

Antibacterial Activity of ZnO Nanoparticle Coated Textiles Against *Staphylococcus Aureus*

M. Anish Sharmila, R.I. Sathya

Department of Home Science, Textiles and Clothing
Gandhigram Rural Institute- Deemed University
Dindigul, Tamil Nadu



Abstract — *Staphylococcus aureus* (S.aureus) is the most common skin affected bacteria. Staph skin infections are common, particularly among children, teenagers, young adults and even healthy people, but more serious infections tend to affect those who have a weakened immune system because of an underlying medical condition or a side effect of treatment, skin, deep wound or major burn such as chemotherapy. The textiles used for the hospital purpose are contacted with bad bacteria and cause the illnesses and death. So the effective antimicrobial finish has been essential for the safeguard of human beings from harmful microorganism to prevent them from cross infections. Zinc oxide nanoparticles (ZnO-NPs) exhibit attractive antibacterial properties. This paper focuses on the antimicrobial activity on 100% Cotton 40s count fabric with natural and synthetic dye used as hospital usable colors, such as Green and blue. ZnO-NPs was synthesized by a wet chemical process with (1:1) concentration of the NaOH solution (pH=12) under optimized temperature 60, 70, 80, 90° C. The ZnO-NPs characterization was analyzed by XRD, SEM/EDAX, FTIR, UV absorption. Higher zone inhibition (ZE4) was selected for further textile coating process and evaluated the activity on against staphylococcus aureus. ZE4 coated green and Blue Vat fabrics have 20 mm, 22 mm Zone, respectively, which are larger than 18 mm, 19 mm for ZE4 coated Eclipta and Indigo fabrics.

Keywords— Nano Zinc Oxide; *Staphylococcus aureus*; Antibacterial activity; Cotton Fabric.

I. INTRODUCTION

A bacterial disease that causes serious health problems has drawn the public attention on global health threats. Increasing outbreaks and infections of pathogenic strains, bacterial antibiotic resistance, emergence of new bacterial mutations, lack of suitable vaccine in underdeveloped countries and hospital-associated infections, are affecting to human, chiefly in children. [1] Staphylococcal infections (often shortened to staph) mainly affect the skin. *Staphylococcus* and *staphylococcus aureus* are the two types usually cause mild infections, but these can be more serious in people who have cancer. [2] The potential sites for infection of S.aureus are the anterior nares (the nostrils) [3] respiratory tract, open wounds, intravenous catheters and the urinary tract. [4] The skin and mucous membranes are usually an effective barrier against infection. However, if these barriers are breached (e.g. Skin damage due to trauma or mucosal damage due to viral infection) S. aureus may

gain access to underlying tissues or the bloodstream and cause infection [5].

Consumer's attitude towards hygiene and active lifestyle has created a rapidly increasing market for a wide range of functional textiles, which in turn has stimulated intensive research and developments. [6] Microbial invasion in textile technology has been much demanded in the world. Among these, development of antibacterial textiles is extremely essential because clothes are in direct contact with the human body. [7], [8] Textile materials are used for several medical purposes. Medical textiles are utilized in forms of fiber, yarn, woven fabric and non woven. [9] Cotton fabrics offer an ideal environment for microbial growth. Increasing global competition in textiles has created many challenges for textiles researchers and industries. Therefore, the textile finishes with antibacterial finish is highly demanded in global. So it is necessary for human to wear antibacterial finished textile products for healthy life style. A wide range

of commercial textile products based on synthetic antibacterial agents such as triclosan, metal and their salts, phenols and quaternary ammonium compounds have been developed by researchers, some of them are also commercially available [8].

Nanotechnology is one of the researches in modern materials science. This technology is capable to provide miscellaneous novel applications that range from innovative fabric compounds, and sophisticated medicinal techniques, [10] which is a rapid, multidisciplinary advancement of contemporary science and technology. [11] It is considered as the synthesis, characterization and exploration of materials in the nanometer region (1-100 nm). At this level, the properties and functions of living and anthropogenic systems are defined. [12] This nanoscale size generally confers larger surface areas to nanoparticles (NPs) compared with micro-sized particles [13] NPs are known as controlled or manipulated particles at the atomic level (1-100 nm) [14]. Synthesis of noble metal nanoparticles for applications in catalysis, electronics, textiles, environmental Protection, and biotechnology is an area of constant interest. [11] The intrinsic properties of metal and metal oxide NPs such as silver, ZnO and TiO₂, are mostly characterized by their size, composition, crystalline and morphology. Reducing the size to nanoscale can modify their chemical, mechanical, electrical, structural, morphological and optical properties. These modified features allow the NPs to interact in a unique manner with cell bio molecules and thus facilitate the physical transfer of NPs into the inner cellular structure [15].

