mm) |
|---|---|---|---|
| 5 mL | 1 | 0.5 | 13.25 ± 0.33 |
| | 2 | 0.05 | ne |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 10 mL | 1 | 0.5 | 10.5 ± 0.66 |
| | 2 | 0.05 | ne |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 15 mL | 1 | 0.5 | 13.75 ± 0.57 |
| | 2 | 0.05 | 10.0 ± 0.51 |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |

***Values are mean inhibition zone (mm) ± S.D of three replicates***
***Note: 'ne' indicates no effect***

***Control***



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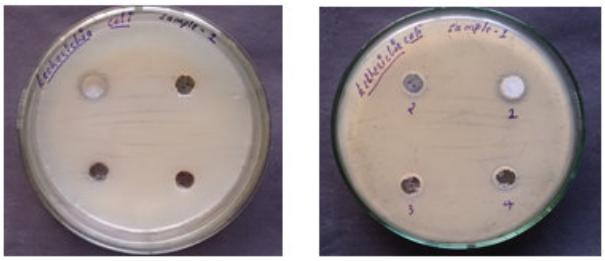

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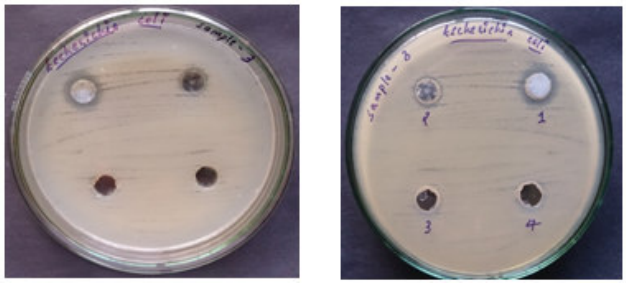

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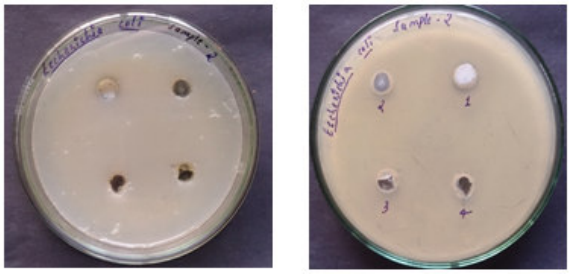


**Figure 2**
***Photographs showing antibacterial activity in zone of inhibition method with Escherichia coli 0.0005 to 0.5 (mg/100μL) of 1,2,3 samples respectively.***

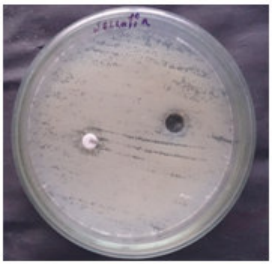
The pathogenic bacterial strains of *Escherichia coli with various concentration* 0.0005 to 0.5 (mg/100μL) of 1,2,3,4 samples respectively showed the zone of inhibition shown in Figure 3 and the data was shown in Table 3.

**Table 3**
***Zone of inhibition of spherical ZnO nanoparticles against Serratia marcescens.***

| Sample | Sl.No. | Concentration (mg/100μL) | Zone of inhibition (mm) |
|---|---|---|---|
| 5 mL | 1 | 0.5 | 14.5 ± 0.52 |
| | 2 | 0.05 | 13.0 ± 0.79 |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 10 mL | 1 | 0.5 | 14.0 ± 1.15 |
| | 2 | 0.05 | 11.0 ± 1.20 |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 15 mL | 1 | 0.5 | 13.0 ± 0.33 |
| | 2 | 0.05 | 10.5 ± 0.88 |
| | 3 | 0.005 | 10.5 ± 0.57 |
| | 4 | 0.0005 | ne |

***Values are mean inhibition zone (mm) ± S.D of three replicates***
***Note: 'ne' indicates no effect***

***Control***



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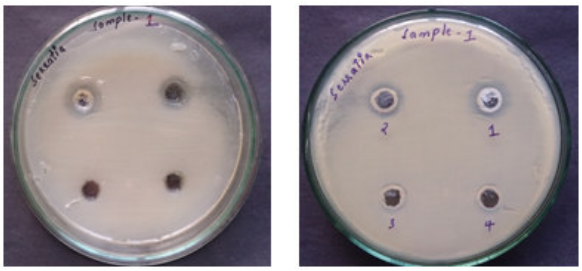

