CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY STUDIES OF CALCITE-ZINCATE NANOPARTICLES BY GREEN SYNTHESIS

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Abstract: Nanoparticles (Nps) are extremely useful in a extensive choice of industries, together with electronics, the environs, cosmetics, material science, and medicinal systems, among others. Calcium Carbonate ($CaCO_3$) and Zinc Oxide (ZnO) nanomaterials have long been popular research topics. It is frequently used in dentistry applications due to its biocompatibility. The purpose of this study was to produce and describe calcium carbonate/zinc oxide nanoparticles ($CaCO_3$/ZnO-NPs) prepared via precipitation method. The precipitation method has many advantages, including being more controllable and reproducible, as well as allowing for easy particle size control. Temperature and the calcination process both have an impact on the formation of nanoparticles. The calcination at high temperatures resulted in spherical-shaped particles, a reduction in aggregate size, and an increase in the crystalline nature of the NPs. Nitrates of calcium and zinc, sodium hydroxide were used, along with silk washed waste water containing sericin as a capping agent. $CaCO_3$/ZnO-NPs were produced at 60°C using calcination (500°C). The antibacterial activities of the nanoparticles against gram-positive bacteria Staphylococcus aureus and Enterococcus faecalis, as well as gram-negative bacteria Escherichia coli and Klebsiella pneumoniae, also, Fungus - Candida albicans were investigated, via the disk diffusion method. $CaCO_3$/ZnO-NPs were successfully produced and exhibited a crystalline nanostructure, confirmed the elements of $CaCO_3$/ZnO-NPs, and revealed that the $CaCO_3$/ZnO-NPs obtained had an irregular spherical shape. The nanoparticles were found to be void of contaminants and organic. The absorption group at nm points out the occurrence of $CaCO_3$, and the absorption group at 380 nm confirm the existence of ZnO. In addition, these $CaCO_3$/ZnO-NPs have improved antimicrobial activity with potential applications in dentistry.
Keywords: precipitation method; green synthesis;characterization; antibacterial;antifungal; disc diffusion method;

I. INTRODUCTION

Nanotechnology is defined as "the meticulous and precise employment, accuracy employment, exhibiting, size, and synthesis of ingredients at the nanoscale to a variety of material, arrangements, and maneuvers by basically unique features and tasks" [1].Humans have long been interested in "nano"-sized existence. Richard P. Feynman, a physicist and well-known Nobel Prize winner was generally credited with developing the idea of nanotechnology [2].

A broad range of technologies is now included in the branch of applied science known as nanotechnology. NPs have seen substantial development and have numerous applications in the fields of medicine, catalysis, sensors, electronics, the environment, and biology [3,4]. For damaged soils, groundwater, wastewater, air, and surfaces, modern nanomaterials offer a variety of environmental treatments and remediations [5,6]. Nanomaterials can provide solutions to technical and environmental issues in the areas of catalysis, medicine, solar energy conversion, and water treatment [7]. Due to their special qualities and versatility in several disciplines, metal and metal oxide nanoparticles are currently the subject of intensive research. Determining the biocompatibility of nanostructured materials is the subject of a lot of current research breakthroughs. They have been discovered to be cytocompatible and harmless. Most of these nanomaterials exhibit unique chemical and biological characteristics that are not seen in nature [8]. There are numerous sectors where ZnO-NPs could be used, In addition to having high catalytic efficiency, good adsorption ability, a high isoelectric point (9.5), biocompatibility, and quick electron transfer kinetics for biosensing purposes, these materials also include optical piezoelectric, magnetic, and gas sensing. [9-17]. The US Food and Drug Administration (FDA) has designated five compounds containing zinc as generally recognized as safe (GRAS) [18]. ZnO is a zinc supplement used by the food industry. Zinc is a vital micronutrient that is important for both human and animal growth, development, and health [19]. Calcite, which is thermodynamically stable at room temperature, aragonite, which is less stable at high pressure than calcite, and vaterite, which is the least stable of the three polymorphs but can transform into either of the other two polymorphs, are the three anhydrous crystalline phases of calcium carbonate. [20]. the precipitation of homogeneous solutions [21], water-in-oil-in-water emulsions [22], mechanochemical and sonochemical syntheses [23], and water-in-oil (W/O) microemulsions [24] are only a few of the methods used to create calcium carbonate nanoparticles that have been documented. The origin of silk, and historical information may date back as far as 5000 years in North China [25]. The silkworm, Bombyx mori, a holometabolous insect of the Lepidoptera order and Bombycidae family, produces the protein sericin. At the end of the fifth larval instar, B. Mori produces a significant sum of sericin, which together with fibroin produces the silk yarn required to build the cocoon, a structure that provides the ideal conditions for the larval transformation to adults to occur. [26]. The most significant lepidopteran exploited in scientific research is B. Mori, a genetic resource that can provide light on a variety of biological issues [27]. Recent studies have shown that the B. Mori cocoon and its two primary proteins, fibroin, and sericin, have been used in the production of polymers, biomaterials, cosmetics, and food [28, 29]. Sericin has been ignored in the field of sericulture for a very long period. According to estimates, 400,000 tons of dried cocoons are produced globally each year, yielding 50,000 tons of sericin, most of which is dumped in wastewater [30]. As a result, there is a significant increase in the need for chemical and biological oxygen and water contamination [31]. Particularly in nations where sericulture is dominant, like China, India, and Brazil, the removal and usage of sericin might have a significant negative influence on the economy, society, and ecology. Hazardous chemical species that adhere to surfaces can impair some technology's medical use. To avoid chemical toxicity and a highly active environment of physical and chemical processes, green synthesis has been established. Due to their qualities, they are excellent for biomedical applications. During green synthesis, several variables, including the amount of plant extract used, industrial wastes, the pH, the temperature, the length of the reaction, and the solvent, affect the form of the NPs [32]. The objectives of this study are to synthesize $CaCO_3$/ZnO-NPs using an environmentally friendly method and to evaluate their antibacterial abilities.