Inorganic materials such as metal and metal oxides have attracted a lot of attention over the past decade due to their ability to withstand harsh process conditions [16], [17], [11] Metal oxides NPs offer a wide variety of potential applications in medicine due to the unprecedented advances in nanotechnology research. [18] Among other metal oxide nano-material's, ZnO is famous for their catalytic efficiency, chemical stability and strong absorption ability [19] Nanosized ZnO exhibits varying morphologies and shows significant antibacterial activity over a wide spectrum of bacterial species explored by a large body of researchers. [20] ZnO is currently being investigated as an antibacterial agent in both micro scale and nanoscale formulations. ZnO exhibits significant antibacterial activities when particle size is reduced to the nanometer range. The nanosized ZnO can interact with bacterial surface and the bacterial core where it enters inside the cell, and subsequently exhibits distinct bactericidal mechanisms. [21] The antibacterial activity of ZnO has been found to be due to a reaction of the ZnO

surface with water. ZnO produced increased levels of reactive oxygen species, namely hydroxyl radicals. An exposure of bacteria to the small ZnO nanoparticles results in an increased cellular internalization of the nanoparticles and bacterial cell damage [22] ZnO-NPs are reported to have an extremely good safety profile and no toxicity observed when taken at different nanosize. [23] Chou, ZnO and TiO₂ nanoparticles have been developed to be associated with textiles. [24] ZnO has been reported as presenting a better effect on microorganisms than other metal oxides, such as SiO₂, MgO or TiO₂. The relation between the antibacterial activity versus the ZnO particle size, between 212 and 12 nm. [25], [26] In the present work, discover the effect of Antibacterial activity against *S.aureus* on ZnO-NPs coated cotton fabric has been demonstrated.

II. METHODOLOGY

A. Materials:

100% bleached cotton plain woven fabric was used with following particulars Ends/inch 94, picks per inch 67. The *Eclipta Alba*, *Indigofera tinctoria* Linn leaves were collected from Gandhigram Rural Institute-DU campus, Dindigul, Tamil Nadu, India.

1) *Eclipta Alba* Green Dye Extraction process

Washed the *Eclipta Alba* leaves thoroughly, then dry the leaves remove the water moisture and make it as a paste form into fine particles. 100g of the powder was weighed and taken in a round bottom flask and 400 ml of ethanol in the ratio 1:4 was added to it. The conical flask was heated in a water bath at 60°C for 1 hour. The dye solution was filtered to obtain crude dyestuffs. The crude dyestuffs are distilled and get one third (1/3rd) of the solution using the soxhlet apparatus at 70°C for 3hrs. In this process ethanol is recovered and then concentrated dye is obtained. The solution is kept overnight at room temperature for the precipitation. The precipitated ethanol water was obtained by decanting the solution. The obtained particles are dried in the oven overnight at 60°C.

2) *Indigofera tinctoria* Linn Blue Dye Extract process

Fresh leaves of *Indigofera tinctoria* Linn 100 gm used for preliminary work performance. The plant materials were cut into tiny pieces dried at room temperature (37±2°C) for 5 days. The standard indigo dye was obtained from the extraction with water by fermentation process. Using the at different periods and then added twice in volume of Ca (OH)₂ solution and maintain the pH level below 11, in the air at 15 min to precipitate the indigo dye. The precipitated

indigo was washed twice with $\text{Ca}(\text{OH})_2$ solution and centrifuged at 5000 rpm to 25 min.

B. Natural Dyeing Process

The bleached cotton fabric rinsed several times with de-ionized water before dyeing process. The aqueous extracts of plant extract liquid dyes were used as a direct dye diluted by 10 times. The material to liquor ratio was 1:40. Fabrics were introduced into the dye solution at room temperature. The temperature was then raised to 80°C and dyeing continued at the boil for 60 minutes where the Zinc, then the fabric was kept at 70°C for another 30 minutes subsequently. After dyeing, fabrics were rinsed in de-ionized water, washed using a nonionic detergent and air dried.

C. Synthetic Vat dye Process

In this synthetic vat dyeing process two colors Olive 100, Blue BC-415, where using the same tint as natural dye green and blue. These vat dyestuffs which show their optimum affinity at 20°-30°C and required small additions of caustic soda and hydros. An additions of commons salt is made to improve exhaustion of the dyebath and added in the dissolved from after dyeing for the same time, the dyeing started at 40-70°C and is then completed in the cooling bath in $\frac{3}{4}$ to 1 hour, to dye the fabric squeezed and oxidized in air without rinsing and cool down the dyed fabric and rinsed in de-ionized water.

D. Zinc oxide Nano Chemical Synthesis

1) Chemicals

Pure and analytical grade chemicals were used in all experiments, including synthesis of ZnO of chemical and green chemistry method. Zinc acetate dehydrate (Merck, 99%), Sodium hydroxide (NaOH) (Merck 99%) was purchased from Chemico Glass Pvt. Ltd, Erode, Tamil Nadu, India.

2) Preparatory Process

All glassware was washed with sterile distilled water and dried in a hot air oven before use. The procedure for the synthesis was referenced from literature. [27] In this wet chemical method of synthesis process to sort of procedure followed.

