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Estimation of antimicrobial activity and nano-toxicity with optimized ZnO nanoparticles

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Abstract
The present study was concerned about the optimization and characterization (0.3M, 0.4M, 0.5M, 0.6M, 0.7M, 0.8M) of zinc oxide nanoparticles and their use as antibacterial agent. Chemical synthesized zinc oxide nanoparticles (precipitation) has been used. The synthesized zinc oxide nanoparticles were check by UV- VIS Spectrophotometer and characterized by Fourier transform infrared spectroscopy which has used for detection of functional group present in synthesized zinc oxide nanoparticles. The bacterial species used for examining of the antimicrobial activity of zinc oxide nanoparticles *are Enterobacter aerogenes, Aeromonas hydrophila, Bacillus amyloliquefaciens, Micrococcus luteus.* Similarly, the antibacterial activity of standard antibiotics (Gentamicin, Ciprofloxacin and Ampicillin) was tested against pathogens. Among all, 0.3M showed enhanced activity against all bacteria. Tox trak test was also analyzed on optimized ZnO nanoparticles to check the toxicity against human gut *microflora* microorganism and resulted that 0.3M is less toxic to human gut.

Keywords: optimization, characterization, antimicrobial activity, toxicity, zinc oxide nanoparticles

1. Introduction
Nanotechnology research playing crucial role in modern science. This technology providing novel applications that range from innovative fabric compounds, food processing, and agricultural production to sophisticated medicinal techniques such as diagnostic technique, drug delievery, sunscream, disinfectant, biosensors, Nano medicine, bio nanotechnology and many more [1-5]. It is considered as the synthesis, characterization, and exploration of materials in the nanometer region (1–100 nm) [6]. Among various semiconducting materials, Zinc oxide, with its unique physical and chemical properties, such as high chemical stability, high electrochemical coupling coefficient, broad range of radiation absorption and high photostability, is a multifunctional material [7]. In materials science, zinc oxide (white powder that is insoluble in water) is classified as a semiconductor in group II-VI since Zn and O are classified into groups two and six in the periodic table, respectively, whose covalence is on the boundary between ionic and covalent semiconductors. Zinc oxide (ZnO) is a distinctive electronic and photonic wurtzite n-type semiconductor with a broad energy band (3.37 eV), high bond energy (60 meV) and high thermal and mechanical stability at room temperature make it attractive for potential use in electronics and laser technology [8-9]. Among the metal NPs such as TiO2, silver, gold, iron etc. zinc oxide nanoparticles has been extensively applied due to the antimicrobial and antitumor activities. There are potentially numerous promising applications of ZnO nanoparticles in veterinary sciences due to their wound healing, antibacterial, antineoplastic and antigenic properties. There are various approaches for the preparation of ZnO nanopowders namely, sol-gel, microemulsion, thermal decomposition of organic precursor, spray pyrolysis, electrodeposition, ultrasonic, microwave-assisted techniques, chemical vapor deposition, and hydrothermal and precipitation method [10]. Most of these techniques are not extensively used on a large scale, but chemical synthesis has been widely used due to its simplicity and is less expensive. In this project work, ZnO nanoparticles were synthesized by wet chemical method with optimization and characterized by using UV-VIS spectroscopy (scanning range 350-700nm), FTIR analysis. The analysis of antibacterial activity and toxic effect of synthesized ZnO nanoparticles against human gut *microflora* was evaluated and the study concluded that most effective molarity is 0.3M of ZnO nanoparticles against bacteria.

2. Materials and Methods
2.1 Chemicals
The chemicals used for synthesis of zinc oxide nanoparticles are: Zinc nitrate, soluble starch and sodium hydroxide. The solution of different molarity of zinc nitrate (0.3, 0.4, 0.5, 0.6, 0.7, 0.8 M) were prepared.

2.2 Collection of microbes
Micrococcus luteus, *Aeromonas hydrophila*, *Enterobacter aerogens* and *Bacillus amyloliquefaciens* collected from the microbiology laboratory in Biotechnology, Department of Meerut Institute of Engineering and Technology, Meerut. Three antibiotics (ampicillin, gentamicin, ciprofloxacin) were used.

