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GREEN SYNTHESIS AND ANTIMICROBIAL ACTIVITY STUDIES OF CURCUMINANILINE FUNCTIONALIZED ZINC OXIDE NANOPARTICLES AND COMPARATIVE STUDIES WITH ITS NON-FUNCTIONALIZED FORM

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Keywords:

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ABSTRACT: Biofunctionalization of nanoparticles is an advancement of current nanotechnology and biotechnology fields. Developing biocompatible progressive functional materials possess antimicrobial properties could be hopeful for environmentally benevolent applications. Biofunctionalization of nanomaterials is one such topic to produce the non-toxic and more efficient antimicrobial agents. The present study describes a synthesis method of bioactive curcuminaniline functionalized zinc oxide nanoparticles in the eco-friendly methods and comparative studies of those were undertaken against non-functionalized zinc oxide nanoparticles. The synthesis process was carried out through a simple green methodology. Synthesized nanoparticles were analyzed for antibacterial activity by using disc diffusion method against two gram-positive bacteria and two gram-negative bacteria and antifungal activity by using agar well diffusion method against four funguses. Synthesized materials were characterized by UV- Vis, FT-IR, SEM and TEM techniques. The synthesized zinc oxide nanoparticles were in the range of 46 nm and the biofunctonalized nanoparticles were about 100 nm. The antimicrobial activities of those were showed appreciable inhibition zones against *S.typhi*, *B.subtilis*, *A.niger* and *T.simii* species.

INTRODUCTION: Nanotechnology is achieved incredible motivation due to its competence of controlling metals into their nanosize, which considerably changes the chemical, physical and optical properties of metals. This is typically because nanoparticles have a greater surface area per weight than larger particles which causes them to be more reactive to other molecules. [1, 2] Nanoparticles are widely used due to their multiple potential applications in material science, medicine, and industry.

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Even though nanoparticles possess good potential in variety of applications, people are increasingly concerned about the emergence of possible subsequent diseases caused by nanotechnology and application of nanomaterial.[3, 4] The development of a reliable and green chemistry process for biogenic synthesis of nanomaterial is akeyfeature of current nanotechnology research. [5]

Green synthesis of nanomaterials are of considerable interest in the nano field at present. It deals with the progress of nanotechnology through the design of greener nanoscale materials and finding the green-nano manufacturing methods. [6] Green synthesis focusses mainly avoiding of hazardous wastages, implementation of environmentally benign materials and renewable of reaction materials. Furthermore its low-cost, high

yields and short reaction times under standard reaction conditions are also the impact factor of green-nano preparation.[7, 8]

In the green-nanotechnology, various metal nanoparticles were synthesized through biological methods where microbes, plants, other natural materials and modified natural materials used either as reducing agents or stabilizing agents. Biological methods of nanoparticles preparation could be alternative for conventional methods, ecofriendly and does not cause any harm to human and domestic animals health. [9, 10] Plants are known to possess various healing compounds and due to its huge variety and availability plants have been explored constantly in the wide range of fields such as medicinal, agricultural, industrial etc. Biomolecules present in plant extracts can be used as a reducing agent in the nanoparticles preparation through a simple green synthesis process. This biogenic reduction of metal ion is reasonably rapid, readily conducted at ambient condition, and easily can be synthesized. The plant extracts utilized method is environmentally benign.[11, 12]

Nanomaterials possess prominent antimicrobial activity against several microbes; however, it has some non-specific toxicity. Biofunctionalization of nanomaterials is the advanced solution to find this issue. The selection of therapeutically active biomolecules to design the nanoparticles will positively increase the biological applications.[13] Biofunctionalization of nanomaterial can provide good biocompatibility to restrict the deployment of biomolecules and offering high specificity for biological recognition, which steered to stable biosensing systems with good selectivity and reproducibility.[14, 15]

The interaction of nanoparticles with biomolecules and microorganisms is an expanding field of research. Curcumin is well renowned biologically active natural material. Curcumin is dietary polyphenolic compounds present in the turmeric plant.[16, 17] The pharmacological activities of curcuminoids have been extensively investigated and the anti-cancer [18], anti-inflammatory [19], anti-viral [20] and antimicrobial properties [21] of these molecules are well recognized. The most important aspect of curcuminoids is the toxicity-free as shown in several researches. Thus, the various pharmacological effects and affordable safety profile make curcumin as attractive biomaterial for the biofunctionalization. However, various researches shown appreciable biological improvements while modified curcumin is used for biosynthesis of nanoparticles.[22, 23]

