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Lignin-Mediated Biosynthesis of ZnO and TiO₂ Nanocomposites for Enhanced Antimicrobial Activity

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Abstract: In this work, we report the synthesis of fragmented lignin (FL) assisted zinc oxide (ZnO) and titanium oxide (TiO₂) nanocomposites. The fragmented lignin synthesized from biomass (sugarcane bagasse) was used as a template to generate the morphology and crystallite structure of metal oxide nanomaterial. The nanocomposites were synthesized by a simple precipitation method, wherein fragmented lignin is used in alkaline medium as a template. X-ray diffraction (XRD) analysis shows the phase formation of hexagonal wurtzite ZnO and mixed phase formation of TiO₂ as rutile and anatase. The morphology was studied by using field emission scanning electron microscopy (FE-SEM) and high-resolution transmission electron microscopy (HRTEM). The FE-SEM of pristine ZnO nanocomposites showed a cluster of particles whereas FL-ZnO NPs showed self-aligned nanoparticles in the form of rod shaped having average size 30–70 nm. Pristine TiO₂ nanoparticles showed clusters of particles and FL-TiO₂ nanocomposites showed well crystalline 41nm size nanocomposites. The FL acts as a surfactant which restrict the cluster formations. The band gap determined by diffuse reflectance spectra is 3.10 eV and 3.20 eV for FL-ZnO and FL-TiO₂ nanocomposites, respectively. Photoluminescence spectra of both nanocomposites showed structural defects in the visible region. Further, the antimicrobial activity of pristine ZnO and TiO₂ nanoparticles, and FL-ZnO and FL-TiO₂ nanocomposites against *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC25923) were studied under UV-A (315–400 nm) (8W) for 30min.

Keywords: ZnO; TiO₂; fragmented lignin; *E. coli*; *S. aureus*. Nanorods

1. Introduction

One of the greatest challenges of the twenty-first century is the spread of multidrug resistance in microorganisms [1]. Microbial infections are common in humans, but the development and spread of drug resistance in microorganisms has made the present antimicrobial therapy ineffective [2]. Infections that were once easily treatable have become increasingly more difficult to treat and result in higher morbidity and mortality. Therefore, in order to overcome the inability of antibiotics to deal with the rising issue of resistance in microorganisms, the need for the development of novel, broad spectrum antimicrobials have arisen [3,4]. Various contemporary novel approaches including combination drug therapy, bacteriophage therapy, fecal microbial transplantation, antimicrobial adjuvants, and

antimicrobial peptides have been tried to address the issues of resistance in microbes, however, either these have met with limited success or have failed to achieve the goal [5,6]. Recently, the advancement in field of nanotechnology has contributed towards the synthesis various antimicrobial nanomaterials with the aim to develop meta and metal oxide-based antimicrobials [7]. The nanomaterials are generally considered to be particles with at least one dimension measuring around 1–100 nm and show unique properties which change significantly with size and differ from bulk material properties, due to the great increase in surface area to volume ratio, leading to an increased number of surface atom interactions with their surroundings. Amongst all nanomaterials, semiconductor-based nanomaterials have showed wide applications including antimicrobial agents [8]. Among them, one dimensional (1D) nanostructures such as nanowires, nanorods and nanobelts have shown potentials antimicrobial activities due to their distinctive geometrical morphologies, novel physical and chemical properties [9]. One dimensional ZnO nanomaterials with a characteristic direct wide band gap of about 3.37 eV and large excitation binding energy of 60 meV at room temperature have been shown to be nontoxic and good chemical stabilities were widely explored for antimicrobial applications due to its photocatalytically phenomenon associated with it [10]. To date, several approaches have been developed for the synthesis of 1D ZnO nanostructures, such as sputtering method, physical, chemical, and pulsed laser vapor deposition [11]. However, most of these methods often faced with problems such as complex procedures, high temperature, and cost. The other problem being the aggregations behaviors and difficult dispersibility which reduces the interface compatibility of nanomaterials with other surfaces [12]. Thus, the template-based chemical-based method which has an advantage of simplicity and low cost, has become the most promising methods for synthesis of ZnO nanomaterials [13]. In chemical-based method, ZnO are synthesized on the template of organic polymer to achieve polydispersity and increase the interface compatibility of nanomaterials with other surfaces. Several natural polymers such as DNA, silk, albumen, orange juice, pea starch, peptide structures, etc., have been used as templates for the synthesis of ZnO [14]. However, one of the natural polymers, called lignin, which is available on earth in a huge quantity is relatively unexplored for the synthesis of ZnO nanomaterials [15]. The lignin is a “renewable chemical resource” formed from the assembling of functionalized aromatic entities with phenolic hydroxyl, alcoholic hydroxyl, carboxyl, or methoxy groups [16,17]. Lignin has many potential value-added applications with significant impact on industry. For example, derivation of lignin leads to functional polymers with a role of dispersant for pesticide, surfactants, additive in oil drilling, stabilizers in colloidal suspensions, antioxidants, antiviral, antibiotic, and/or anticarcinogenic agents, etc. [18–20]. Importantly, over 50 million tons of industrial lignin is produced from the paper-making industry as a by-product every year [21]. However, only 2% of lignin is effectively unitized and more than 98% of lignin is combusted as fuel, which not only causes environmental problems but also causes a huge waste of the resources [22]. Therefore, in the present work, a simple and environmentally friendly in-situ template fragmented lignin (FL)-assisted synthesis method is reported for controllable preparation of FL–ZnO and FL–TiO₂ nanocomposites. The FL–ZnO and FL–TiO₂ nanocomposites were characterized by UV-Visible, high resolution mass spectrometry (HRMS), X-ray diffraction (XRD), field emission scanning electron microscopy (FE-SEM), TEM and photoluminescence (PL) spectroscopy. Finally, the antimicrobial potential of the FL–ZnO and FL–TiO₂ nanocomposites were explored by using model organisms, *Escherichia coli* (ATCC25922) and *Staphylococcus aureus* (ATCC25923). The main objective of this research is to determine the functional significance of fragmented lignin (template) assisted synthesis of ZnO and TiO₂ nanocomposites for improving the antimicrobial property of ZnO and TiO₂ nanoparticles.