2.3 Chemical synthesis of zinc oxide nanoparticles (CH-ZnONPs)
The ZnO nanoparticles were prepared by wet chemical method using zinc nitrate and sodium hydroxides as precursor and soluble starch was used as the stabilizing agent. Soluble

starch (0.5%) was dissolved in 500 ml of distilled water. Zinc nitrate (0.1 M), was added in the solution. The solution was kept under constant stirring at room temperature using magnetic stirrer. 300ml (0.2 M) of sodium hydroxide solution was added under constant stirring via pipetting. The reaction was allowed to proceed for 2 hrs after complete addition of sodium hydroxide. The solution is allowed to settle for overnight and then supernatant solution was discarded. The remaining solution was centrifuged for 10 min at 4 ℃ and the supernatant was discarded. The pellet was washed using distilled water and ethanol. After washing, the nanoparticles were dried at 80°C for overnight using oven. During drying, complete conversion of $Zn(OH)_2$ into ZnO takes place. Same steps will be repeated for different molarity of zinc nitrate.

2.4 Characterization of CH-ZnONPs

The ZnO nanoparticle presence were analyzed by using JASCO-V-530 UV-VIS spectrophotometer. The absorption range for the samples is 350-700 nm. FTIR analysis was also done to characterize the ZnO nanoparticles using SHIMADZU FTIR instrument. The FTIR analysis help in characterization of chemical component and peak assessment.

2.5 Antibacterial Activity

Antibacterial activities of ZnONPs were investigated against four bacteria. Antibacterial activities were determined via disc diffusion method. Solidified plate were required for antimicrobial activity. The plates containing the test organism with ZnONPs and were incubated at 37℃ for 24 hrs. The plates were examined for evidence of zones of inhibition, which appear as a clear area. The diameter of such zones of inhibition was measured using a meter ruler in centimeter.

2.6 Toxicity Analysis

The test tubes of broths for 48 h containing *B.subtilis and E coli* were used for Toxtrak. Test for determining toxicity in chemically synthesized zinc nanoparticles on human gut *microflora*. Two test tube was marked as control and other two test tubes were incubated with 3 ml chemically synthesized zinc nanoparticles, respectively for 4 h. The concentration of the zinc nanoparticles ranges from 25 µg/ml in both solutions. Resazurin dye is added in the volume of 40µl per test tube and incubated from 0 to 4 h. The absorption was recorded just after adding the dye (0 h) in all the all test tube and then the absorption is recorded after every 1 h intervals for 4h. The absorbance test is carried out at a wavelength of 603 nm, which is specific for the blue color.

The percentage inhibition (PI) is expressed equation is as follow:

$PI= [1-(\Delta As / \Delta Ac)] \times 100$

Here, ΔAs and ΔAc represent the changes/differences (decrease) in absorbance for the sample and the control, respectively.

Δ is the initial-final value [11].

3. Results and Discussion

In this study, optimization of zinc oxide nanoparticles, comparative analysis of antibacterial activity of chemically synthesized zinc oxide nanoparticles (0.3-0.8M) and commercially available antibiotics such as gentamicin, ciprofloxacin and ampicillin was done. Toxicity analysis was also investigated.

3.1 Chemical synthesis of ZnO nanoparticles

Fig 1: Chemical synthesis of zinc oxide nanoparticles by wet chemical method.

3.2 Characterization of CH-ZnONPs

3.2.1 UV-Vis spectra for chemical synthesis of ZnO nanoparticles

UV Vis spectra for chemically synthesized ZnO nanoparticles have spectrum range between 390-420nm. Here, the data of UV VIS graphs confined the synthesis of ZnO nanoparticles. All the peak was approximately at the range of 400nm.