Many investigations have been focused on metal nanoparticles concerning antimicrobial applications to develop new and effective antimicrobial reagents free of resistance and cost. Recent studies have proved that metal oxide nanoparticles have good antibacterial activity and antimicrobial formulations consist of nanoparticles could be attractive bactericidal materials. Metal nanoparticles containing magnesium oxide, silver, iron, copper and nickel oxides are of great interest, because of each having different properties and its well established antimicrobial activities.[24, 25]

Zinc is an essential constituent for cell growth and in inhibiting bacterial enzymes like dehydrogenase and certain protective enzymes such as thiolperoxidase and glutathione reductase.[26] Various researches have shown that zincoxide nanoparticles could be used as potential bactericidal materials. The antimicrobial effect of Zn nanoparticles has been attributed due to their excellent reactivity, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution. Zn nanoparticle performs efficiently to resist microorganisms and has a longer life than organic based disinfectants and antimicrobial agents. Furthermore, the surface functionalization of zinc oxide nanoparticles with biomolecules may give highly improved antimicrobial agents.[27, 28]

Based upon the above discussion this study was carried out to find out the enhancement of antimicrobial activity by functionalization of bioactive molecule, curcuminaniline with zinc oxide nanoparticles. Firstly, curcuminaniline was synthesized from the curcumin thenzinc oxide nanoparticles were prepared by reducing zinc acetate with the help of lemon extract. Finally, curcuminaniline was functionalized with synthesized zinc oxide nanoparticles. The antimicrobial results of biofunctionalized zinc

oxide nanoparticles revealed efficient inhibition activity against bacterial strains and funguses tested.

MATERIALS AND METHODS:

The chemicals are of zinc acetate, aniline and ethanol were purchased from Merck (India) Ltd and turmeric curcumin was obtained from Agricultural College and Research Institute, Madurai, India. All the chemicals and solvents used were of analytical reagent grade. The modified biomaterial used for functionalization, i.e., curcuminaniline was prepared by using curcumin and aniline in the laboratory.

Collection of extracts:

In this study fruits of citrus lemon were used as a bioreductant for the synthesis of zinc oxide nanoparticles. The main reason for selecting this, since lemons are a rich source of citric acid and ascorbic acid and phytochemicals present in which acts as an effective reducing agent.[29, 30] Lemon fruits were cut into pieces and squeezed well to get about 10 ml pure extract. The extract was filtered and the filtrate was collected and stored.

Synthesis of curcuminaniline (CA):

The synthesis process was carried out according to our previous work.[31] Initially curcumin was isolated from turmeric sample and the isolated pure curcumin was used to prepare 10mM ethanolic solution which was mixed with aniline at the same mole concentration with constant stirring. The mixture color was changed to orange color and the refluxation was started at 50°C for 5 to 6 hours. Finally, the mixture was changed to orange colored fine precipitate, it was cooled, filtered and washed well with double distilled water thrice to remove unreacted chemicals and dried in vacuum oven at 100°C for 1 hour and stored in a desiccator over silica gel for further analyses.

Synthesis of Zincoxide nanoparticles (ZONP):

1mM aqueous solution of zinc acetate was prepared and used for the synthesis of ZONP. The lemon extract (10 ml) was mixed with freshly prepared zinc salt solution (10 ml) with constant stirring and kept in the magnetic hot stirrer at 50-60°C for a particular time to obtain reduced metal ions. The color changes from pale green to colorless was indicated the metal ion reduction. Freshly prepared curcumin extract (1mM) was mixed with zinc solution and the stirring was continued for 1 to 2 hours. The changes in color observed during the reaction from yellow to yellowish brown indicating the stabilization process and finally a permanent dark brown color was obtained which indicated the complete stabilized ZONP. The reaction pH was maintained constantly in between 3 to 4 throughout the experiment. The mixture was centrifuged and washed several times to obtain the pure ZONP. The supernatant was decanted and kept in oven to dryness.

Biofunctionalization of zinc oxide Nanoparticles (ZNCA): In this scheme biofunctionalization of curcuminaniline with zinc oxide nanoparticles were carried out under constant stirring and heating at 60°C. The synthesized ZONP (1 mM) were mixed with 1mM of curcuminaniline with constant stirring and heating for an hour. The mixture color was started to change from reddish brown color slowly and further continued for an hour. Finally, the brown color mixture was obtained which denoted the strong functionalization of curcuminaniline with zinc oxide nanoparticles. The solution was centrifuged and the upper layer was removed and dried for further analyses.