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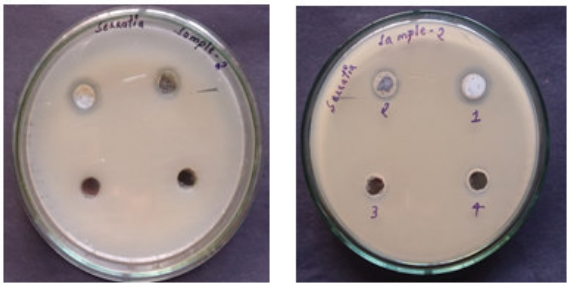

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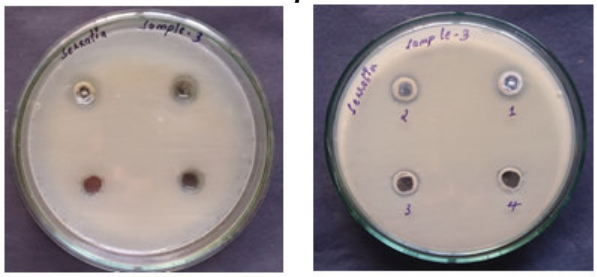


**Figure 3**
***Photographs showing antibacterial activity in zone of inhibition method with Serratia marcescens 0.0005 to 0.5 (mg/100μL) of 1,2,3 samples respectively.***

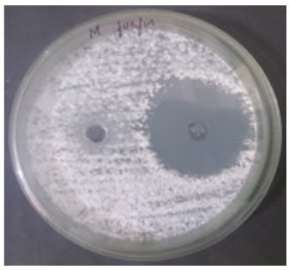
The pathogenic fungal strains of *Malassezia furfur with various concentration* 0.0005 to 0.5 (mg/100μL) of 1,2,3,4 samples respectively showed the zone of inhibition shown in Figure 4 and the data was shown in Table 4.

**Table 4**
***Zone of inhibition of spherical ZnO nanoparticles against malassezia furfur.***

| Sample | Sl.No. | Concentration (mg/100μL) | Zone of inhibition (mm) |
|---|---|---|---|
| 5 mL | 1 | 0.5 | ne |
| | 2 | 0.05 | ne |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |
| 10 mL | 1 | 0.5 | 15.0 ± 0.66 |
| | 2 | 0.05 | 14.0 ± 0.33 |
| | 3 | 0.005 | 12.5 ± 0.00 |
| | 4 | 0.0005 | ne |
| 15 mL | 1 | 0.5 | 13.0 ± 0.23 |
| | 2 | 0.05 | ne |
| | 3 | 0.005 | ne |
| | 4 | 0.0005 | ne |

***Values are mean inhibition zone (mm) ± S.D of three replicates***
***Note: 'ne' indicates no effect***

***Control***



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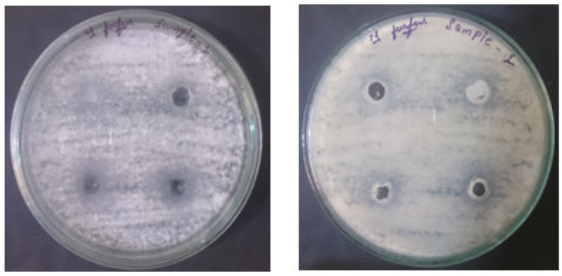

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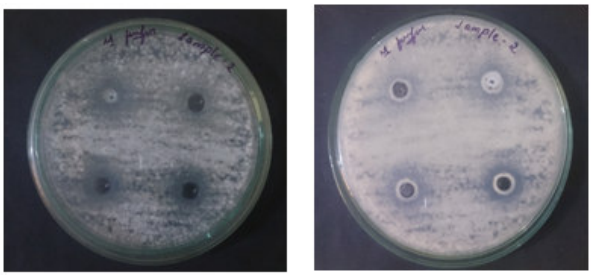

