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In-vitro evaluation of Antibacterial Activities of ZnO and NiO Nanoparticles against Gram-Positive and Gram -Negative Bacteria

Abasaheb R. Padol[a], Madhukar E. Navgire[b], Mahananda A. Raut[c]

[a]Dr. Rafiq Zakaria College for Womens, Aurangabad, India, 431003

[b]Jijamata College of Science and Arts, BhendeBk, Ahmednagar, India

[c]Pratisthan Mahavidyalaya, Paithan, Aurangabad, India

Email of Corresponding author: mahanandaraut@yahoo.in

Abstract:

Nanostructured Nickel oxide (NiO) and Zinc oxide (ZnO) nanoparticles were synthesized by using co-precipitation method. The calcination of material was done at various temperatures. The crystal structure as well as morphology of the synthesized materials was studied by XRD, FTIR UV–Visible spectroscopy and SEM analysis. These materials was then successfully tested for antibacterial activities against Gram-positive and Gram -negative bacteria. On the basis of result obtained it was recorded that, the maximum zones of inhibition was noticed in ZnO Nanoparticle against *Escherichia coli* 16.6mm followed by *Bacillus substilis* 13.3mm. In case of NiO it was recorded that maximum zones of inhibition was recorded against *Escherichia coli* 13.0 mm followed by *Bacillus substilis* 11.3 mm overall it is concluded that, maximum zones of inhibition was recorded in ZnO and NiO nanoparticles against *Escherichia coli* grams Positive bacteria was maximum followed by *Bacillus substilis* grams negative bacteria.

Keywords: Zinc Oxides; Nickel Oxides; Nanoparticles; Antibacterial Activities;

INTRODUCTION:

In Recent years Nano materials have considerable attractions due to their astonishing characteristics properties such as adequately surface area and higher reactivity. The nanomaterials are prepared over a wide range of shapes and sizes and become utilized in production of different industrial and medicinal productions [1][2]. Metal oxide nanomaterial due to its electronic, thermal, biological, catalytic and photocatalytic, optical properties in comparison with their bulk concentrate [3][4]. Over recent year due to applications such as, catalyst [5][6][7] gas sensor [8], electro chromic film, fuel cell, magnetic materials [9]. The main properties taken into account are Antimicrobial and antibacterial activities.

Zinc Oxide NPS is naturally known to have strong inhibition activity towards microbes [10]for this reason ZnO NPS are extensively used for Biological labeling, Sensor, Drug dosage treatment, Genetherapy and Nano medicine [11]. Nanosized ZnO exhibits varying morphological characters and shows significant antibacterial activity over various wide ranges of bacterial species. The ZnO NPS currently founded as antibacterial agent in nano level concentration, it exhibits significant antibacterial activities when particle size is reduced to few nanometer. Nanosize ZnO reacts with the surface of Bacterial region or inner core shell of bacteria where it introduced into cell and exhibits specific bactericides reaction inter-linkage between these distinctive material and bacteria shows most lethal which have been utilized for antimicrobial properties studies such as in food manufacturing and food processing industry. Various skin diseases (infection diseases) due to harmful bacteria are serious health issues that it has drawn considerable attention towards awareness to public

health in overall the world, as living organisms health which enhance to economic and social health problems.it is a nanomaterial with a potential application as semiconducting material. The nanoparticals of nickel oxide have low toxic effect and compatible in biomedical field. The nickel oxides due to its considerable characteristics properties it's mostly applied in different scientific technologies. As nickel oxide shows stable oxidation number they have corrosion inhibitor, temperature resistance catalytic activity. Different methods have been applied to preparation and synthesis of nickel metal in nano scale formulation such as co-precipitation, micro emulsion, sol-gel chemistry, chemical methods. To obtain consistent and controlled preparations of stable and well defined metal nanocompounds with small particle sizes is very important its utilization in medicine technologies. Its small size and good surface activity to volume ratio it is mainly applied in biomedical treatment [12][13][14] metal oxide nanoparticles were applied as photocatalytic activity, environmental studies such as sewage and waste material treatment [14][15]. The nickel oxide NPS have stronger bacterial activity against gram positive and gram negative bacteria.

In this work, we synthesize ZnO, NiO nanoparticles. Simple co-precipitation method was used to prepared pure material due to its low cost. The characterization of the synthesized nanocomposites has been done using various analytical techniques. The antibacterial activities of these synthesized nanoparticles were tested against gram-positive and gram-negative bacterial strain.