2. Materials and Methods

2.1. Material

The materials in this work are Bagasse, Zinc acetate, Titanium isopropoxide, Sodium hydroxide, and *Escherichia coli* (ATCC25922), and *Staphylococcus aureus* (ATCC25923).

2.1.1. Fragmentation of Lignin

Commercial lignin (Sigma-Aldrich, St. Louis, MO, USA) was used to prepare fragments of lignin. 5 g of purified and air-dried lignin was dissolved in 50 mL 0.1M sodium hydroxide (NaOH) solution (pH 12) then stirred for 30 min at temperature 40–50 °C. After this, 100 mL 1% hydrogen peroxide (H₂O₂) solution was added to above solution in a drop wise manner over the period of 1 h. The resultant solution was filtered, cooled and acidified with sulphuric acid (H₂SO₄) up to pH 4.5–5 and the ivory colour precipitation used as FL.

2.1.2. Synthesis of FL–ZnO Nanocomposites and Pristine ZnO Nanoparticles.

All reagents are of analytical grade without further purification, supplied by Loba Chemie Pvt. Ltd. (Mumbai, India). 0.1 g FL and 0.1 M 100 mL NaOH were sonicate for 1 h to get the homogenous solution. After sonication the solution was heated as well as stirred and maintaining temperature between 80 to 90 °C. Then 2 g of zinc acetate was added to the solution over a period of 30 min. again stirred for 1 h. A white color precipitate was obtained. It was wash with water followed by absolute ethanol to remove the residual fragmented lignin and sodium hydroxide. The precipitate was dried in an oven at 90 °C for 1 h, followed by calcination at 450 °C in muffle furnace for 3h. Similar procedure was followed to prepare pristine ZnO nanoparticles except the addition of FL.

2.1.3. Synthesis of FL–TiO₂ Nanocomposites and Pristine TiO₂ Nanoparticles

4 mL Titanium isopropoxide and 25 mL isopropanol mixture was added slowly in the solution of 1 N 100 mL NaOH and 0.1 g of FL at 0 °C up to 3 h. A precipitation formed was washed several times with 0.01 N hydrochloric acid, water and finally with ethanol. Then it was dried in an oven at 110 °C for 1 h followed calcination at 450 °C for 3 h. Similar procedure was followed to prepare pristine TiO₂ nanoparticles except the addition of FL.

3. Characterization

The phase purity and crystallinity of the nanocomposites and nanoparticles were performed by X-ray diffraction studies by using Cu K α 1 (1.5406 angstrom) radiation scanned in the 2 θ range from 10° to 80°, and photoluminescence (PL) spectroscopy optical properties by using UV-visible spectrophotometer in the range from 300–800 nm, morphology by using field emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM).

Antimicrobial Property of Biosynthesis FL–ZnO and FL–TiO₂ Nanomaterial with Pristine ZnO and TiO₂ Material

The standard strain of *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) obtain from Microbiology Laboratory Fergusson College Pune were used for antimicrobial studies. The antimicrobial activity was performed as per the guideline of Clinical and Laboratory Standards Institute [23]. It was performed by measuring the minimum bactericidal concentration (MBC) values of composites and pristine nanoparticles against standard strains of microorganisms. In short, 0.1 mL of 1×10^4 cells/mL was inoculated into 1 mL volume of nutrient broth containing different concentrations of nanocomposites and pristine nanoparticles (0.5–1.5 mg/mL). After inoculation all sets were irradiated in UV-A for 30 min. Then each sample was spread on nutrient agar plate and incubated at 35 ± 2 °C for 24 h. Colonies appeared were counted to calculate the MBC value. Nutrient agar plate with microorganism and without nanoparticles served as control. Similar procedure with standard antibiotics, amoxicillin, served as positive control. The experiments were performed in triplicate and the average measurement were reported.