3) Synthesis of ZnO Nanoparticles

The ZnO nanoparticles were prepared by wet chemical method, 0.02M aqueous Zinc acetate dehydrate was added to ethanol medium solvent under vigorous stirring at room temperature using magnetic stirrer for 30 minutes. After complete dissolution of Zinc acetate dehydrate, 0.1 N of

Sodium hydroxide solution was added drop wise to touching the walls of the vessel under vigorous stirring at room temperature. The reaction was allowed to proceed for 2 h formed a transparent white solution. The ratio of zinc acetate dehydrate and; sodium hydroxide (pH=12) is 1:1, and the synthesis temperature were varied from room temperature, 60, 70, 80, 90 °C. These solutions were slowly reacted to produce the precipitate of ZnO nano particles. After the completion of reaction, the solution was allowed to settle for overnight and the supernatant solution was discarded carefully. The remaining solution was centrifuged at 5000 rpm for 20 min and the supernatant was discarded. Thus produced nanoparticles were washed three times using ethanol. After washing, the nanoparticles were dried at 60°C for 12 h, during drying particles were crushed with mortar and pestle, thus obtained particles was named at ZE0, ZE1, ZE2, ZE3, ZE4.



Fig. 2.4. The ZnO-NP preparation processes a) ZE0- Room temperature, ZE1-60°C, ZE2-70°C, ZE3- 80°C, ZE4- 90°C. b) Shows particles settle down c) Nanopowder

4) Fabric Coating process

ZnO-NPs were embedded on cotton using pad-dry-cure method. The cotton fabric cut to the size of 40×40 cm was immersed in the solution containing (ZE4) (2%) and acrylic binder (1%) for 5 min and then it was passed through a padding mangle. A complete wet pick-up was maintained for all of the treatments. After padding, the fabric was air-dried and then cured for 3 min at 140°C . The fabric was then immersed for 5 min in 2 g/L of sodium lauryl sulfate to remove unbound nanoparticles. Then the fabric was rinsed at least 10 times to completely take out all the soap solution. The washed fabric was air-dried, simultaneously; bulk-ZnO (ZE4) coating was carried out for comparison. [11]

5) Characterization Technique

The Phase evolution of calcined powder as well as that of sintered samples was studied by X-ray diffraction technique (XRD, Panalytical X-ray Diffractor) using Cu K α radiation. The generator voltage and current were set at 35 KV and 25 mA respectively. The ZnO wet chemical and Green synthesis samples were scanned in the 2θ range in continuous scan mode. The scan rate was $0.04^\circ/\text{sec}$. To study the adhesion of nanoparticles onto the fabrics, particles dispersion and evenness of spreading on fiber surface play a key role. Thus scanning electron microscopy was used for observing the surface of the fabric samples. Any changes of the abundance of NPs on the fabrics can be checked and analyzed. In this research work, VEGA 3 TESCAN machine was used to characterize mean particle size, morphology of ZnO-NP. The binding properties of ZnO nanoparticles were investigated by Fourier transform infrared red spectroscopy (FTIR) analysis. The characterization involved (FTIR) analysis of the dried powder of the synthesized ZnO nanoparticles was carried out by Perkin Elmer Spectrum 1000 spectrum in attenuated total reflection mode, and using the spectral range of $4000\text{--}400\text{ cm}^{-1}$ with the resolution of 4 cm^{-1} . The ZnO-NPs synthesized by wet chemical methods these NPs were characterized by perkin-Elmer UV-VIS spectrophotometer, lambda -19 to known the ZnO-NPs. The scanning range of the samples was $200\text{--}800\text{ nm}$ at a scan speed of 480 nm/min . The spectrophotometer was equipped with "UV Winlab" software to record and analyze data. Bases line correction of the spectrophotometer was carried out by using blank reference. The UV-Vis absorption spectra of all the samples were recorded and the numerical data were plotted.

6) Method of antimicrobial testing

The agar diffusion test was used for this research, which included the testing method of AATCC 147-1998. It is a qualitative test method and suitable for a large number of samples. In this test method agar solution was prepared with nutrient and then microbial cells were inoculated. The solution was transferred into the Petri dish where the nanoparticle samples, the nano treated dyed fabrics and the untreated dyed fabrics were laid over the solution for intimate contact. The plate was incubated at 37°C for 18 to 24 hours and the growth of bacteria was then examined. A zone of inhibition becomes apparent with the diffusion of antimicrobial agent in the agar plate. [9], [28].

III. RESULTS AND DISCUSSION

A. XRD

The XRD pattern of ZnO-NPs define line broadening of the XRD peaks indicates that the prepared material consist of particles in nanoscale range. From this XRD pattern analysis, we determined peak intensity, position and width, full width at half maximum (FWHM) data. The diffraction peak located at 31.84° , 34.52° , 36.33° , 47.63° , 56.71° , 62.96° , 68.13° , and 69.18° have been keenly indexed as a hexagonal quartzite phase of ZnO. [29],[30] In this study observed diffraction peaks of ZnO at $2\theta = 31.93^\circ$, 34.53° , 36.32° , 47.82° , 56.62° , 62.88° and 68.26° are associated with (100), (002), (101), (102), (110), (103) and (112). All the reflections can be assigned to the standard powder pattern for the pure hexagonal phase of ZnO with lattice constants $a = 3.2516\text{ \AA}$, $c = 5.2000\text{ \AA}$.