Experimental:

Chemicals and reagents:

All chemicals nickel (II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), zinc (III) sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) are purchased from LOBA Chemicals. The Cetyl Trimethyl Ammonium Bromide ($C_{19}H_{42}N.Br$), Sodium Hydroxide (NaOH), Poly vinyl alcohol $(C_2H_4O)_n$, ammonia solution (NH_4OH), were bought from SD fine Chemicals. All of the chemicals were of analytical quality and were employed without further purification, with solutions prepared by dissolving in double-distilled water (DDW).

Synthesis of NiO nanoparticles:

The synthesis of nickel oxide nanoparticles were synthesized by taking nickel (II) chloride hexahydrate (26.4 g), dissolving in 100 mL of DDW. The 1:1 ammonia solution was dropwise added into the above solution at room temperature under constant stirring. The mixture was kept at the room temperature for 24 hrs for aging. After the reaction was complete, the resulting light-green solution was filtered using Whatman filter paper No 41. It was then washed with DDW and ethanol for 8 to 10 times until removes unreacted materials. The precipitate was then dried at 100 °C for 4hrs in oven. Finally material was calcined at various temperature and time such as 400 °C for 1 hrs, 500 °C for 2 hrs, 500 °C for 4 hrs, 500 °C for 5 hrs, and 600 °C for 3 hrs which gives NiO nanoparticles further named as 4N1, 5N2, 5N4, 5N5 and 6N3, respectively.

Synthesis of ZnO nanoparticles:

The synthesis of Zinc oxide nanoparticles were synthesized by taking zinc (II) sulphate heptahydrate (26.4 g), dissolving in250 mL of DDW. The 1:1 ammonia solution was dropwise added into the above solution at room temperature under constant stirring. The mixture was kept at the room temperature for 12 hrs for aging. The precipitate obtained was then filtered using Whatman filter paper No 41. It was then washed with DDW and ethanol for 8 to 10 times until removes unreacted materials. The precipitate was then dried at 100°C for 4hrs in oven. Finally material was calcined at 500 °C for 4 hrs which gives ZnO nanoparticles.

Characterization techniques:

By using various analytical techniques, the synthesized materials were characterized to find out the some important information such as crystalline size, surface structure, morphology, particle size etc.The X-Ray Diffraction (XRD) analysis of the synthesized nanocomposites is recorded on a powder X-ray diffractometer (Rigacu MiniFlex 600) using the radiation source Cu Kα with λ = 0.154 nm with scanning range between angle

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2θ (°) = 20 to 80. Fourier Transform Infra-red Spectroscopy (FT-IR) of prepared nanocomposite was analyzed by using Perkin Elmer (Spectrum 2) using a Diamond ATR in the region between 4000 to 450 cm^{-1}. The UV-Visible Diffused Reflectance Spectra UV-DRS) of the nanocomposites were measured using a Lab India UV-Vis spectrophotometer (UV 3092) in the wavelength range of 200 to 800 nm. Surface morphology and elemental analysis of the samples was carried out using scanning electron microscopy with electron dispersion spectroscopy (SEM-EDS) characterization conducted using a JEOL-JED 2300 (*LA*) instrument.

Characterization of the Synthesized Nanomaterial:

X-Ray Diffraction Analysis (XRD):

Figure 1 shows XRD pattern of pure NiO. It shows highly intense peaks at 2θ (°) at 37.19, 43.22, 62.81, 75.34 and 79.32 corresponding for the planes (111), (200), (220), (311), (222) indicating cubic crystal structure. It was noted that all the XRD peaks are identified as NiO peaks from the JCPDS card no. 78-0643 with lattice parameter a=b=c= 4.176Å [16]. The strong and sharp peaks suggest that the prepared material has highly crystalline cubic in nature.

The average crystal size (D), of the samples can be calculated by following Scherrer equation:

$$D= k\lambda/ (\beta\cos\theta)$$

Where β = FWHM (Full Width at Half Maximum = 0.240), λ= wavelength (0.154 nm), K= 0.89 (0.9), θ = diffraction angle. For NiO, (200) diffraction peak of height intensity at 2θ =43.22 ° was taken for the particle size determination. The average crystal size (D) was calculated to be 35.60nm.