Biological Assay:

The antibacterial activity of the samples were tested by disc diffusion method against two gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), two gram negative bacteria (*Escherichia coli* and *Salmonella typhi*) and antifungal activity was carried out by agar well diffusion method against four funguses (*Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii*).

The antibacterial activity of samples was measured by disc diffusion method.[32] In this method, the stock cultures were incubated in nutrient agar and transferred to Muller-Hinton broth (MHB) contained test tube for bacteria that were incubated for 24 hours at 37°C. The cultures were diluted with fresh Muller-Hinton broth to obtain 2.0×10^6 CFU/ml for bacteria. The Muller Hinton Agar (MHA) plates were prepared by pouring 15 ml of molten media into sterile petri plates. The sample

was loaded on the surface of the cultured agar plates and incubated at 37°C for 24 hours. The inhibition zones observed around the disc were measured and the results were compared with standard antibiotic, Chloramphenicol.

The antifungal activity of samples were measured by agar well diffusion method, [33] the fungal strains were suspended in sabouraud's dextrose broth for 6 hours to give concentration 10^5 CFU/ml and then inoculated with the culture medium. A total of 8 mm diameter wells were punched into the agar and filled with the sample and solvent blanks (hydro alcohol and hexane). Standard antibiotic, Fluconazole (concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hours. The diameters of zone of inhibition observed were measured.

RESULTS:

UV-Vis spectroscopy is the most convenient technique for nanoparticles characterization. The UV-Visible absorption spectra of the samples were measured on a Shimadzu UV-Vis V-530A spectrophotometer in the range of 300 to 900 nm. **Fig. 1** and **2** shows a UV spectrum of zinc oxide nanoparticles and curcuminaniline functionalized zinc oxide nanoparticles respectively. ZONP were observed two important peaks at 300 nm and 225 nm which are very clearly shown in the **Fig.1**. Biofunctionalized zinc oxide nanoparticles exposed two small broad peaks at 301 nm and 248 nm which are slightly higher in the wavelength compared with non-functionalized form.

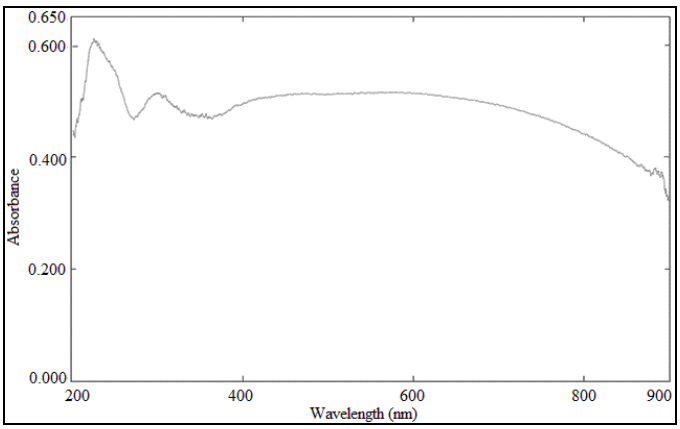

FIG. 1: UV SPECTRA OF ZONP

FT-IR spectroscopy was used to investigate the interactions between different species and changes in chemical compositions of the mixtures. FT-IR spectra analysis was recorded on a Jasso FT-IR/4100 spectrophotometer with 4 cm^{-1} resolution in the range of 4000 to 400 cm^{-1}. **Fig. 3** and **4** shows the FT-IR spectrum of zinc oxide nanoparticles and biofunctionalized ZONP respectively. The reasonable difference was observed between these in the ranges from 1700 cm^{-1} to 800 cm^{-1} due to the functionalization of curcuminaniline.