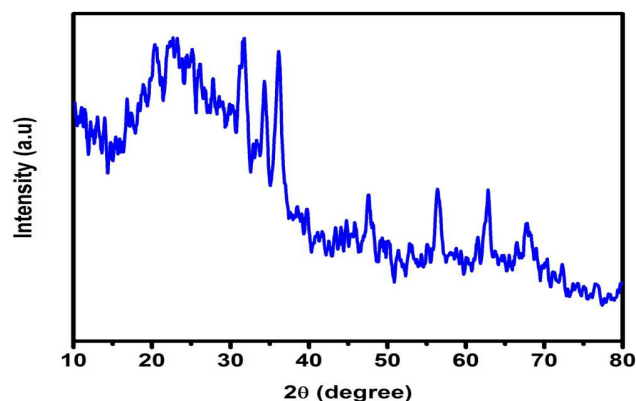


Fig. 3.1. XRD pattern of the as prepared ZnO-NPs (ZE4)

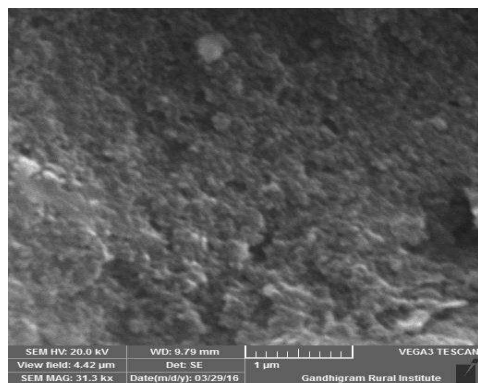
The (hkl) values are agreed well with the standard card of ZnO powder sample (JCPDS file No: 36- 1451). The

crystallite size (t) calculated on the basis of Scherrer's equation1:

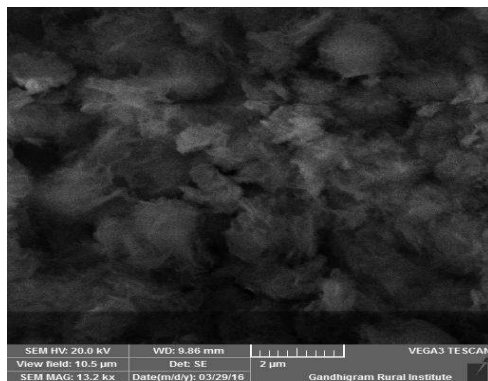
$$(t = 0.9\lambda/\beta \cos \theta)$$

Where λ is the wavelength of X rays used (1.54060 Å), β is the full width at half maximum (FWHM) and θ is the angle of diffraction. The crystallite size of ZE4-NPs is found average particle size as 12.30 nm, which is considered to be more beneficial for the antibacterial activity against *Staphylococcus aureus*.

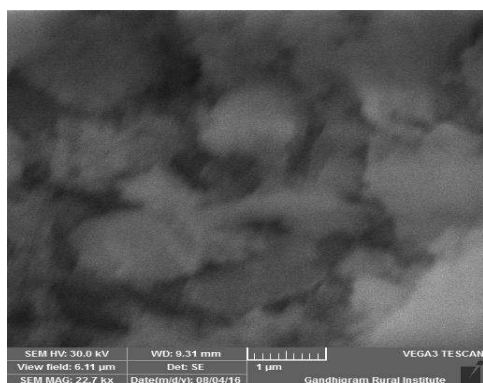
B. Scanning Electron Microscopy (SEM)/EDAX Analysis