II. MATERIALS AND METHOD

SD Fine Company provided the calcium nitrate tetrahydrate [$Ca(NO_3)_2$*4H2O], zinc nitrate hexahydrate [$Zn(NO_3)_2$*$6H_2O$] and sodium hydroxide (NaOH). From the microbiology department at Sri Krishnadevaraya College of Dental Sciences, bacterium cultures of gram-positive bacteria's Staphylococcus aureus and Enterococcus faecalis, gram-negative bacteria'sEscherichia Coli and Klebsiella pneumoniae. Also, Fungus - Candida albicanswere acquired. The aqueous solution is made by mixing 500 ml of deionized water with 0.075 M of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$) and 0.025 M of Zinc Nitrate Hexahydrate ($Zn(NO_3)_2 \cdot 6H_2O$) for an hour to create a homogenous combination of nitrates. After that, agitate for another 30 minutes while adding 30 ml of the wastewater from the silk-washing process. Then, under magnetic stirring, the 0.2 M of sodium hydroxide (NaOH) in 100 ml of deionized water was combined and concurrently added dropwise to the mixture. The heat was applied to the solution until a white precipitate was produced. Then it is allowed to settle down the nanoparticles then it was heated in a crucible, to obtain the powder of samples. To create nanoparticles, the powder was finally transferred to the crucible and calcined at 500°C for 3 hours. The schematic representation of precipitation processes is shown in the figure. 1. The crystal-like $CaCO_3$/ZnO-NPs obtained were characterized by X-ray Diffractometer (XRD) using CuKα, Scanning Electron Microscopy (SEM) with Energy Dispersive X-ray (EDX), and Fourior Transform Infrared Spectroscopy (FTIR) were performed to study and determine the fabricated nanoparticles' functional groups and Ultraviolet Visible (UV Vis) Spectroscopy to check the absorption wavelength of $CaCO_3$/ZnO nanoparticles.

III. ANTIMICROBIAL ASSAY USING THE DISC DIFFUSION TECHNIQUE

Mueller-Hinton Agar media (Himedia) was prepared and autoclaved at 120°C for 20 minutes. Mueller-Hinton agar liquid (20 ml; pH 7.3 0.2; 25 °C) was added to sterilized Petri plates and allowed to harden. Sterile discs (Himedia) were saturated in different concentrations of green synthesis nanoparticles in sterile conditions overnight. 100 µl of microbial broth culture was spread uniformly on Mueller-Hinton Agar medium by spread plate method.

Then the different concentrations of $CaCO_3$/ZnO-NPs 05µl, 10µl, 15µl, and 20 µl discs and antibiotic disc were aseptically placed. After 24 hours of incubation at 37°C, the experiment was performed in triplicate, and the diameters of the zones of inhibition were assessed. The standard antibiotic Streptomycin (10 mg/ml) was used. The antimicrobial activities were studied by disc diffusion method for gram-positive bacteria's Staphylococcus aureus and Enterococcus faecalis, gram-negative bacteria's Escherichia Coli and Klebsiella pneumonia, on Fungus - Candida albicans.