4. Results and Discussions

4.1. Structural Study

Crystalline phase and lattice parameters of FL–ZnO and FL–TiO₂ nanocomposites, and pristine ZnO and TiO₂ nanoparticle were obtained from recorded XRD spectra (Figure 1). The crystal size was calculated by using the Debye–Scherrer equation:

$$\beta(2\theta) = \frac{K\lambda}{L\cos\theta},$$

where K—Scherrer constant. K varies from 0.68 to 2.08. K = 0.94 for spherical crystallites with cubic symmetry, L—volume average of the crystal thickness in the directional normal to the reflecting planes, λ —X-ray wavelength. For Mini XRD, Cu K α average = 1.54178 Å, θ —XRD peak position, one half of 2 θ .

The major peaks in XRD of FL–ZnO nanocomposites and pristine ZnO do not show any difference in peak positions but an appreciable difference in peak intensities were observed. The observed peak at 2 θ = 31.610, 34.320, 36.140, 47.370, 56.480, 62.710 and 68.280 correspond to (100), (002), (101), (110), (103) and (201) planes these are characteristic peaks of hexagonal crystalline structure of ZnO (JCPDS file 36-1451 of ZnO). Sharp and intense peaks in the XRD analyses of both F–ZnO nanocomposites and pristine ZnO indicate high crystallinity and the polycrystalline nature of ZnO nanoparticles (curves a and b Figure 1). Moreover, no extra peaks other than hexagonal crystalline phase of ZnO were found in XRD. Pristine ZnO has average crystallite size 43.76. High intensity of peak at (101) plane shows a weak preferential growth in FL–ZnO nanoparticles along c-axis [24] with an average crystallite size 25.34 nm. The XRD of FL–TiO₂ nanocomposites showed a mix phase of anatase and rutile (JCPDS anatase 33-1381, and Rutile 34-0180) which shows the major peaks at 2 θ = 25.30, 27.380, 36.08 37.730, 41.24 48.020, 54.320, 55.068, 62.840, 68.780, and 70.250 (curve c in Figure 1). The observed peaks (27.23, 36.08 41.24, and 54.32) at (110) (101), (111) and (211) can be indexed is a typical characteristic of rutile phase TiO₂. The peaks 25.30, 37.730, 48.020, 53.068, 62.11, 62.840, and 68.780, and 70.250 at (101), (004), (200), (105), (211), (213), (116) and (220) is typical of anatase phase TiO₂ (curve d in Figure 1). The pristine TiO₂ showed pure anatase phase (curve d in Figure 1) with major peaks 25.380, 37.730, 48.910, 53.980, 55.980, 62.840, 68.830, 70.160 and 75.030 indexed at (101), (004), (200), (105), (211), (213), (116), (220), and (215) planes. The FTIR and HRMS spectra of FL gives the information of functional groups present in the FL such as 679, 850, 1029–1150, 1629, 2829–2926, and 3354 cm^{−1} for aromatic ring, aromatic ring (Para Substituted), CNO stretching, C–O–C stretching, aromatic compound, CH₂ stretching, hydroxyl group broad band (Intramolecular Hydrogen Bonding), HRMS showed five different fragments as (1) m/z 276 gives 4-(4-(hydroxymethyl)-3-methoxyphenoxy)-2-methoxyphenol, (2) m/z 290 suggest the β -etheral linkage of phenolic structure, (3) m/z 322 is for Sinapic acid (3-(4-hydroxy 3, 5-dimethyl phenyl), (4) m/z 405 is for dimeric fragments, (5) 534m/z for β -O-4 dimer with attachment of different carbon atom or –CH₂ group present in the molecule, ¹H NMR of FL. ¹H NMR (400MHz, MeOD): δ 0.84, δ 1.2, δ 1.52, δ 2, δ 2.3, δ 3.8, and δ 6.5–7.8 showed presence of methylene proton, ethylene proton, acetylene proton and aromatic proton (SEI-1 FTIR of Fragmented Lignin, SEI-2 ¹H-NMR of Fragmented Lignin and SEI-3 HRMS of Fragmented Lignin in Supplementary Materials).