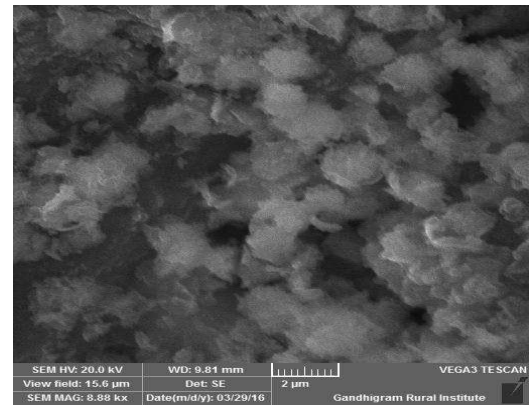
a- ZE0



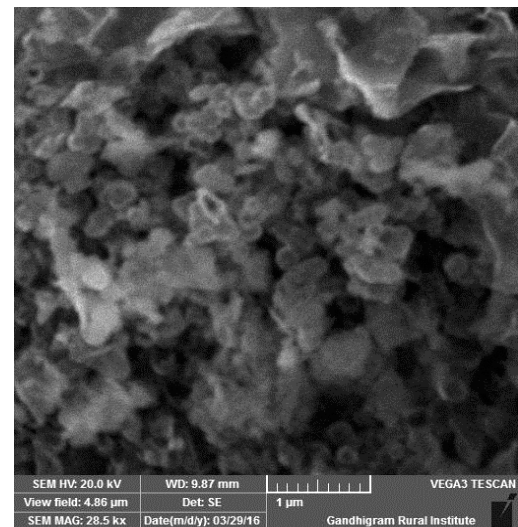
b- ZE1



c- ZE2



d- ZE3



e- ZE4

Fig. 3.2. Scanning Electron Microscopic picture of ZE0, ZE1, ZE2, ZE3, ZE4.

The SEM images confirm the morphology of ZnO-NPs ZE0, ZE1, ZE3 have aggregated morphology, while ZE3 and ZE4 show nano flake like structure. The average particle size of ZnO-NPs was estimated to be 12.30 nm.

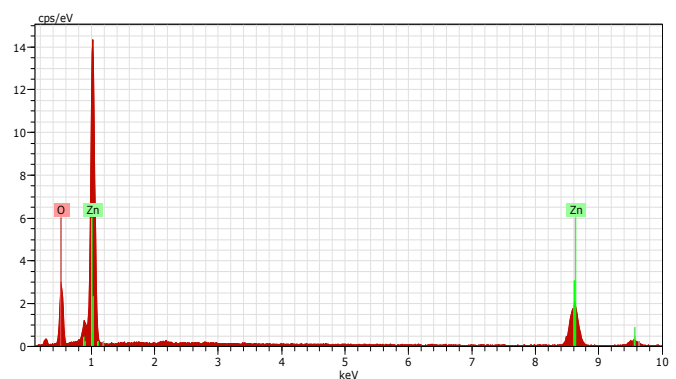
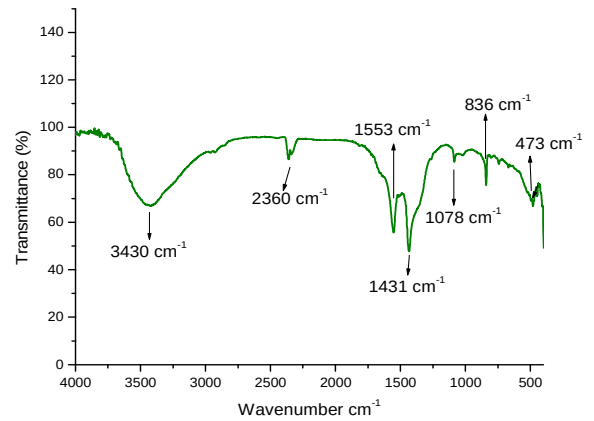


Fig. 3.2. EDAX picture of EZ4

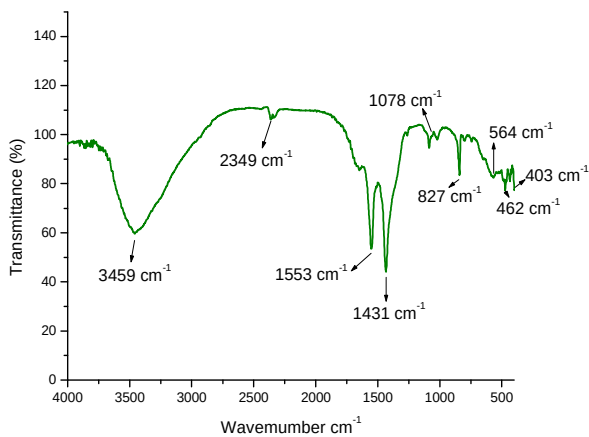
Figure 3.2 (b) EDAX spectrum shows four peaks which were identified as zinc (72.87%) and oxygen (27.13%). The optical absorption peaks of ZnO-NPs and these absorption peaks of ZE4 show confirm the presence of Zn and O composition.

C. FTIR Spectra

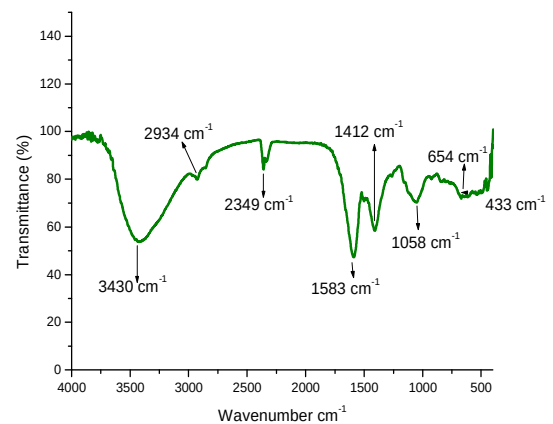
Figure 3.3 is the typical FTIR spectra of ZnO-NPs. (ZE0-ZE4 bands obtained at 654, 564, 403, 433, 462, 452, 473 cm^{-1} could be attributed to the characteristic metal oxygen stretching vibrations in ZnO nanoparticles. The bands at 540- 417 cm^{-1} point out ZnO-NPs [31].