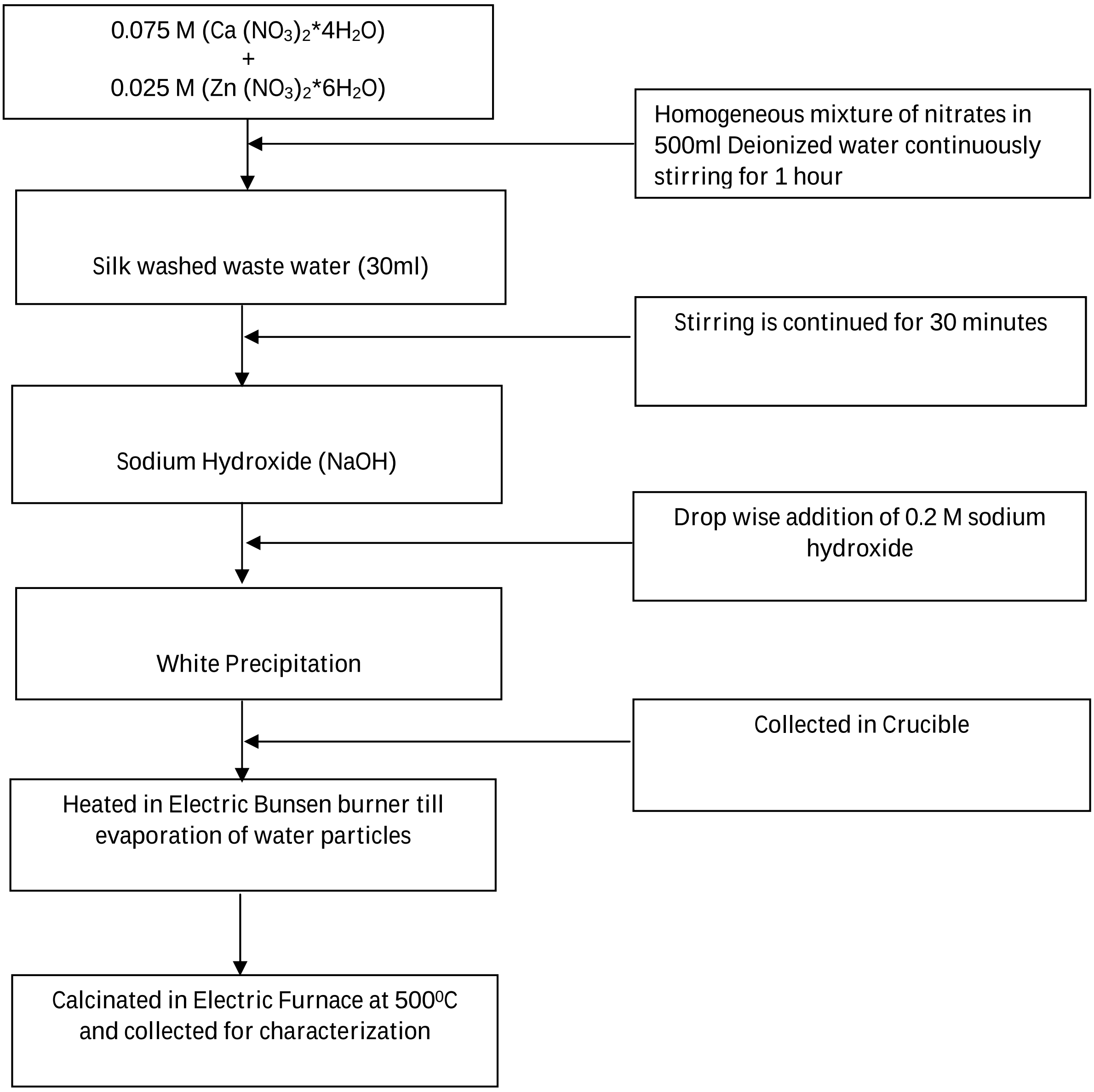

Fig .1 Schematic representation of green synthesis to obtain $CaCO_3$/ZnO nanoparticles.

IV. RESULTS AND DISCUSSION

Physical appearances of the produced Calcium-Zincate nanoparticles

The X-ray diffractograms of $CaCO_3$/ZnO-NPs after calcination at 500°C are depicted in Figure.2. The results are consistent with the reported zincate [34] and calcite [33] phases, which correspond to 20.6% and 79.4% of the displayed pattern for $CaCO_3$/ZnO-NPs produced following calcination, respectively. The diffraction peaks of the green synthesis precipitation method for $CaCO_3$/ZnO-NPs have two theta values and are Bragg reflections of $CaCO_3$ 23.01°, 29.46°,39.48°, 43.22°, 47.58°, 48.56°, 56.66°,57.46°, 62.94°, 69.16° to their corresponding Miller Bravais indices (012), (104), (202), (016), and (018) planes of hexagonal structure phases of standard Calcite (JCPDS#47-1743). For ZnO 31.84°, 34.48°, 36.32°, 47.58°, 56.66°, 62.94°, 66.44°, 68.02°, 69.16° matching the appropriate Miller Bravais indices (100), (002), (101), (102), (110), (103), (200), (112), and (201) of the standard JCPDS card 36-1451's hexagonal structure phases. It was clear from the synthesized nanoparticles' sharp and strong peaks that $CaCO_3$/ZnO-NPs with extremely crystalline structures had been effectively created. The average particle size of $CaCO_3$/ZnO-NPs is determined using Debye–Scherrer's formula [35] given in equation 2 and Crystallinity [47] was determined by equation 3.