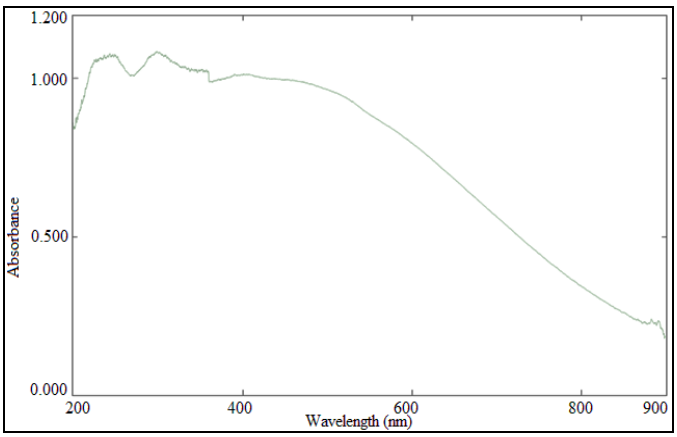

FIG. 2: UV SPECTRA OF ZNCA

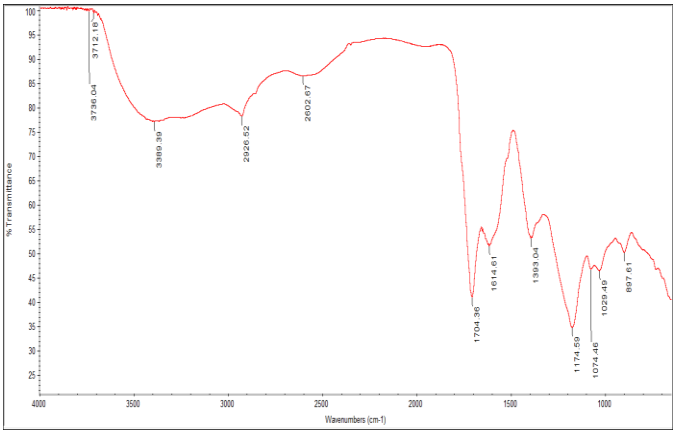

FIG. 3: IR SPECTRA OF ZONP

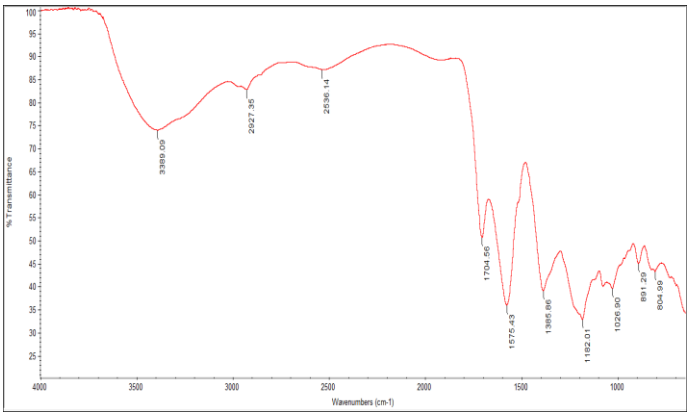

FIG. 4: IR SPECTRA OF ZNCA

Morphology of synthesized zinc nanoparticles was characterized by SEM analysis. Scanning electron microscopy (SEM) images were recorded by using JEOL Model JSM - 6390LV scanning electron microscope. The samples were kept in an evacuated chamber and scanned in a controlled pattern by an electron beam. Interaction of the electron beam with the specimen yields a variety of physical phenomenon that detected, were used to form images and provide information about the specimens. The SEM image of ZONP and ZNCA were shown in **Fig. 5a** and **5b** respectively.

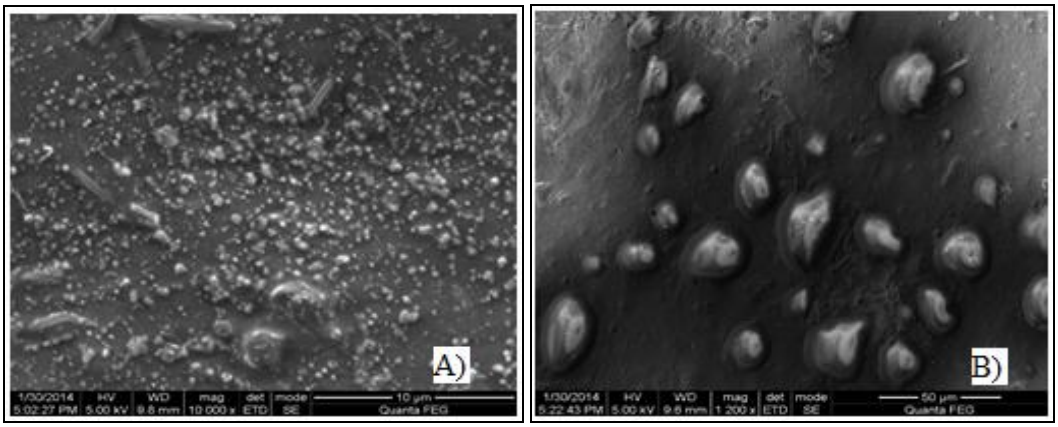

FIG. 5: SEM IMAGES OF ZONP (A); ZNCA (B)