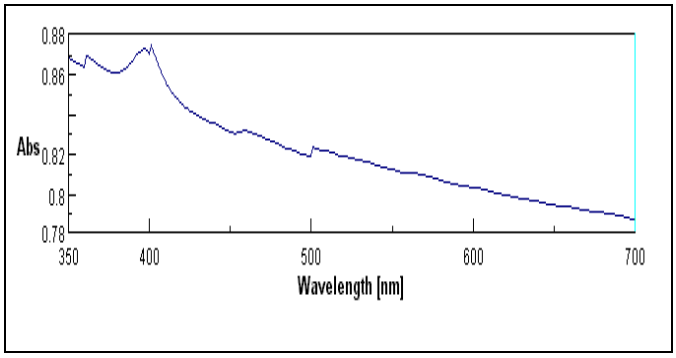


Fig 2: UV Spectroscopy of Chemically synthesized 0.3M of ZnO nanoparticle

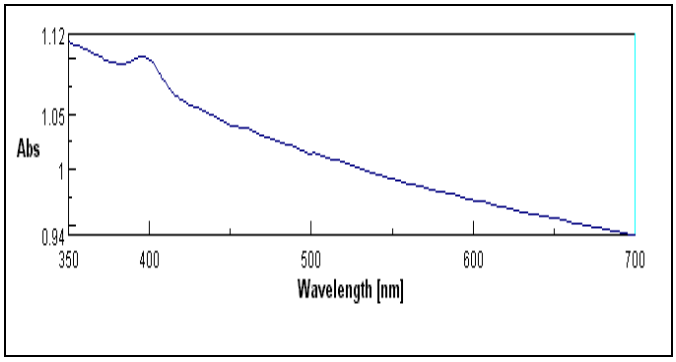


Fig 3: UV Spectroscopy of Chemically synthesized 0.4M of ZnO nanoparticle

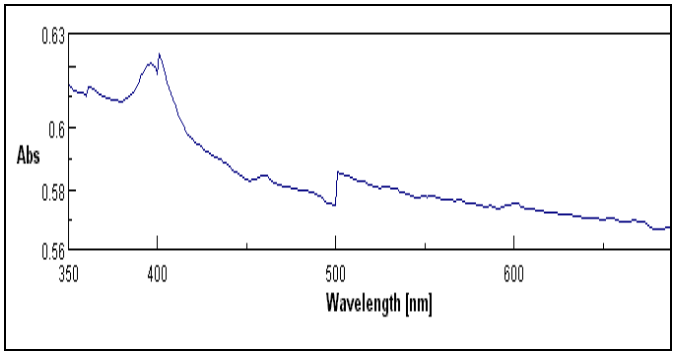


Fig 4: UV Spectroscopy of Chemically synthesized 0.5M of ZnO nanoparticle

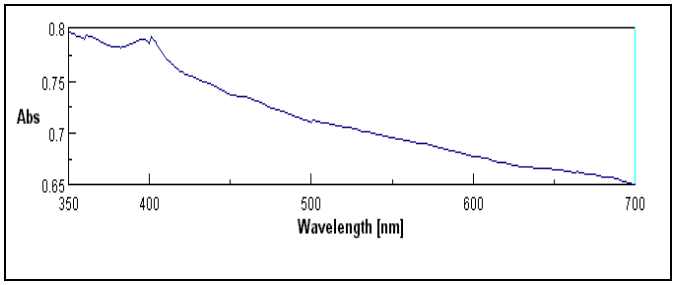


Fig 5: UV Spectroscopy of Chemically synthesized 0.6M of ZnO nanoparticle

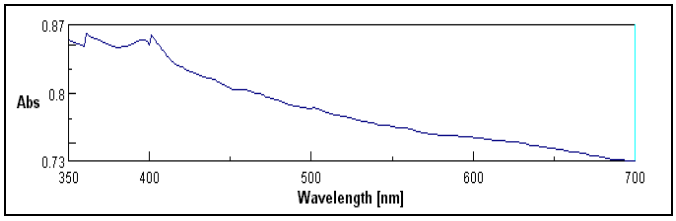


Fig 6: UV Spectroscopy of Chemically synthesized 0.7M of ZnO nanoparticle

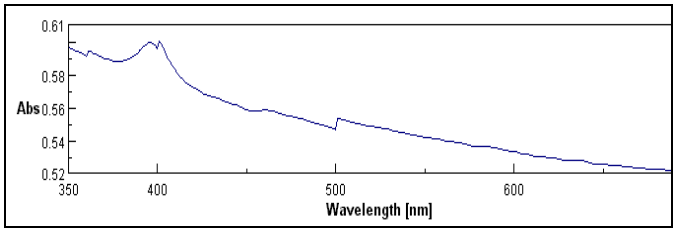


Fig 7: UV Spectroscopy of Chemically synthesized 0.8M of ZnO nanoparticle

3.2.2 FTIR analysis of ZnO nanoparticles

The spectrum of interference pattern by FTIR analysis is in the wavelength of 500-4000 cm^{-1}.