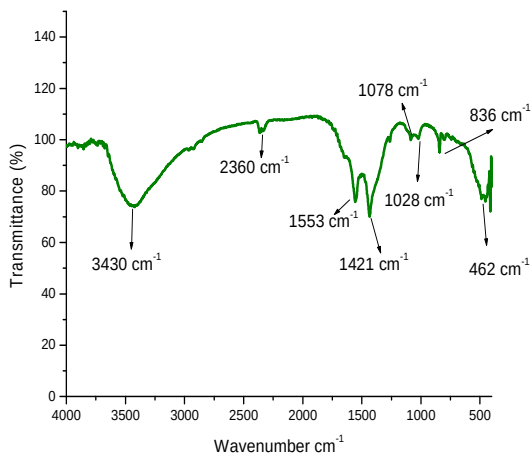
c- ZE2



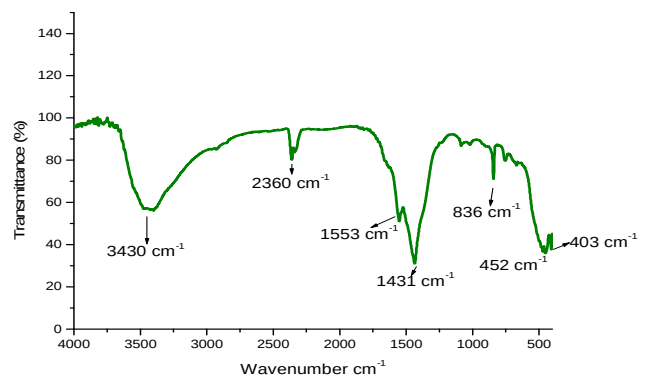
a- ZE0



d- ZE3



b- ZE1



e- ZE4

Fig. 3.3. FTIR Spectra

The broad strong absorption peak at 3430, 3459 cm^{-1} can be attributed to the characteristic functional alcohol O-H group stretch, FT-IR spectrum of the synthesized ZnO nanoparticles showed (fig. 3) the peaks at 3258.97, 2928.3 and 1365 cm^{-1} indicates the presence of O-H, C-H and C=O residues.

D. UV absorption

The size of the nanoparticles plays an important role in changing the entire properties of materials. Thus, size evolution of semiconductor nanoparticles becomes very essential to explore the properties of the materials. UV – visible absorption spectroscopy technic is widely used technique to examine the optical properties of Nano sized particles. Earlier studies reported as absorption spectrum of ZnO nanopowder exhibits a strong absorption band at about 355 nm [32], [7]

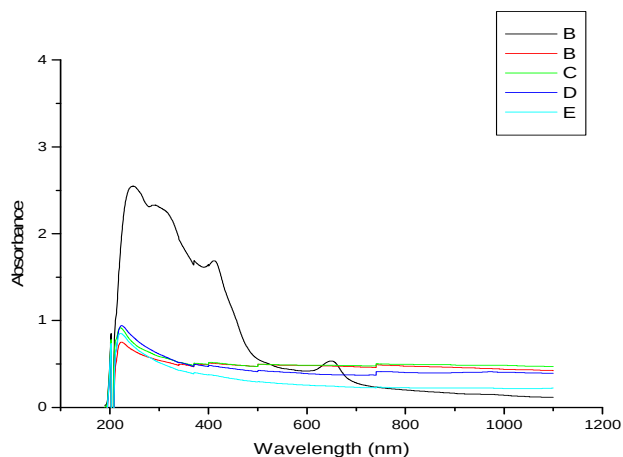


Fig. 3.4. UV absorption

Figure 3.4 shows all the absorption wavelength of ZnO particles confirm the presence of ZnO Nanoparticles. ZE0-ZE4 (B-E) bands at 247 nm, 224 nm, 221 nm, 224 nm and 222 nm respectively.

E. Antimicrobial Activity *Staphylococcus aureus*

There are many disinfectants that are composed of metals or organic compounds for antibacterial treatment of fibres and polymers, [30] ZnO has been found to have several advantages, including marked antibacterial activity. The in vitro antibacterial assessments have been performed using the ZnO-NPs synthesis temperature conditions. It consists of determining the number of viable bacteria by plating in suitable agar medium, microorganisms incubated in culture broths containing ZnO-NPs at temperature variations. The antibacterial activity of ZnO-NPs has been tested against *Staphylococcus aureus*. ZE4 showed excellent antibacterial activity better than ZE0. Earlier work reported that the increased production of hydrogen peroxide with an increase in the c lattice parameter and hence the enhanced antibacterial activity. [33] Recent in vitro studies showed that ZE4 inhibits the growth of *S aureus* and *Escherichia coli* [34] also the finding that Gram-positive bacteria are