The synthesized zinc oxide nanoparticles were characterized by TEM technique in depth to detect accurate morphology and size. High resolution transmission electron microscopy (HRTEM) was carried out using a 300 KV JEOL-3011 instrument with an ultrahigh resolution pole piece to determine the size and morphological changes. **Fig. 6a** and **6b** shows the TEM images of the non-functionalized and biofunctionalized zinc oxide nanoparticles respectively.

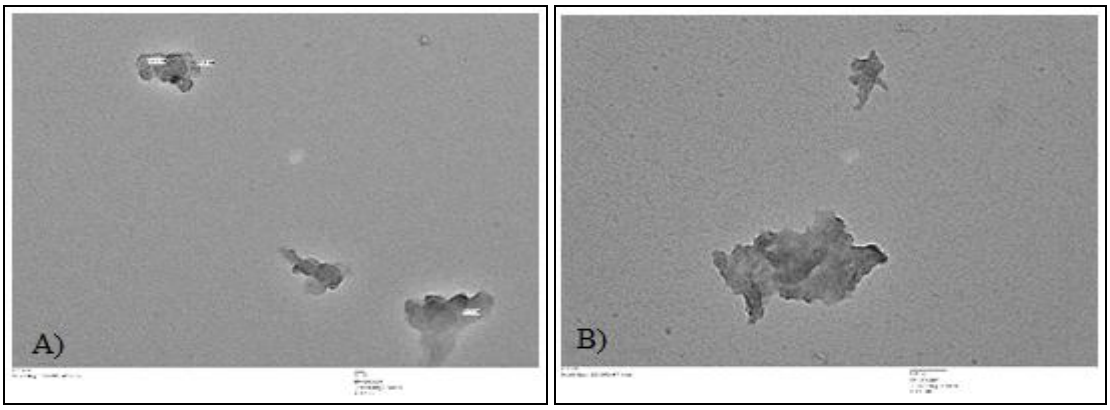

FIG. 6: TEM IMAGES OF ZONP (A); ZNCA (B)

Antibacterial Activity:

The antibacterial activities of curcuminaniline, ZONP and ZNCA against two gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) were evaluated and their activity was compared to a well-known commercial antibiotic Chloramphenicol. The results are reported in **Table 1**.

TABLE 1: ANTIBACTERIAL ACTIVITY EVALUATION

Bacterial Species	Zone of inhibition diameter (mm sample^{-1})			
	Standard drug (C)	Curcuminaniline (CA)	Zinc oxide nanoparticles (ZONP)	Biofunctionalized zinc oxide nanoparticles (ZNCA)
S. aureus	13	09	14	04
B.subtilis	15	14	15	17
E. coli	11	07	08	10
S.typhi	14	10	13	15

Antifungal Activity:

Curcuminaniline, ZONP and ZNCA were determined for their antifungal activity against four fungal strains *Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii* and their activity was compared with standard antifungal drug Fluconazole. The results were shown in the **Table 2**.

TABLE 2: ANTIFUNGAL ACTIVITY EVALUATION

Fungal Species	Zone of inhibition diameter (mm sample^{-1})			
	Standard drug (C)	Curcuminaniline (CA)	Zinc oxide nanoparticles (ZONP)	Biofunctionalized zinc oxide nanoparticles (ZNCA)
C.albicans	16	09	12	14
C.lunata	15	11	13	12
A.niger	15	14	17	20
T.simii	13	13	15	16

DISCUSSION: The formation of zinc oxide nanoparticles can be confirmed by the easy view of color change during the reaction. The color change from pale green to colorless was obtained due to zinc metal reduction and yellowish color changes to brown color which denoted the nanoparticle stabilization by the stabilizing agent.

The UV absorption spectra of zinc oxide nanoparticles (**Fig. 1)** exhibited at 300 nm corresponding to the absorption of zinc oxide nanoparticles and the other peak observed at 225 nm could be due to the presence of zinc oxide nanoparticles aggregation which was observed at the below range of 300 nm. The absorption spectra of ZNCA (**Fig. 2**) exhibited a broad peak at 301 nm corresponding to the absorption of zinc oxide nanoparticles. The sharp peak observed on the spectra of ZONP was broadened and exhibited at 248 nm which could be due to the combination of azomethine compound presence in the curcuminaniline with zinc oxide nanoparticles.