$$D = 0.9\lambda/\beta \cos\theta \text{ ------------------ } (2)$$

Where, D - Average crystalline size, λ – monochromatic Cu-Kα wavelength is 1.5414 Å, β - full-width at half maximum in radians and θ - scattering angle in degree.

$$\text{Crystallinity} = \frac{\text{Area of Crystalline Praks}}{\text{Area of all peaks(Crystallinie+Amorphous)}} * 100 \text{ -- } (3)$$

The average crystal-like dimension of the produced $CaCO_3$/ZnO-NPs is 29.18 nm after calcination at 500°C. The Crystallinity of the material is 71%.

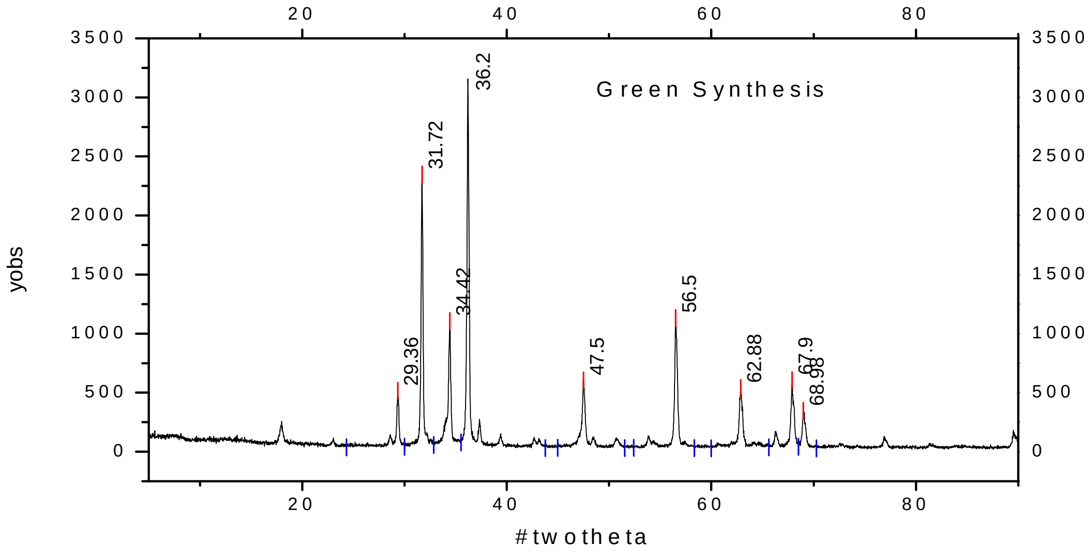

Fig .2 XRD pattern of Calcite-Zincate NPs.

V. MORPHOLOGY OF THE SYNTHESIZED CALCIUM-ZINCATE NANOPARTICLES

Morphological studies of the samples were examined through a scanning electron microscope (SEM). The SEM appearance of $CaCO_3$/ZnO-NPs after calcination is shown in Figure.3. $CaCO_3$/ZnO-NPs is existing as irregular sphere-shaped employing the average basic dimension of approximately 30 nm was confirmed by Debye–Scherrer's formulae. Spongy-shaped, non-uniformed, and non-homogeneous nanostructured $CaCO_3$/ZnO-NP powders were observed. EDX study shows the chemical composition of produced $CaCO_3$/ZnO-NPs. Table 1 shows the proportion of substances still present in nanoparticles following calcination. $CaCO_3$/ZnO-NPs is indicated by the presence of the elements calcium, zinc, carbon, and oxygen, according to EDX, and this analysis revealed crests that matched the optical absorption of the created nanoparticle, as illustrated. (Figure.3, Figure.4) and agreed with the earlier studies [36,37].