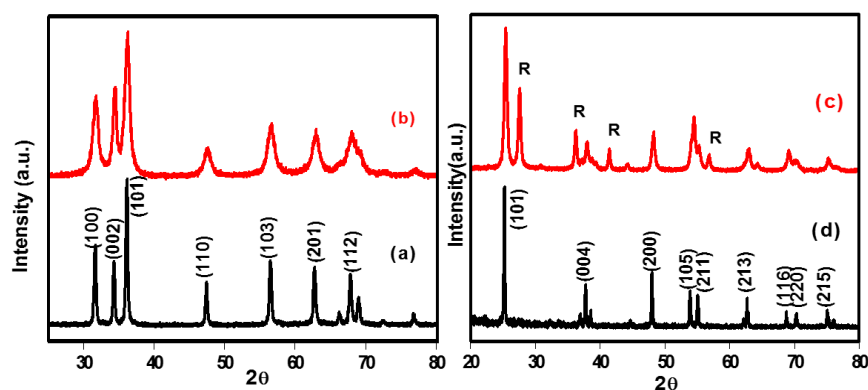


Figure 1. X-ray diffraction (XRD) of (a) Pristine ZnO, (b) FL-ZnO, (c) FL-TiO₂ and (d) Pristine TiO₂.

4.2. Surface Morphological Studies

The surface morphology was studied by FE-SEM. The FE-SEM image of FL-ZnO exhibits rod shape morphology given in Figure 2c,d, it is possible due to the inhibition of the growth of nanoparticle in third dimension by fragmented lignin.

The FE-SEM images of pristine ZnO (Figure 2a,b) showed an agglomeration. FL contain many polar functional groups mainly -OH , -OCH_3 , >C=O and C-O-C which might play an important role in the formation of ZnO rod nanoparticle. Due to the presence of these functional groups lignin fragments may have acted as a complexing, capping or stabilizing agent for Zn^{+2} ions. The polymeric nature of lignin fragments can create protective and functionalized surrounding for metal ions which might be playing a structure directing role in the formation of rod shaped ZnO nanoparticles [25]. Difference in particle morphology of of pristine TiO₂ and FL-TiO₂ was observed in Figure 2e,f and Figure 2g,h. FL-TiO₂ showed uniformity in grain size and shape. The grains were roughly spherical to spherically elongate in shapes. The average grain size for this FL-TiO₂ was 41 nm. Pristine TiO₂ showed grains of large and varied size with roughly spherical in shape with an average grain size of 277 nm. Here the anisotropic effect was observed in both samples. The peak of FL-ZnO and FL-TiO₂ show broad peak width due to the decrease in crystal size as compare to the pristine ZnO and TiO₂.

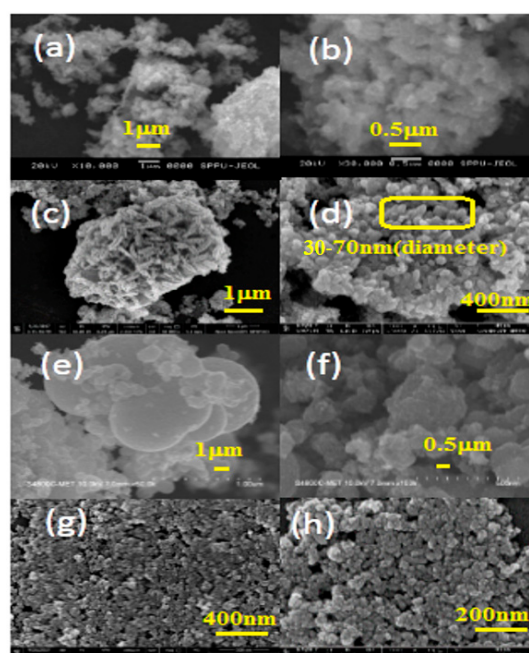


Figure 2. Field emission scanning electron microscopy (FE-SEM): (a,b) Pristine ZnO, (c,d) FL-ZnO, (e,f) Pristine TiO₂ and (g,h) FL-TiO₂.

4.3. TEM

Under TEM, FL-TiO₂ showed spherical morphology with average particle size is 14 nm (Figure 3a). FL-ZnO has rod shaped structure composed of spherical particles with self-aligned nature having average size 20 nm diameter. This is one directional growth along (101) plane [26]. XRD showed the highest intensity peak (101) plane of the FL-ZnO, which may be favored by biosynthesis method using fragments lignin as a template given in Figure 3b.

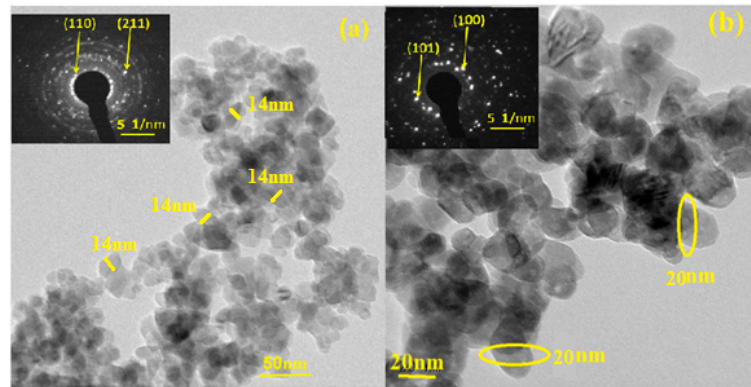


Figure 3. TEM of (a) Spherical morphology of FL-TiO₂, (b) self-aligned in one direction Spherical particle of FL-ZnO.