From the IR data obtained (**Fig. 3** and **4**), the weak broad band observed in the range of 3500-3200 cm^{-1} which is assigned to ph-OH group of curcumin moiety in both ZONP and ZNCA. The peak observed at 2913 cm^{-1} which can be assigned to the –OH stretching of water or ethanol present in the system. The C=O stretching of curcumin at 1625 cm^{-1} was shifted to a higher wave number at 1700 cm^{-1} in ZONP and 1714 cm^{-1} in ZNCA due to interaction with zinc nanoparticles. The important sharp peak of biofunctionalized form exhibited at 1557 cm^{-1} was assigned to azomethine compound of curcuminaniline which was interacted with zinc oxide nanoparticles and reached higher wave number. The characteristic peaks in the range of 1520–1350 cm^{-1}in both formulations conforms the aromatic unsaturation (C=C) of stabilized curcumin system and the (C-O) band presence was assigned by the peaks found at 1000-1250 cm^{-1}.

From the SEM images of Zn oxide nanoparticles, it can be view that ZONP shown an agglomerated crystal and rod shaped morphology of material (**Fig. 5a**). In the case of biofunctionalized zinc oxide nanoparticles, the image exhibited a clear dot or spherical shaped morphology with slight agglomeration due to the nanoparticles oxidation (**Fig. 5b**).

From TEM images, both zinc oxide nanoparticles and biofunctionalized nanoparticles are moderately dispersed and the average crystallite size of particles is about 40 nm (**Fig. 6a**) and 100 nm (**Fig. 6b)** respectively. They have exhibitedthe images in the different shaped morphology and slightly agglomerated. ZNCA shown the particles size which is higher than the non-functionalized form due to might be the interaction of curcuminaniline. From this we can conclude that the functionalization of curcuminaniline with zinc oxide nanoparticles was carried out well.

The antibacterial and antifungal results of biofunctionalized zinc oxide nanoparticles shown satisfactory improvement than the inhibition results observed by non-functionalized nanoparticles as well as curcuminaniline. From **Table 1** results, non-functionalized zinc oxide nanoparticles displayed reasonable antibacterial activity against *S.aureus*, *B.subtilis* and *S.typhi* species. But remarkably zinc oxide nanoparticles exposed an

excellent inhibition activity when functionalized with curcuminaniline against *B.subtilis, E.coli* and *S.typhi* bacterial strains. The enhancement in the activity is due to the biomolecule functionalization which increases the antimicrobial efficiency to the nanoparticles.

Especially, it showed higher inhibition zone results than standard drug chloramphenicol against *S.typhi* and *B.subtilis* strains. From **Table 2** results, zinc oxide nanoparticles showed affordable antifungal activity against *A.niger* and *T.simii* fungal strains, it has displayed higher inhibition results when functionalized with curcuminaniline against the same fungal species which meant by biofunctionalization is the vital role in the result increment. Interestingly, it showed higher activity than the standard drug against *A.niger* and *T.simii* funguses.

CONCLUSION: In summary, this investigation has been carried out in the green process manner to synthesize biologically well improved curcuminaniline functionalized zinc oxide nanoparticles by interacting zinc oxide nanoparticles which was synthesized by reducing the metal with citrus lemon extract with the modified curcumin biomaterial, curcuminaniline which was prepared by the refluxation of curcumin and aniline. Synthesized zinc oxide nanoparticles and biofunctionalized zinc oxide nanoparticles morphology studies are investigated and itshowed the particle size of ZONP was about46 nm and biofunctionalized nanoparticles was in the range of100 nm.

The antimicrobial activity studies were displayed that synthesized ZONP were shown reasonable inhibition activity but the biofunctionalized zinc oxide nanoparticles were showed better activity than the non-functionalized nanoparticles against *S.typhi, B.subtilis, A.niger* and *T.simii*. Particularly, ZNCA shown appreciable activity than the standard drug against all the above species. Therefore, our report reveals that zinc oxide nanoparticles exposed excellent antimicrobial activity while functionalized with modified curcumin biomaterial, i.e., curcuminaniline than the natural curcumin which gives the new way in the biomaterial utilized nanoparticle preparation.

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CONFLICT OF INTEREST: The authors declare that they have no competing interests.

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