Figure (8, 9, 10, 11, 12 and 13) clearly, shows that the peak spectrum ranges nearer to 1400 cm^{-1}.

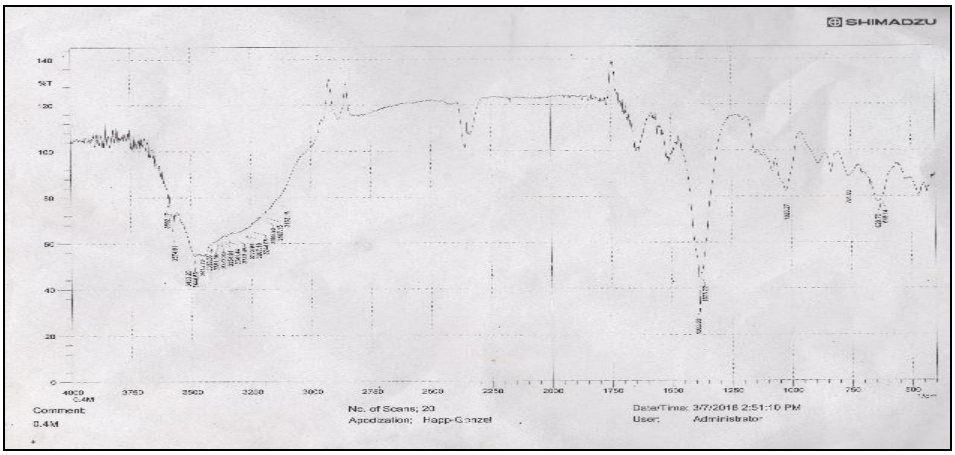


Fig 8: FTIR analysis of 0.3M ZnO nanoparticles

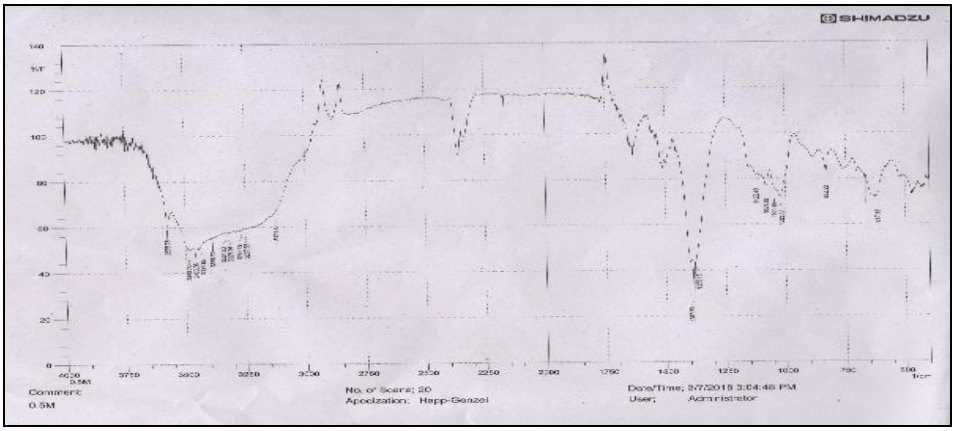


Fig 9: FTIR analysis of 0.4M ZnO nanoparticles

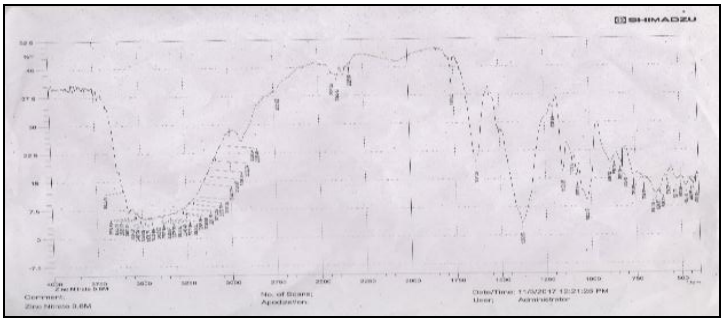