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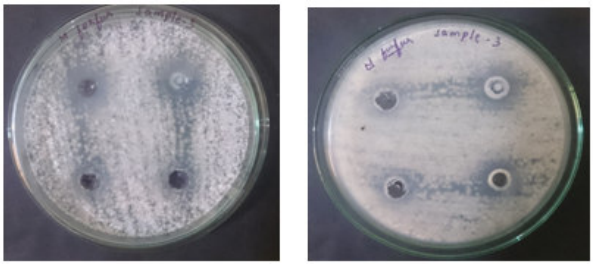

**Figure 4**
***Photographs showing antifungal activity in zone of inhibition method with Molassezia furfur. 0.0005 to 0.5 (mg/100μL) of 1,2,3 samples respectively.***

Minimum inhibitory concentration(MIC) of pathogenic bacterias like *Escherichia coli, Serratia marcescens, Bacillus cereus* and pathogenic fungus like *Molassezia furfur* is summarized in Table 5.

**Table 5**
***Minimum inhibitory concentration of bacterias and fungus.***

| Test organism | Sample | Zone of inhibition (mm) | MIC (mg/100µL) |
|---|---|---|---|
| *Bacillus cereus* | 1 | 11.5 ± 0.66 | 0.5 |
| | 2 | 12.0 ± 0.51 | 0.05 |
| | 3 | 12.0 ± 0.57 | 0.5 |
| *Escherichia coli* | 1 | 13.25 ± 0.33 | 0.5 |
| | 2 | 10.5 ± 0.66 | 0.5 |
| | 3 | 13.75 ± 0.57 | 0.5 |
| *Serratia marcescens* | 1 | 13.0 ± 0.79 | 0.05 |
| | 2 | 11.0 ± 1.20 | 0.05 |
| | 3 | 10.5 ± 0.57 | 0.005 |
| *Malassezia furfur* | 1 | ne | ne |
| | 2 | 12.5 ± 0.00 | 0.005 |
| | 3 | 13.0 ± 0.23 | 0.5 |

***Values are mean inhibition zone (mm) ± S.D of three replicates***
***Note: 'ne' indicates no effect***

Minimum inhibitory concentration 0.5 mg/100µL (Nps prepared from 5mL of crude sample) is enough for antibacterial effect against *Bacillus cereus*.
Minimum inhibitory concentration 0.05 mg/100µL (Nps prepared from 10mL of crude sample) is enough for antibacterial effect against *Bacillus cereus*.
Minimum inhibitory concentration 0.5 mg/100µL (Nps prepared from 15mL of crude sample) is enough for antibacterial effect against *Bacillus cereus*.
Minimum inhibitory concentration 0.5 mg/100µL (Nps prepared from 5mL of crude sample) is enough for antibacterial effect against *Escherichia coli*.
Minimum inhibitory concentration 0.5 mg/100µL (Nps prepared from 10mL of crude sample) is enough for antibacterial effect against *Escherichia coli*.
Minimum inhibitory concentration 0.5 mg/100µL (Nps prepared from 15mL of crude sample) is enough for antibacterial effect against *Escherichia coli*.
Minimum inhibitory concentration 0.05 mg/100µL (Nps prepared from 5mL of crude sample) is enough for antibacterial effect against *Serratia marcescens*.
Minimum inhibitory concentration 0.05 mg/100µL (Nps prepared from 10mL of crude sample) is enough for antibacterial effect against *Serratia marcescens*.
Minimum inhibitory concentration 0.005 mg/100µL (Nps prepared from 15mL of crude sample) is enough for antibacterial effect against *Serratia marcescens*.
Nps prepared from 5mL of crude sample is not enough for antifungal effect against *Malassezia furfur*.
Minimum inhibitory concentration 0.005 mg/100µL (Nps prepared from 10mL of crude sample) is enough for antifungal effect against *Malassezia furfur*.
Minimum inhibitory concentration 0.5 mg/100µL (Nps prepared from 15mL of crude sample) is enough for antifungall effect against *Malassezia furfur*.