High-Resolution Transmission Electron Microscopy (HRTEM)

The interplanar distance of FL-ZnO is 0.247 nm for (101) plane. Both the technique XRD and HRTEM shows the mixed phase of FL-TiO₂ nanoparticles. For anatase plane (110) with an inter planer distance of 0.266 nm and for the rutile phase (211) the inter planer distance is 0.324 nm. The mixed phase formation of FL-TiO₂ and one directional growth of nanoparticle were due to the use of FL which act as a surfactant as shown in Figure 4a,b.

The fast Fourier transformation (FFT) gives the information of the lattice fringes on HRTEM image and it can use to index the observed spots. In given Figure 4c,d corresponding to the fast Fourier transformation (FFT) pattern of the nanoparticle is closed to single crystalline. From these observations, it is reasonable that the as-prepared ZnO and TiO₂ nanoparticle is of nanocrystalline structures, which means that they are constituted by well-crystallized ZnO and TiO₂ nanoparticles.

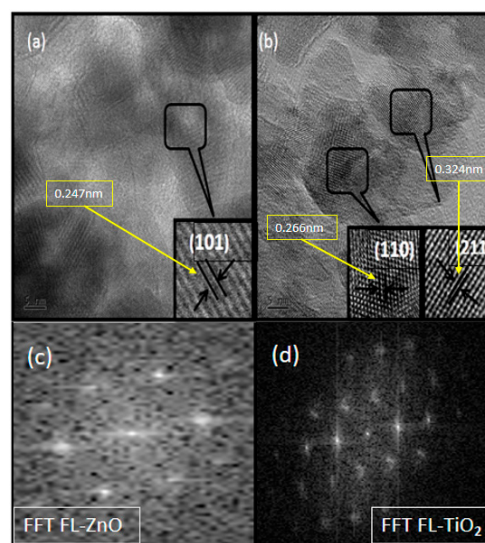


Figure 4. High-resolution transmission electron microscopy (HRTEM): (a) FL-ZnO, (b) FL-TiO₂, (c) FFT FL-ZnO and (d) FFT FL-TiO₂.

4.4. Optical Study

The Ultra-Violet diffuse reflectance spectra (UVDRS) of pristine ZnO, TiO₂, FL-ZnO and FL-TiO₂ were shown in set of Figure 5. It was calculated from a UV-Visible spectrum and Tauc's plot. All these materials showed absorbance in a range of 350–400 nm. The calculated band gap of FL-ZnO nanoparticles in presence of FL at 3.10 eV and pristine ZnO is 3.26 eV. More is the oxygen defects lower is the band gap [27]. Deviation in the band gaps of FL-TiO₂, and pristine TiO₂ was observed in curves b and d in Figure 5. FL-TiO₂ showed the band gap of 3.20 eV while pristine TiO₂ has band gap of 3.37 eV.

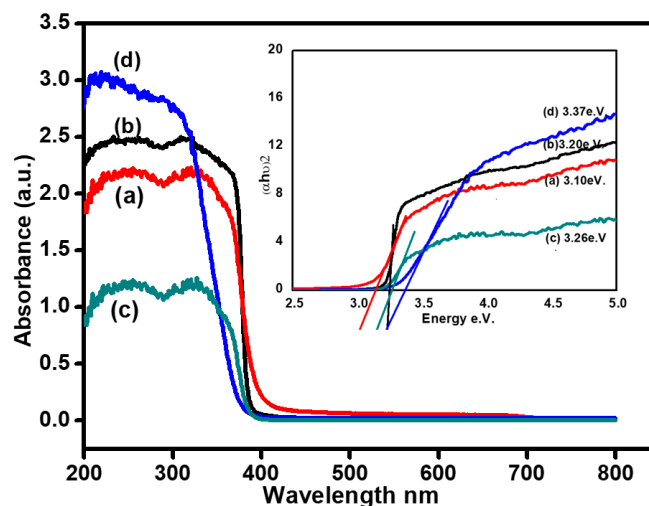


Figure 5. UV-Visible spectrum and UV-diffuse reflectance spectrum of ZnO and TiO₂ as (a) FL-ZnO 3.10 eV, (b) FL-TiO₂, 3.20 eV. (c)Pristine ZnO 3.20 eV and (d) Pristine TiO₂ 3.37 eV.