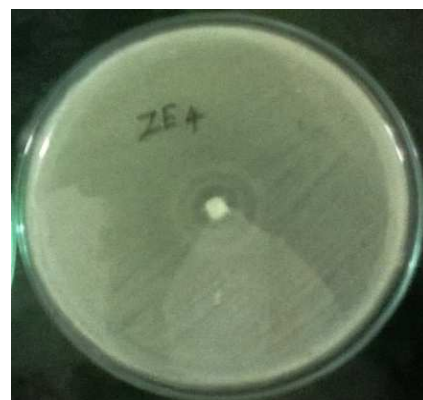
more susceptible to ZE4 than Gram –negative bacteria [35] supports our results. Thus we concluded that nano ZE4 might have a bactericidal action effect on *S.aureus*. This could be attributed to the damage of the bacterial cell membrane and extrusion of the cytoplasmic contents, thereby resulting in the death of the bacterium. On the basis of the research it can be concluded that the inhibition of bacterial growth by ZE4 nanoparticles as could be attributed to the damage of the bacterial cell membrane reported same as the reference [36], and [37].



a-ZE0



b-ZE1, ZE2, ZE3



c- ZE4

Fig. 3.5. Antibacterial Activity against *Staphylococcus aureus* Zone
a) ZE0 b) ZE1, ZE2, ZE3 c) ZE4

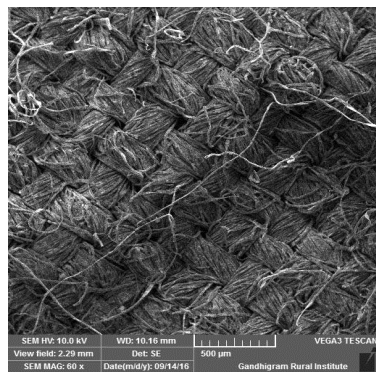
Table I. indicate the antibacterial activity of ZnO-NPs, ZE4 has good Zone inhibition shown as the higher diameter of inhibition zone against *S. aureus* (Gram +) bacteria.

TABLE I. THE ANTIBACTERIAL ACTIVITY OF ZnO-NPs

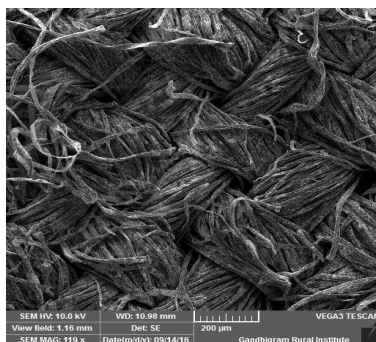
Sl. No	Sample Code	Temperature	<i>Staphylococcus aureus</i> Zone in mm (Gram +)
1	ZE0	Rt	4
2	ZE1	60°C	6
3	ZE2	70°C	10
4	ZE3	80°C	12
5	ZE4	90°C	19

F. Scanning Electron Microscopy of ZE4 Coated Fabric

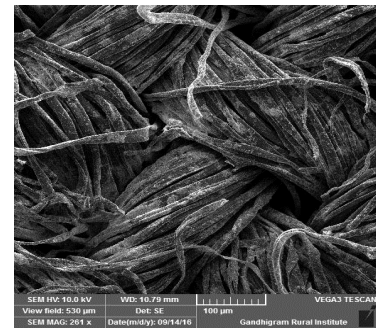
The surfaces of the treated fabrics were observed by SEM microscopy. In Figure 3.6, SEM micrographs show the nannoscaled ZE4 particles on cotton fabric under different magnifications from 500, 200, 100, 50, 20 and 10 μm .



a



b



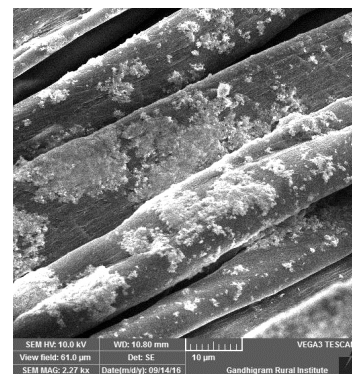
c



d



e



f

Figure 3.6 Scanning Electron Microscopy Picture of ZE4 Coated Fabric a) 500 μm b) 200 μm c) 100 μm d) 50 μm e) 20 μm f) 10 μm

G. Antibacterial *Staphylococcus aureus* Activity of ZE4 Nano treated Fabric

The antibacterial activity of ZnO-NPs coating on wearable natural and synthetic dyed cotton fabrics discussed as follows:

Fig 3.7 and Table 2 indicate Anti-bacterial *Staphylococcus aureus* zone in mm effect of Nano treated and untreated natural and synthetic dyed fabrics. Only nanotreated fabric gives good antimicrobial activity towards

Staphylococcus aureus due to the reason of good diffusion property compared with the rest of the dyed and zinc mordant samples compare to natural dyed fabrics synthetic dyed and bleached samples have a better activity. The Zone size is the 21 mm, 19 mm and 18 mm for ZE4 nanotreated bleached cotton, naturally dyed nanotreated fabrics green and Blue, respectively. The vat dyed nanotreated fabrics green is 22 mm, and Blue is 20 mm.