## CONCLUSION

We have successfully synthesized spherical ZnO nanoparticles via the green combustion method using 5, 10 and 15 mL, 10% citrate containing *Artocarpus gomezianus* solution as a fuel. We have utilized the natural, renewable sources for the synthesis of nanoparticles. ZnO nanoparticles exhibit significant antibacterial activity against pathogenic bacterial strains *Bacillus cereus, Escherichia coli, Serratia marcescens* and antifungal activity against pathogenic fungus *Malassezia furfur*. Hence the prepared samples may show the applications in antimicrobial activity. The study fruitfully reveals simple, economical and eco-friendly method of synthesis of multifunctional spherical ZnO nanoparticles.

## CONFLICT OF INTEREST

Conflict of interest declared none.

## REFERENCES

1. Ravindra Singh P, Vineet Shukla K, Raghavendra S, Yadav K, Sharma P, Singh P K, Avinash, Pandey C. Biological approach of zinc oxide *nanoparticles* formation and its characterization. Advanc. Mater. Lett. 2011 Mar;2(4):313-17.
2. Singh A K, Viswanath V, Janu V C. Synthesis, effect of capping agents, structural, optical and photoluminescence properties of ZnO nanoparticles, J Luminescence. 2009 Mar 21;129(8):874-78.
3. Ates T, Tatar C, Yakuphanoglu F. Preparation of semiconductor ZnO powders by sol-gel method : Humidity sensors. Sensors Actuators A. 2012 Nov 26;190:153-60.

4. Chakraborty S, Kumbhakar P. Observation of exciton–phonon coupling and enhanced photo luminescence emission in ZnO nano twins synthesized by a simple wet chemical approach. Mater. Lett. 2013 Mar 20;100:40-43.
5. Premanathan M, Karthikeyan K, Jeyasubramanian K, Manivannan G. Selective toxicity of ZnO nano partIcles toward Gram positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomed. 2010 Oct 12;7:184-92.
6. Floresa G, Carrilloa J, Lunaa J A, Martinezb R, Sierra-Fernandezc A, Milosevicd O, Rabanale M E. Synthesis, characterization and photocatalytic properties of nanostructured ZnO particles obtained by low temperature air-assisted-USP. Adv.Powder Tech. 2014 Sep 02;25(5):1435-41.
7. Ahmad M, Ahmed E, Fezza Zafar, Khalid N R, Niaz N A, Abdul Hafeez, Ikram M, Ajmal Khan M, Zhanglian HONG. Enhanced photocatalytic activity of Ce-doped ZnO nanopowders synthesized by combustion method. J. Rare Earths. 2015 Mar 17;33(3):255–62.
8. Karimi A K A, Arefi-Oskoui S, Soltani R D C, Hanifehpour, Soltani B, Joo S W. Sono chemical synthesis of Pr-doped ZnO nanoparticles for sonocatalytic degradation of Acid Red 17. Ultrason. Sonochem. 2014 Jun 03;22(5):371-81.
9. Garima Singhal, Riju Bhavesh, Kunal Kasariya, Ashish Ranjan Sharma. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. J. Nanoparticle Research. 2011 Jul 11;13(7):2981-88.
10. Prashanth G K, Prashanth P A, Utpal Bora, Manoj Gadewar, Nagabhushana B M, Ananda S, Krish naiah G M, Sathyananda H M. In vitro antibacterial and cytotoxicity studies of ZnO nano powders prepared by combustion assisted facile green synthesis. K. Int. J. Modern Sci. 2015 Oct 20;1(2):67-77.
11. Chandrasekhar M, Nagabhushana H, Sharma H C, Sudheerkumar K H, Dhananjaya N, Sunitha D V, Shivakumara C, Nagabhushana B M. Particle size, morphology and color tunable ZnO:$Eu^{3+}$nanophosphors via plant latex mediated green combustion synthesis. J. Alloys and Compd. 2014 Jan 25;584:417–24.

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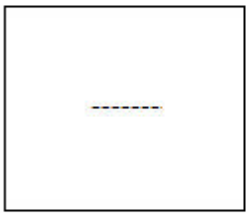


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