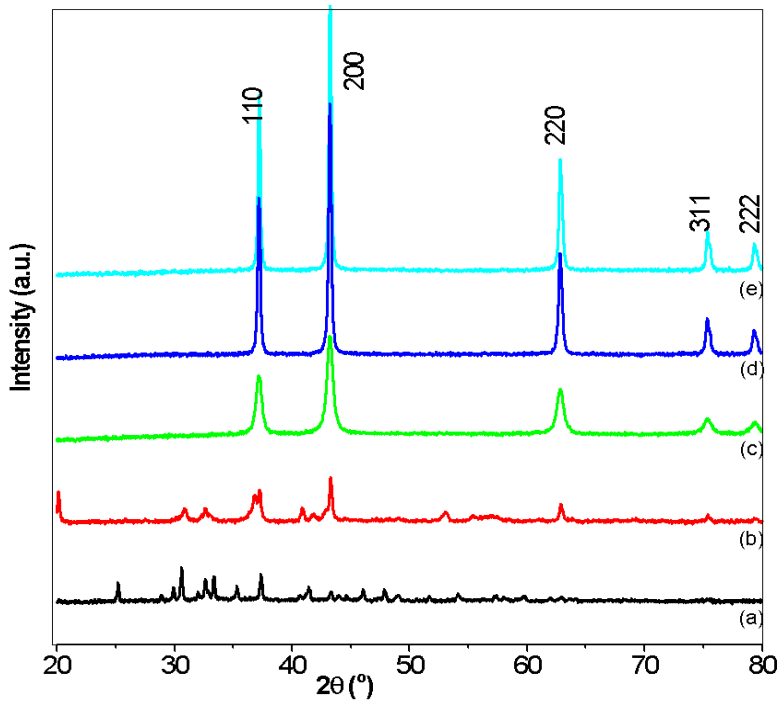

Figure 1: XRD spectrum of NiO nanoparticles calcined at (a) 4N1, (b) 5N2, (c) 5N4, (d) 5N5 and (e) 6N3

Fourier Transform Infra-Red Spectroscopy (FT-IR):

Figure 2(a) show the FT-IR spectrums of NiO nanoparticle over the range of 4000-400 cm^{-1}. A sharp band appears at 558 cm^{-1} for bending vibration of Ni-O-Ni bond. The band at 760 cm^{-1} is due to stretching vibration of Ni-O bonding. The band around 1610 cm^{-1} is attributed due to bending vibrations of H-O-H bond. Similarly, the broad peak between 3500 - 3400 cm^{-1} is due to O-H stretching vibrational modes of the absorbed

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water on metal oxide surface. Almost same type of spectrum is also observed for ZnO shown in Figure (2b). The peak at around 574 $cm^{-1}$for bending vibration of Zn-O-Zn bond. The band at 730 cm^{-1} is due to stretching vibration of Zn-O bonding. The band around 1620 cm-1 is attributed due to bending vibrations of H-O-H bond. The broad peak between 3550 - 3370 cm^{-1} is due to O-H stretching vibrational modes of the absorbed water.

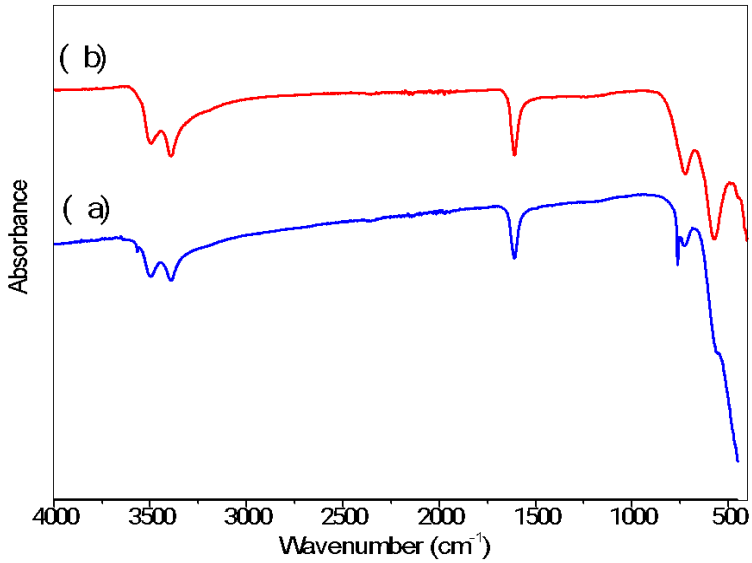

Figure 2: FT-IR Spectra of (a) NiO and (b) ZnO Nanoparticle

Scanning Electron Microscopy (SEM) Studies:

The surface morphology of NiO and ZnO nanoparticles were tested by SEM analysis to know the dispersion of the active species on the support. The Figure.3 (a -c) shows the SEM images for NiO with the different resolution, which shows the nanocrystalline structure of composite material. It is clearly seen the increasing porosity and improvement in the morphology of material as compared to ZnO.