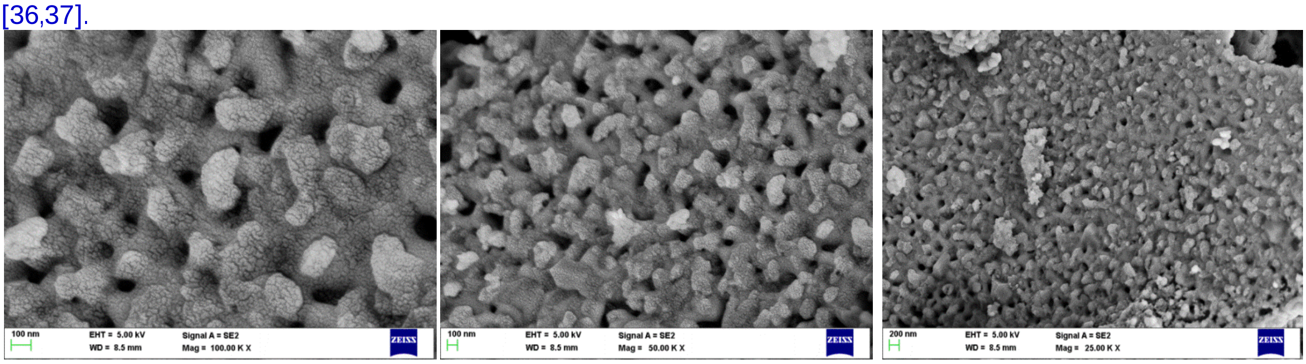

Fig .3 SEM images of $CaCO_3$/ZnO-NPs in 100nm and 200nm magnifications.

TABLE 1: PERCENTAGE OF COMPOUNDS AFTER CALCINATION AT 500°C

Element	Weight%	Atomic%	Standard :
C K	9.67	20.33	C CaCO3
O K	35.19	55.54	O SiO2
Ca K	11.60	7.31	Ca Wollastonite
Zn K	43.54	16.82	Zn Zn

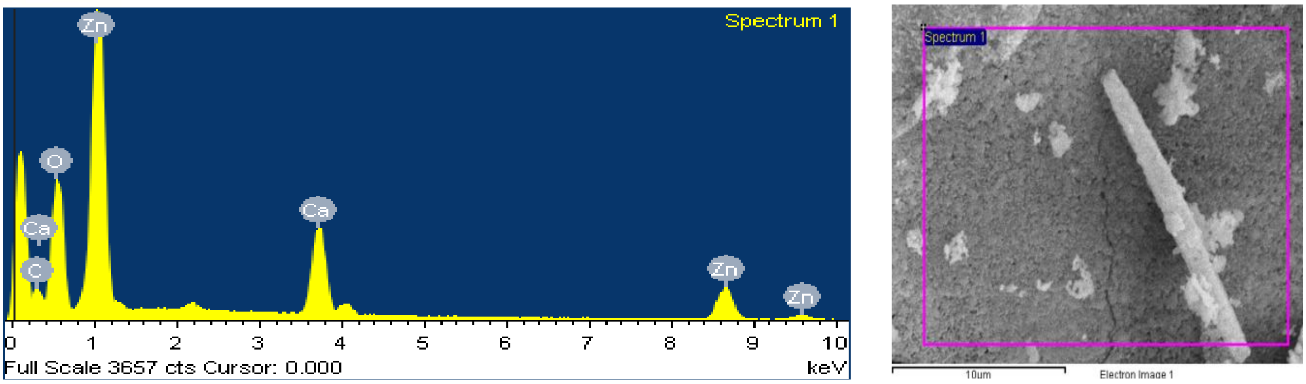

Fig .4 EDX profiles and image of calcite-zincate nanoparticles.

FTIR Analysis

The transmission band is visible in the FTIR spectrum in the 400–4000 cm-1 range as shown in the figure. 5. The vibrational bands in the spectra at 1417.33 cm-1, 862.44 cm-1, and 700.53 cm-1, indicate that carbonate is vibrating in a plane-bending manner [38]. Calcite was identified in the $CaCO_3$ NPs produced via green synthesis due to a strong peak at 876.72 cm-1. The powder's composition as $CaCO_3$ itself was further demonstrated by FT-IR analysis. The peak at 1621.66 cm-1, which is a characteristic of proteins and enzymes, is brought on by the amide regions. The high-intensity band at 419.16 cm-1 is produced by the zinc and oxygen bond's stretching mode. The spectra showed a clear signal at 3428.60 cm-1 that was the -OH group from the phenol present in the sample. The bands at 3646.32 and 2507.46 cm-1 are hypothesized to be caused by amine stretching vibrations, alcohol O-H stretching, and alkane C-H stretching. Similar band patterns have been reported by Sangeetha et al. [39-43].