4.5. Photoluminescence Study of Pristine ZnO, FL-ZnO, Pristine TiO₂ and FL-TiO₂

Photoluminescence study gives us the information of the structural defects in nanoparticles. The Figures 6 and 7 showed photoluminescence property of nanoparticles of pristine ZnO, FL-ZnO, pristine TiO₂, and FL-TiO₂ synthesized. ZnO samples were excited at 390 nm excitation wavelength and TiO₂ samples were excited at 395 nm excitation wavelength. The emission spectrum of pristine ZnO, FL-ZnO, Pristine TiO₂ and FL-TiO₂ samples were showed in Figures 6 and 7. The different types of peaks were observed in visible region from 400 to 600 nm, such as 400–436 nm (violet region), 436–497 nm (blue region), 497–568 nm (green region) and 568–592 nm (yellow region).

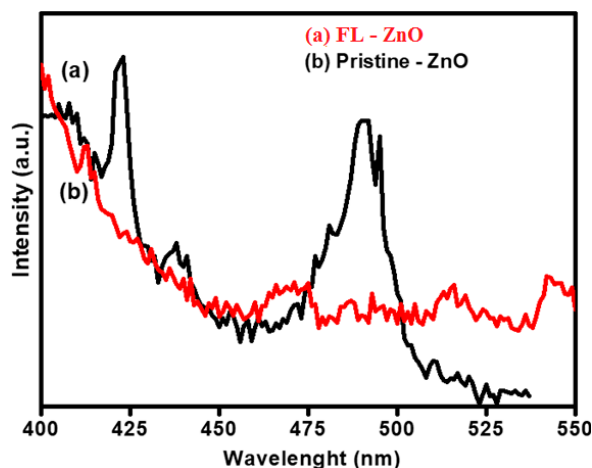


Figure 6. Photoluminescence spectra of ZnO materials excited at 390 nm excitation wavelength (a) FL-ZnO and (b) Pristine ZnO.

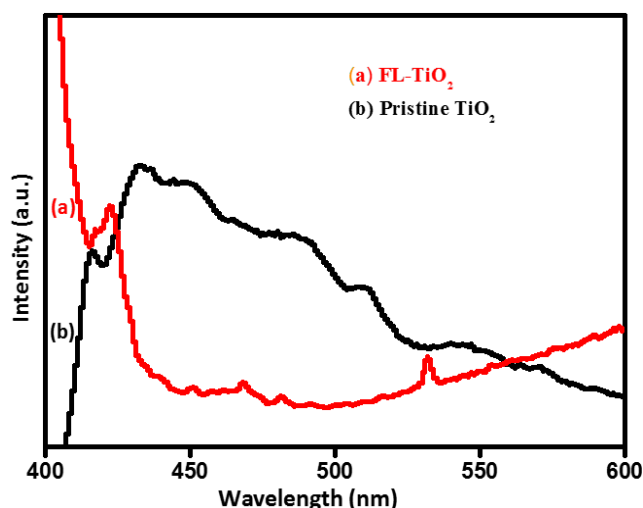


Figure 7. Photoluminescence spectra of TiO_2 material excited at 395 nm excitation wavelength (a) FL- TiO_2 and (b) Pristine TiO_2 .

FL-ZnO has highest peak intensity than pristine ZnO where pristine ZnO has no specific peak shoulder in spectrum (Figure 6). For FL-ZnO the peak intensity area was 400–436 nm (violet region), 436–497 nm (blue region) which gives more oxygen defect in visible region. The blue green region are due to the lattice defects which can produce compressive strain at intrinsic crystal lattice [28]. It causes the transition of energy from conduction band or zinc interstitials, which results the recombination of holes and electrons in the valences band and conduction band [29]. Pristine TiO_2 showed broad emission band because agglomerated lattice sites and peak intensity shift towards 568–592 nm (yellow–red region) which is significant for increase in particle size (Figure 7) caused by oxygen interstitials zinc vacancies and oxygen interstitials. It may conclude that O^-/O_2^- ion concentration increases intrinsic strain in the lattice of the materials, which gives transition from conduction band to the oxygen interstitial position yellow emission [30,31]. In FL- TiO_2 only two peaks were observed, one at 400–436 nm (violet region), and the other at 497–568 nm (green region) due to structural defects related to the deep level emission [32].