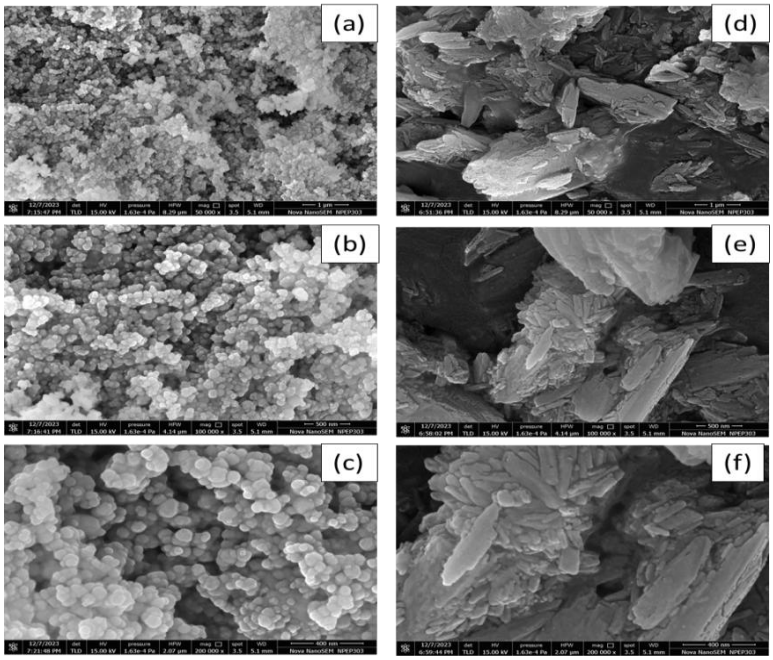

Figure 3: SEM images of NiO nanoparticles (a,b,c) N2P and (d,e,f) N1P

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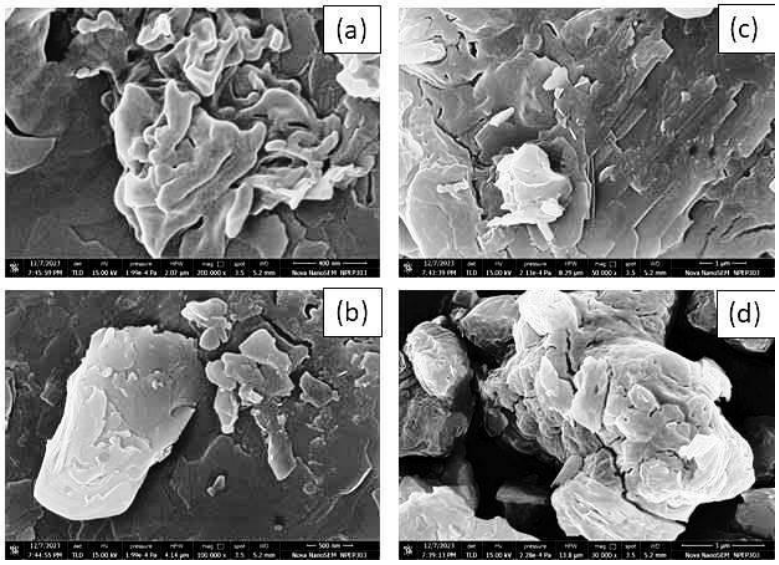

Figure 4: SEM images of ZnO nanoparticles

UV Visible Spectrophotometer:

The absorbance of the nanomaterial was studied with the help of the AU-2701 UV-VIS Spectrophotometer in the range of 800-200 nm wavelength. The optical absorption behavior and band gap energy of synthesized material were studied by means of UV-Vis spectroscopy.