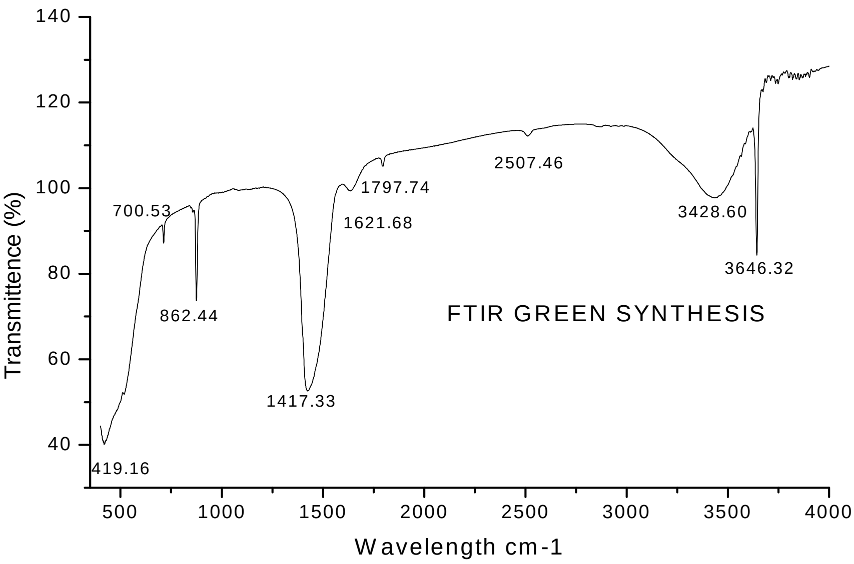

Fig .5 FTIR plots of synthesized Calcite-Zincate nanoparticle without calcination.

UV Absorption Spectroscopy

To measure absorbance, dimethyl sulfoxide (DMSO) was placed in a UV spectrometer. Before identifying the UV absorption peaks for $CaCO_3$/ZnO-NPs in powder form, the powdered $CaCO_3$/ZnO-NPs were slightly dissolved in DMSO solution and sonicated. To determine the absorbance, the specific constituent solutions should be placed in a quartz cuvette and mounted on the spectrophotometer. The obtained spectra, which are depicted in Figure 5, show peaks associated with the produced $CaCO_3$/ZnO-NPs. In the UV area, proteins often contain two absorbance peaks, one between 215-240 nm and the other 260-290 nm. UV radiation in the 215-240 nm range is absorbed by the peptide bonds of amino acids. Tryptophan, tyrosine, and phenylalanine are aromatic amino acids that may absorb UV radiation between 260 and 290 nm [44]. The silk sericin solution exhibits absorbance peaks at 216 and 275 nm, demonstrating sericin's capacity for UV resistance [45]. The CaCO3 NPs produced a high absorbance peak in the 538, 685, 780, 865, and 963 nms range when nanopowders were partially dissolved in distilled water [38]. The energetic absorbance crest accessed for CaCO3-NPs was observed at 812 nm when nanopowders were partially dissolved in DMSO solution. Distinctive UV peak of ZnO NPs, the absorption peak occurs at 380 nm. UV radiation is more effectively absorbed when ZnO NPs are included in the sericin matrix. Increased ZnO NPs concentration led to higher UV absorbance values between 300 and 400 nm [46].

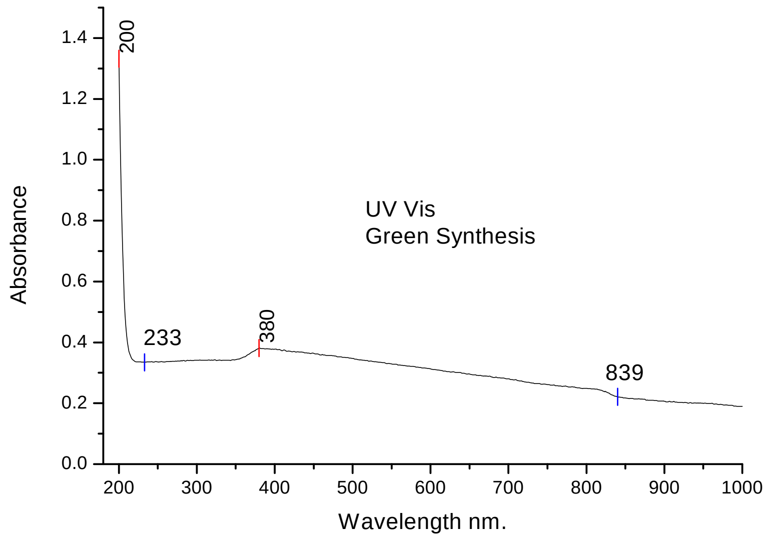

Fig.6 UV Absorption Spectroscopy plot.