Fig 10: FTIR analysis of 0.5M ZnO nanoparticles

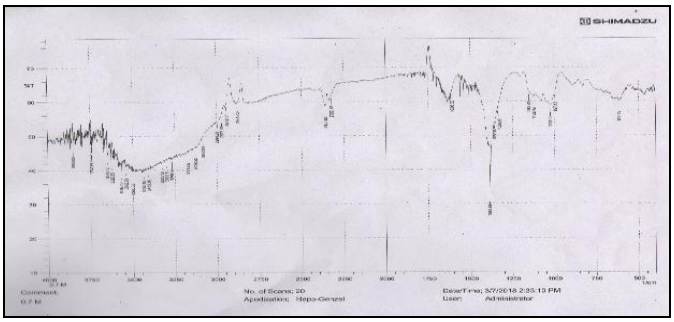


Fig 11: FTIR analysis of 0.6M ZnO nanoparticles

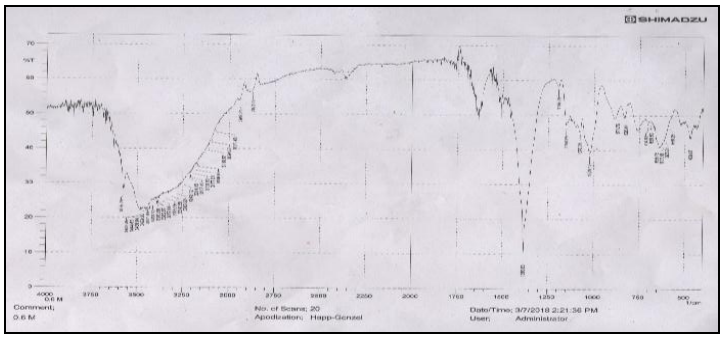


Fig 12: FTIR analysis of 0.7M ZnO nanoparticles

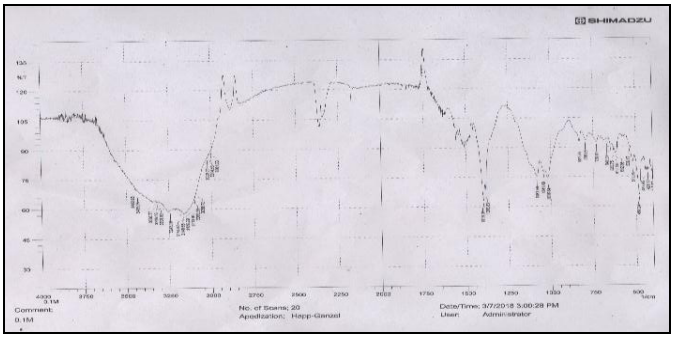
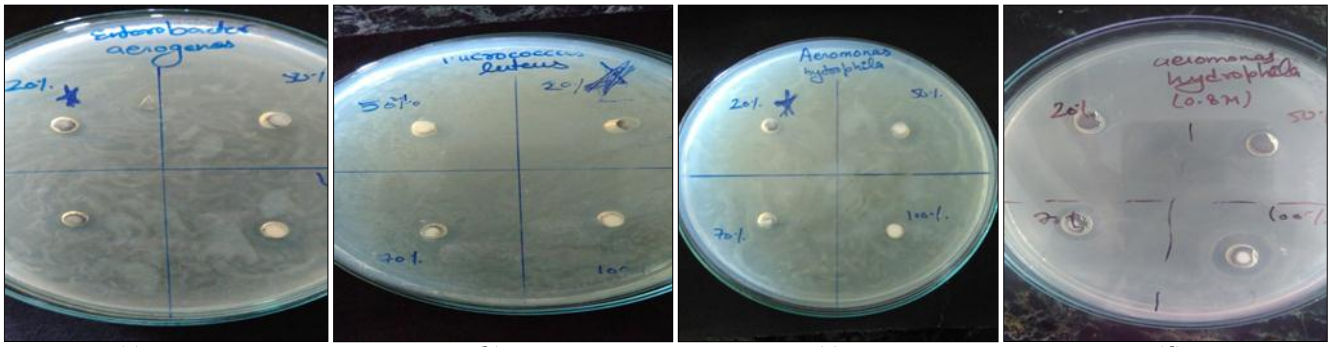