The pure NiO absorbs light of wavelength that 370nm and has band gap around 3.35 eV for 400 °C, For 500 o C greatest wavelength at 300 nm and it has band gap around4.1 eV and For 600 o C greatest wavelength at 730 nm and has band gap around 1.6 eV. Pure NiO shows red shift for light absorption and can absorbs light beyond 44 nm because of its lower band gap. The pure ZnO absorbs light of wavelength at 380 nm and has band gap around 3.2 eV for 400 °C, For 500 oC greatest wavelength at 340 nm and has band gap around 3.6 eV and For 600 oC greatest wavelength at 310 nm and has band gap around 4.0eV. Pure ZnO shows red shift for light absorption and can absorbs light beyond 44 nm because of its lower band gap.

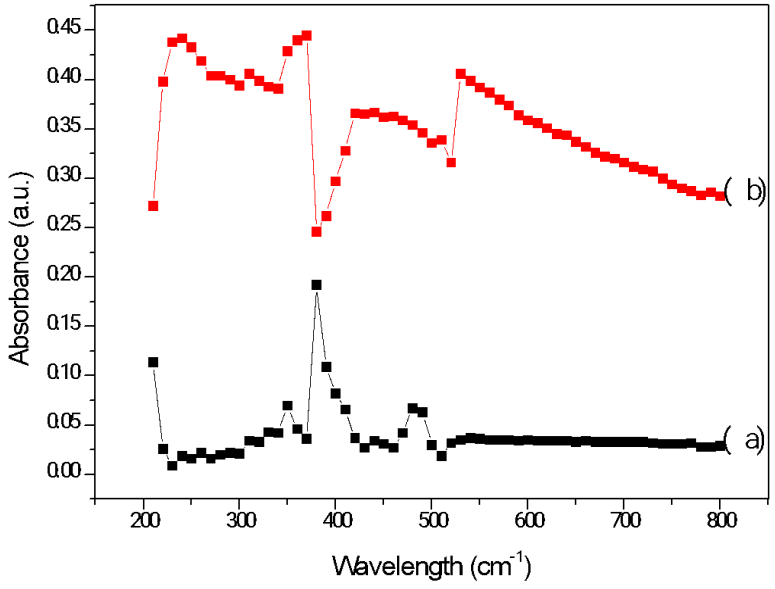

Figure 5: UV-spectra of (a) NiO and (b) ZnO nanoparticle

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Antibacterial activity (Kirby-Bauer Method):

Antibacterial activity of the metal oxide nanoparticles was determined using the agar well diffusion assay method [17]. Approximately, 25 ml of molten and cooled nutrient agar media were poured in the sterilized petri-dishes. The plates were left over night at room temperature to check for any contamination to appear. The bacterial test organism S. aureus and E. coli were grown in nutrient broth for 24 h. A 200µg/ml nutrient broth culture of each bacterial organism was used to prepare bacterial lawns. Agar wells were prepared with the help of a sterilized stainless steel cork borer. The wells in each plate were loaded with 200µg/ml of different concentration of metal oxide nanoparticles. Antibiotic gentamycin and streptomycin used as positive control for each bacterium to compare the inhibition of growth of bacteria with metal oxide nanoparticles. The plates containing the bacteria and solutions of metal oxide nanoparticles were incubated at 37 °C. All the tests were repeated in quadripulates. The antibacterial activity was taken on the basis of diameter of Zone of Inhibition which was measured at cross angles after 24 hours of incubation and the mean of three readings are shown in Table 1.The inhibition of the bacterial growth on the agar plates by using different concentration of metal oxide nanoparticles. It is well known that if the diameter of antibacterial circle of one sample is larger than 9 mm, it means that the sample has better antibacterial activity, however, if the diameter of antibacterial circle is equal to or less than 9 mm, it means that the sample has poorer antibacterial activity. From the results it can be seen that all samples have better antibacterial activity because their antibacterial circle diameter is much larger than 9 mm as well as the DMSO used as control.

In addition, a 200µg/ml concentration of nano-particles is better as compared to 50 ll for inhibiting growth of bacterial test organism. The metal oxide nanoparticles proved to be very active on the tested Gram-positive strains, this differential sensitivity of Gram-negative and Gram-positive bacteria toward nanoparticles could be explained by the fact that the liquid medium is probably favoring the close interaction between the suspended nanoparticles and the Gram-positive microbial cells, which could be better attach and anchor to the surface of the microbial cell, causing structural changes and damages leading to cell death [18]. The Gram-positive bacteria have a relatively thick wall composed of many layers of peptide glycan polymer, and only one membrane (plasma membrane).The Gram-negative bacteria have only a thin layer of peptidoglycan and a more complex cell wall with two cell membranes, an outer membrane, and a plasma membrane. The addition of the outer membrane of the Gram-negative bacteria cells influences the permeability of many molecules. Under certain conditions, the Gram-negative bacteria are more resistant to many chemical reagents than Gram-positive cells. In addition, the cell walls of Gram-negative bacteria are more prone to mechanical breakage because of the low amount of peptidoglycan [19].