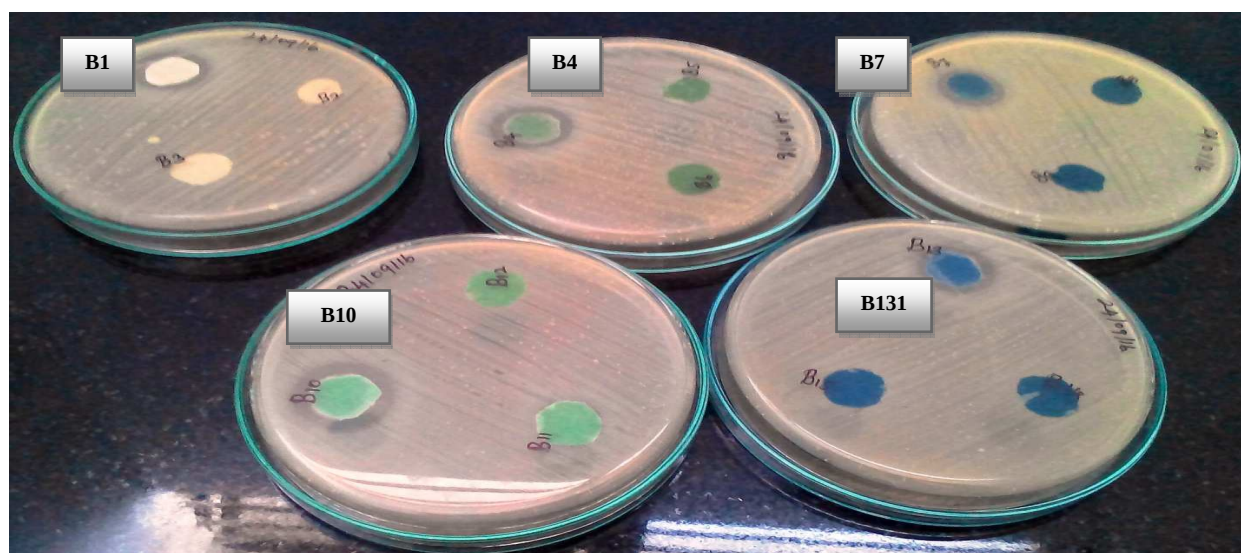


Fig 3.7 Antibacterial activity against *Staphylococcus aureus* ZE4 Coated Fabrics

TABLE II. ANTI-BACTERIAL STAPHYLOCOCCUS AUREUS ZONE

Sl.No	Sample Name	<i>Staphylococcus aureus</i> Zone in mm	
1	Cotton Bleached ZnO Nano Fabric	B1	21
2	Cotton Bleached Fabric 1	B2	N.D
3	Cotton Bleached Fabric 2	B3	N.D
4	Cotton Eclipta Green Dyed ZnO Nano Fabric	B4	19
5	Cotton Eclipta Green Dyed Fabric 1	B5	N.D
6	Cotton Eclipta Green Dyed Fabric 2	B6	N.D
7	Cotton Indigo Blue Dyed ZnO Nano Fabric	B7	18
8	Cotton Indigo Blue Dyed Fabric 1	B8	N.D
9	Cotton Indigo Blue Dyed Fabric 2	B9	N.D
10	Cotton Vat Green Dyed ZnO Nano Fabric	B10	22
11	Cotton Vat Green Dyed Fabric 1	B11	N.D
12	Cotton Vat Green Dyed Fabric 2	B12	N.D
13	Cotton Vat Blue Dyed ZnO Nano Fabric	B13	20
14	Cotton Vat Blue Dyed Fabric 1	B14	N.D
15	Cotton Vat Blue Dyed Fabric 2	B15	N.D

N.D: not determined (It means that no antibacterial activity effect observed at the Zinc mordant and dyed fabrics)

IV. CONCLUSION

The surgical gown hospital purpose clothing textiles are quite new to Indian market. The high profile doctors, whose number is increasing day by day, prefer safety of themselves and the patients. Bacterial infections, especially *Staphylococcus aureus* skin affected bacteria used to get quickly absorbed and held by the cloths. This nano finish coating application gives better protection than other finishing processes. ZE4-NPs can be embedded in cotton natural and synthetically dyed fabrics for functional with potential applications of hospital usable coloured textiles and antibacterial finish against *Staphylococcus aureus* in medical textiles and inner wears. We successfully dispersed ZE4-NPs inside a fabric via a simple water based technique. The average size of ZE4 is 12 nm, which shows excellent antibacterial activity against *S. aureus*; after being impregnated onto cotton textiles. The ZE4-NPs coated fabric samples exhibit more effective bactericidal efficacy than pure ZE3-ZE0, which can be attributed to the effect of enhanced surface bioactivity.

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