Fig 13: FTIR analysis of 0.8M ZnO nanoparticles

3.3. Antibacterial test for zinc oxide nanoparticles and antibiotics against bacteria

3.3.1. Antibacterial activity of CH-ZnONPs

ZnO nanoparticles bactericidal effect was evaluated against four bacteria *Micrococcus luteus*, *Aeromonas hydrophila*, *Enterobacter aerogens* and *Bacillus amyloliquefaciens.* The effect of different molarity concentration were performed against the bacteria and graph -14 shows the decreasing order of zone of inhibition against the bacteria. Figure-15, also determining the decreasing effect of ZnO nanoparticles against the group of bacteria.



(a) (b) (c) (d)

(e) (f) (g) (h)

Fig 14: Here, (a, b, c, h) belongs to ZnO nanoparticles of 0.3M which have the maximum zone of inhibition and (d, e, f, g) belongs to 0.8M which have minimum zone of inhibitio

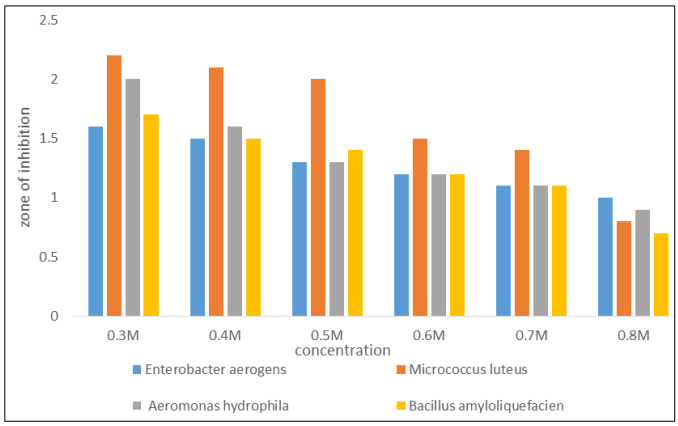
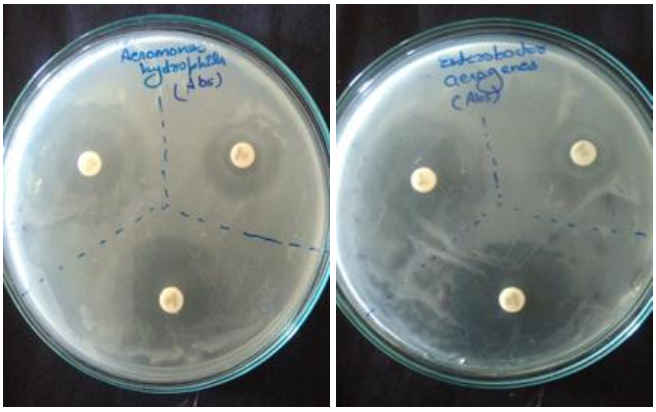


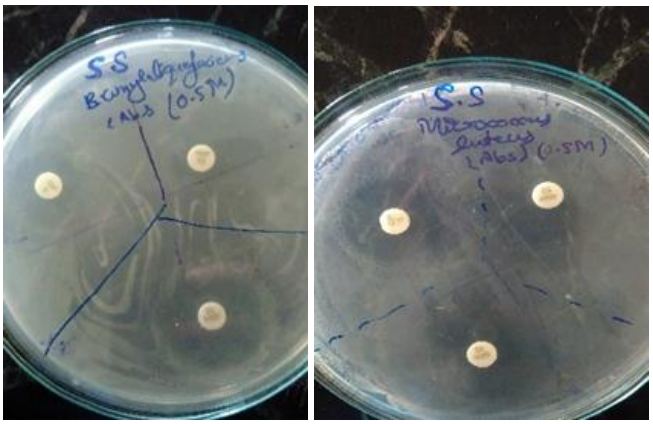
Fig 15: Showing relationship between pathogenic bacteria and zone of inhibition (cm) of chemically synthesized CH-ZnONPs