5. Antimicrobial Property

Nanoparticles have emerged as an effective antimicrobial-agent alternative to traditional organic based drugs, primarily due to actions that specifically target and minimize toxicity [33–35]. Most studies reported bacteriostatic or bactericidal effect of nanoparticles due to disruption of their cell membrane. The nanoparticle can accommodate a large number of ligands present on microbial cells due to its large surface area to volume ratio. Several types of metal and metal oxide nanoparticles have been already reported to possess anti-microbial property like titanium oxide, gold, silver, copper, iron, zinc oxide, copper oxide and iron oxide nanoparticles [36–42]. However, of all nanoparticles, TiO_2 and ZnO nanoparticles have gained considerable attention because of their unique electronic, optical and medicinal properties [43,44]. These nanoparticles are highly biocompatible and therefore have found various applications in biological field [45]. Recently, a green chemistry approach has been reported to synthesis various nanoparticles including ZnO and TiO_2 wherein natural products such as silk fibroin, cellulose, starch, humic acid, carbohydrates, lignin, etc. have been used as reducing and stabilizing agents. Among them, lignin is interesting natural products because: (i) it comes from the wood pulp industry; (ii) it is the second most abundant on earth, just second to cellulose; (iii) it is a nanocrystalline and heterogeneous polymer with a network structure; (iv) it is the only kind of biomass constituent that belong to the aromatic compounds; (v) structurally, it contains many aliphatic and aromatic hydroxyls, several aromatic methoxy, carboxyl, carbonyl and etheral moieties and (vi) it has many aliphatic hydroxyl groups, therefore, it can be used in reduction of metal salt to metal nanoparticles [46–48], for

changing surface morphology and nanoroughness of metal oxide, it can affect on for cell adhesion and proliferation [49]. Antibacterial activity of pristine ZnO and TiO₂ nanoparticles and FL–ZnO and FL–TiO₂ nanocomposites on *E. coli* and *S. aureus* were as shown in Figures 8 and 9, respectively.

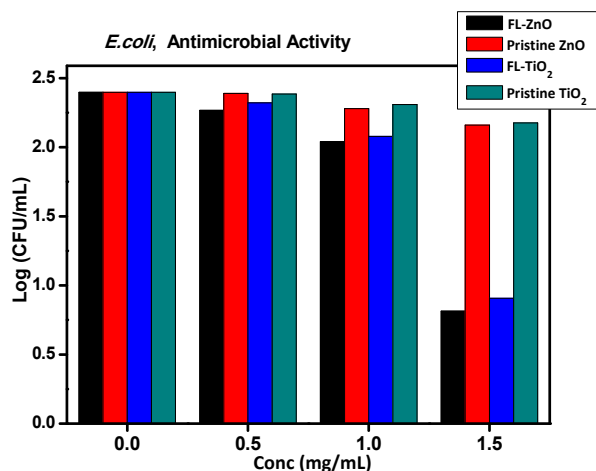


Figure 8. Antimicrobial activity of *E. coli* against pristine ZnO (48%), FL–ZnO (5.2%), pristine TiO₂ (52%) and FL–TiO₂ (6%).

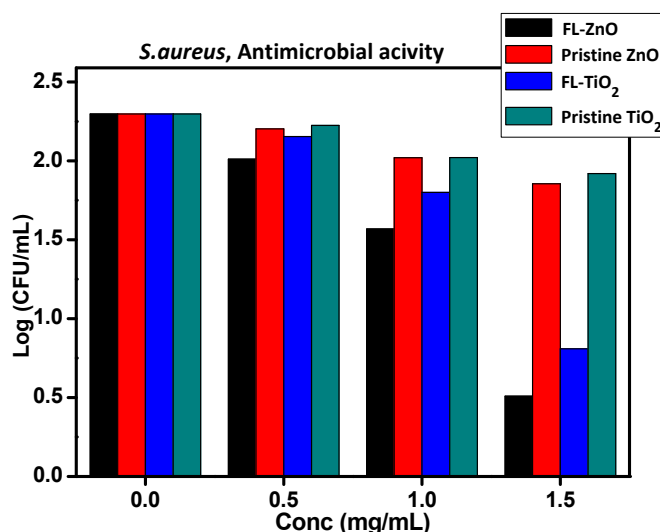


Figure 9. Antimicrobial activity of *S. aureus* against; growth shown for Pristine ZnO (32%), FL–ZnO (3.2%), Pristine TiO₂ (36%) and FL–TiO₂ (5%).

As can be observed, FL–ZnO and FL–TiO₂ nanocomposites showed a better antimicrobial activity than pristine ZnO and TiO₂ nanoparticles. The nanocomposites were more effective on cells of *S. aureus* than on cells of *E. coli*. The antimicrobial action of FL–ZnO and FL–TiO₂ nanocomposites were concentration dependent. When the cells of *E. coli* and *S. aureus* were independently subjected to various concentrations of ZnO and TiO₂ nanoparticles and FL–ZnO and FL–TiO₂ nanocomposites, there was a decrease in the number of viable cells. An initial 2.4 log CFU/mL was reduced to less than 0.5 log CFU/mL in presence of FL–ZnO and 0.7 log CFU/mL in presence of FL–TiO₂ nanocomposites. However, the log CFU/mL in presence of pristine ZnO and TiO₂ nanoparticles were respectively, 2.2 and 2.1. The reduction was more prominent in presence of FL–ZnO and FL–TiO₂ nanocomposites. The colony count of *E. coli* and *S. aureus*, in presence of FL–ZnO and FL–TiO₂ nanocomposites also, was less (Figure 10). Small particles size of material showed enhancement in the bioactivity because increased in surface area to the volume ratio. The smaller particles can easily bind higher number of bacterial colonies which result in the large number of active oxygen species to burst the cell wall

of the bacteria also the structural defects, oxygen defect and oxygen interstitials help to increase the antimicrobial activity of nanomaterial of FL-ZnO and FL-TiO₂. Whereas, pristine ZnO and TiO₂ nanoparticles have agglomeration, bulky size and less surface to the volume ratio can decrease contribution to the antimicrobial activity [50].