ANTIBIOTIC SUSCEPTIBILITY TESTING BY DISC DIFFUSION METHOD

The antimicrobial activity of Calcium Carbonate/Zinc oxide nanoparticles ($CaCO_3$/ZnO-NPs) was performed by the disc diffusion method. As mentioned in Fig.7 and table 3. The different concentration of $CaCO_3$/ZnO-NPs 05µl, 10µl, 15µl, and 20 µl was saturated in sterile discs and placed in a bacterial medium. The maximum zone of inhibition of 36 mm diameter was observed in Escherichia coli at 15µl of $CaCO_3$/ZnO-NPs and a low concentration of 05µl of $CaCO_3$/ZnO-NPs inhibited the Klebsiella pneumonia growth and observed 13mm diameter of inhibition. We also observed no inhibition in 05µl of $CaCO_3$/ZnO-NPs on Staphylococcus aureus, enterococcus faecali, and Klebsiella pneumonia. The other concentrations of 10µl, 15µl, and 20µl of $CaCO_3$/ZnO-NPs inhibited the growth of bacteria and fungi results shown in Fig.7 and table 2.

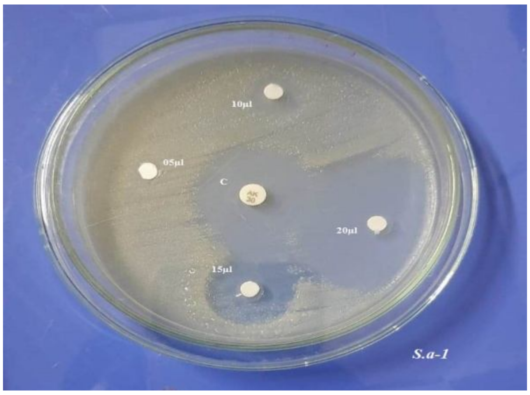

Fig 7.a Staphylococcus aureus

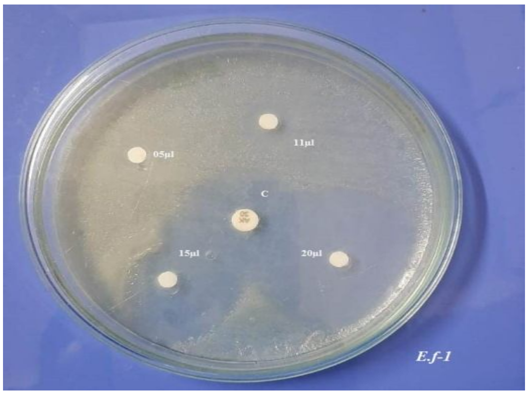

Fig 7.b Enterococcus faecalis

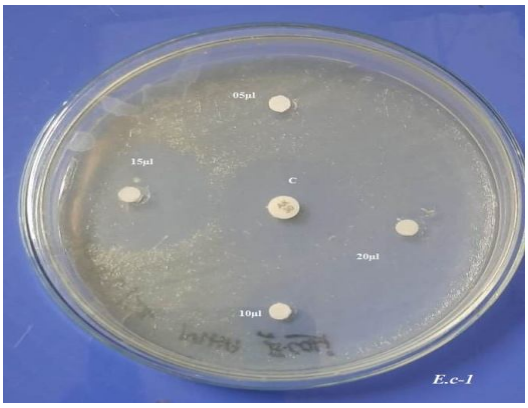

Fig.7.c Escherichia coli

Fig 7.d Klebsiella pneumoniae

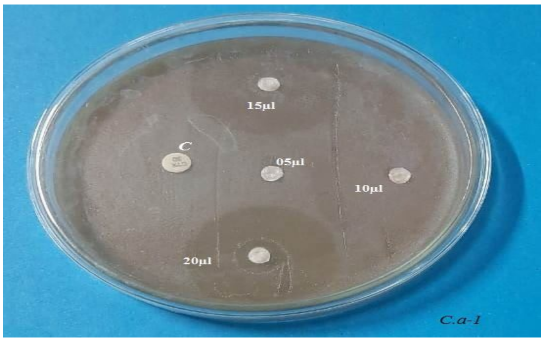

Fig 7.e Candida albicans

Fig .7 Antimicrobial activity of $CaCO_3$/ZnO-NPs at concentrations of i - (5 µl), ii - (10 µl), iii – (15 µl) and iv (20 µl) against Gram-positive and Gram-negative bacteria using disc diffusion method. Photographs of $CaCO_3$/ZnO-NPs nanocomposites on Staphylococcus aureus, enterococcus faecali, Escherichia coli, Klebsiella pneumonia and Candida albicans.