3.3.2 Antibacterial activity of antibiotics against bacteria

The antibacterial activity were shown by antibiotic against bacteria as represented in the figures and graphs. Ampicillin shows the least activity against the all four bacteria and Gentamycin have high zone of inhibition.



(a) (b)



(c) (d)

Fig 16: (a, b, c, d) represented the zone of inhibition using antibiotics against the bacteria.

+

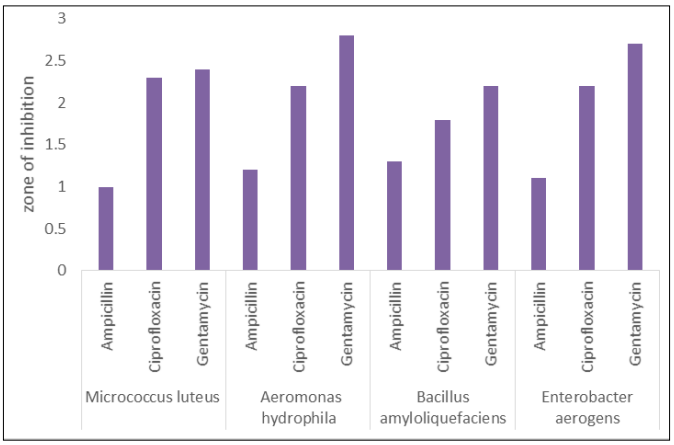


Fig 17: Showing relationship between pathogenic bacteria and zone of inhibition (cm) of for different antibiotics

3.4 Tor trak test

Toxicity was analyzed on the human gut *micro flora* especially *E coli and B. subtilies*. It was observed that ZnO nanoparticles of 0.3M have least toxicity against human gut *microflora*.

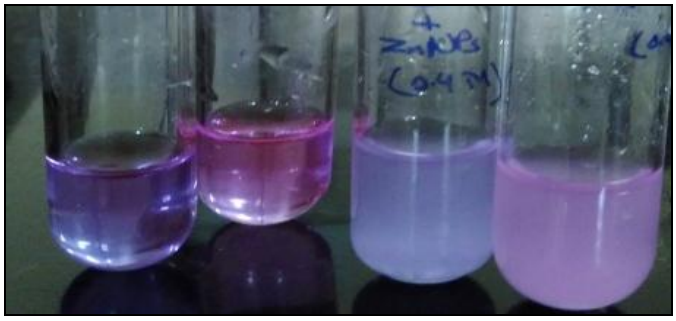


Fig 18: Determining the toxicity of ZnO nanoparticles against human gut *microflora*.

Table 1: representing the final result after the calculation of ΔAs and ΔAc using the PI= [1-(ΔAs / ΔAc)] ×100

	***Ecoli* (concentration in %)**	***B.subtiles* (concentration in %)**
0.3	7.7	74.36
0.4	13	74.75
0.5	48	85.49
0.6	49.80	85.79
0.7	55	87
0.8	60	87.89

4. Conclusion

Zinc Oxide nanoparticles were synthesized and optimization by using starch, sodium hydroxide and zinc nitrate. The chemically synthesized zinc oxide nanoparticles were characterized using UV spectroscopy and FTIR. Antibacterial activity against four bacteria and antibiotic were checked via disc diffusion method. From the above study it was concluded that concentration of 0.3M showed the best result and 0.8M

showed the minimum result in the case of antimicrobial activity. It was also found that 0.3M have minimum effect on human gut *microflora.* Salient feature of this study can used to investigate the antifungal and anticancerous activity and skin related diseases.

5. Acknowledgement

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6. References

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