Result Table: zone of inhibition of bacteria on metal oxides

Sr. No.	**Microorganism**	**Zone of inhibition in (mm)**	
		Ni-O	**Zn-O**
1	*Escherichia coli*	13.0	16.6
2	*Bacillus subtilis*	11.3	13.3

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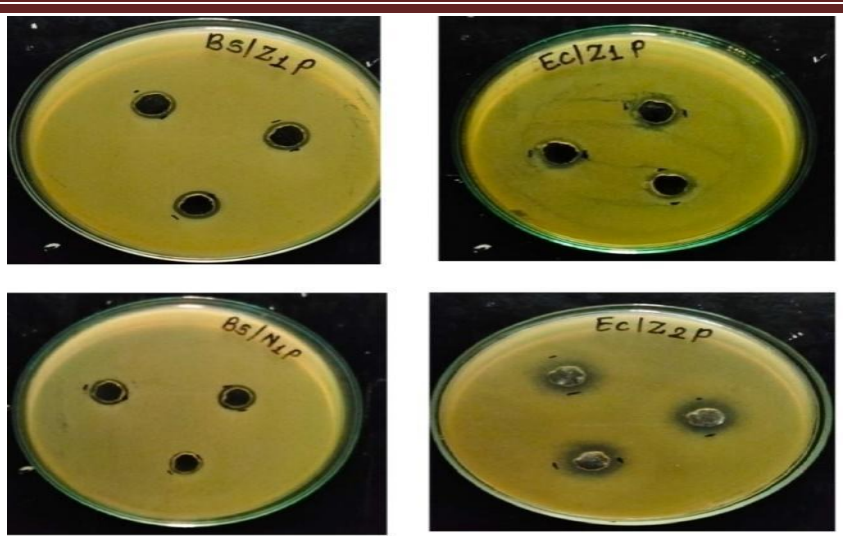

Figure 6: Antimicrobial activities by B. Subtilis and E. coli by NiO and ZnO

Conclusion:

We have successfully synthesized NiO and ZnO nanoparticles using a co-precipitation method. XRD spectra confirmed the formation of single phase NiO and ZnO nanoparticles. Crystallite size was found to increase with the increase in calcined temperature. Minimum crystallite size of 20 ± 1.24 nm was observed in the case of NiO and ZnO nanoparticles calcined at 500 °C. SEM results corroborate well with XRD results. FTIR spectra also validated the purity of NiO and ZnO nanoparticles. Antibacterial activity experiments performed on various microorganisms.

On the basis of result obtained it was recorded that, the maximum zones of inhibition was noticed in ZnO Nanoparticle against *Escherichia coli* 16.6mm followed by *Bacillus substilis* 13.3mm.In case of NiO it was recorded that maximum zones of inhibition was recorded against *Escherichia coli* 13.0 mm followed by *Bacillus substilis* 11.3 mm overall it is concluded that , maximum zones of inhibition was recorded in ZnO and NiO nanoparticles against *Escherichia coli* grams Positive bacteria was maximum followed by *Bacillus substilis* grams negative bacteria.

The higher effectiveness of NiO and ZnO nanoparticles annealed at 500 °C against bacterial growth due to smaller particle size of this sample compared to other samples. The zone of inhibition for all the microorganisms reached a maximum point using NiO and ZnO nanoparticles annealed at 500 °C. Moreover, minimum inhibitory concentration and minimum bactericidal concentration of NiO and ZnO nanoparticles annealed at 500 °C was lowest for all the bacterial strains.

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Ethical approval

Not applicable

Conflicts of interest/Competing interest:

There are no conflicts/competing interest to declare.

Authors Contributions:

First author (ARP) performed all laboratory and experimental work. The authors MEN help in the analysis and processing of characterization data, preparation of Manuscript. The MAR (corresponding author) was supervised during all process. Thus these all authors have equal contribution in this manuscript.

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Not applicable

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Not applicable

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