TABLE 2: ANTIBACTERIAL ACTIVITY RESULTS AFTER 24 HOURS OF INCUBATION PERIOD.

S. No.	PATHOGENS	GREEN SYNTHESIS				
		05µl	10 µl	15 µl	20 µl	C
1	Staphylococcus aureus	Nil	12mm	21mm	32mm	21mm
2	enterococcus faecalis	Nil	11mm	19mm	32mm	24mm
3	Escherichia coli	Nil	25mm	36mm	36 mm	22mm
4	Klebsiella pneumoniae	13mm	15mm	24mm	32mm	23mm
5	Candida albicans	Nil	Nil	34mm	34mm	Nil
* Amikacin AK 30 mcg – Control used for bacteria and * Cefotaxime (Cephotaxime) CTX 30 mcg - Control used for fungus						

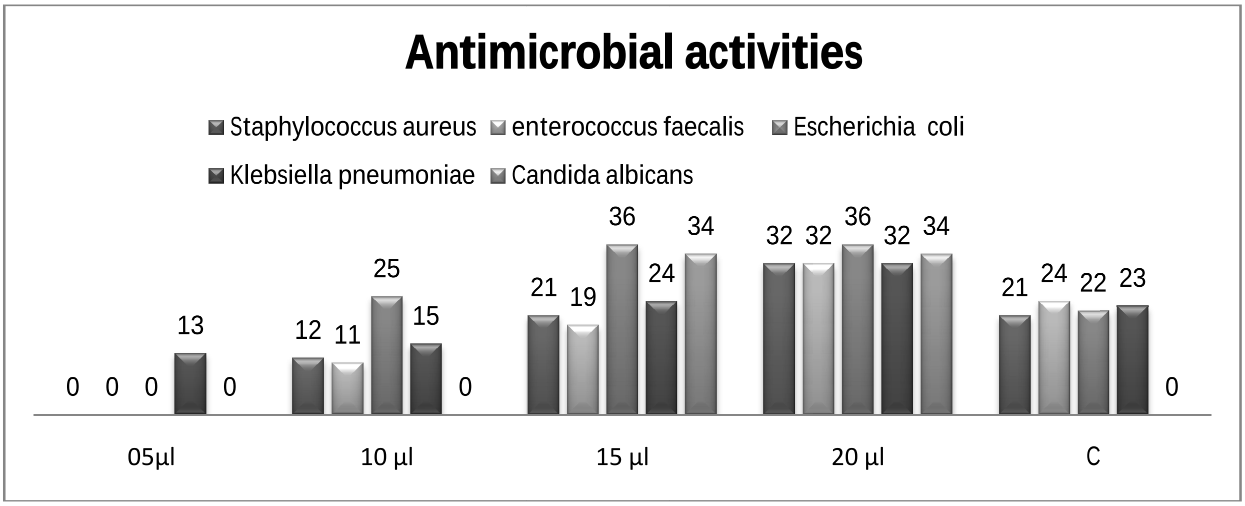

Fig .8 Graphical representation of antibacterial activities after 24 hours of incubation period.

IV. CONCLUSIONS

By using the precipitation process, $CaCO_3$/ZnO-NPs were synthesized. After being calcined at 500ºC, the $CaCO_3$/ZnO-NPs that were created have an average crystal-like size of 29.18 nm. The prepared NPs have a 71% crystallinity. By using Debye-formulas Scherrer's in conjunction with SEM pictures, an average basic dimension of about 30 nm was confirmed, and spongy-shaped, non-uniform, and non-homogeneous nanostructured $CaCO_3$/ZnO-NP powders were noted. According to EDX, $CaCO_3$/ZnO-NPs is suggested by the presence of the elements calcium, zinc, carbon, and oxygen. $CaCO_3$, protein and ZnO NPs are all present in the produced sample, according to the FTIR results. Sericin is present at 233 nm, ZnO NPs are present at 380 nm, and $CaCO_3$ is present at 816 nm, according to a UV absorption spectrum plot. The environmentally friendly synthesis of $CaCO_3$/ZnO-NPs demonstrates the antibacterial action against both bacteria and fungi. This nanomaterial permeates the surface of a cell membrane, damages it, and releases the contents of the cell. The size, shape, and concentration of nanoparticles all affect their antibacterial activity. Smaller particles may interact with bacteria at higher rates relative to larger particles because large nanoparticles allow broad surface areas to come into touch with them. Studies on the antibacterial properties of $CaCO_3$/ZnO NPs against Staphylococcus aureus, enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, and Candida albicans concluded that the results were more effective after 24 hours and that low concentrations of $CaCO_3$/ZnO inhibit bacterial growth.

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