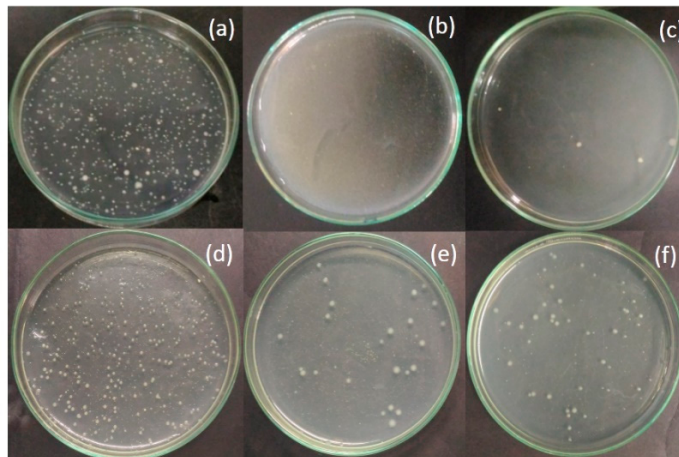


Figure 10. Antimicrobial activity of *E. coli* and *S. aureus* against TiO₂ and ZnO nanomaterials. (a) Control of *S. aureus*; (b) FL-ZnO against *S. aureus*; (c) FL-ZnO against *E. coli*; (d). Control of *E. coli*; (e) TiO₂ against *S. aureus*; (f) TiO₂ against *E. coli*.

The antimicrobial activities of FL-ZnO and FL-TiO₂ were in agreement with previous reports. The powder extract of dry ginger rhizome was used as a reducing material as well as surface stabilizing agent to synthesize ZnO nanoparticles and were shown to possess antimicrobial activity against bacteria and fungi [51]. Recently *Coptidis rhizoma*-mediated ZnO nanoparticles were tested for its antimicrobial activity against four disease-causing pathogens *Bacillus megatherium*, *B. pumilus*, *B. cereus* and *E. coli* [52]. ZnO nanoparticles adopt a series of mechanisms to act as an anti-bacterial agent. Loss of phospholipid bilayer cell membrane integrity is considered as one of the most important mechanisms of ZnO and TiO₂ NPs due to the oxidative stress induced by reactive oxygen species (ROS). This ROS molecule further causes cell death by inhibiting or altering DNA replication, protein synthesis, and membrane potentials [53]. In the present study, FL-TiO₂ and FL-ZnO nanocomposites have shown a prominent action on cells of *S. aureus* because of the difference in the mechanism of attachment of Gram-positive and Gram-negative bacteria to nanoparticles, their transport inside the cell, and differences in their different membrane structure. Gram-positive bacteria have a thick layer of peptidoglycan and have teichoic acid and lipoteichoic acid, the latter serves as binding sites to nanocomposites. They also chelate metal ions from nanoparticles and transport inside the cell. Gram-negative bacteria have a triple layer of peptidoglycan in their cell wall which imparts additional barrier for nanoparticles to enter inside cells [54]. Upon illumination, ZnO and TiO₂ nanoparticles generate the electron-hole pairs, and produce reactive oxygen species (ROS), which oxidizes organic matter and, thus, imparts biocidal property to nanoparticles [55]. However, ZnO and TiO₂ nanoparticles are having a very low efficiency for the separation of electron-hole pairs due to fast recombination of charge carriers, therefore, it is very essential to suppression of the recombination of photogenerated electron-hole pairs in ZnO and nanoparticles. FL which contains different organic function groups, such as aromatic, phenolic, hydroxyl, etheral, alkene and methoxy groups, are hypothesized to prevent this recombination of electron-hole pair. In the present study, the biosynthesized FL-ZnO and FL-TiO₂ nanocomposites have porous structure and low band gap with several defects as was observed in photoluminescence spectrum. FL-TiO₂ nanocomposites have mixed phase morphology of anatase and rutile geometry. Therefore, such an environment is favorable for ejection and stabilization of electrons to create hole and electron pairs and imparts FL-ZnO and FL-TiO₂ nanocomposites as an antimicrobial property.

6. Conclusions

The FL results in five different fragments which contain aldehydic, etheral, alcoholic, aromatic, hydroxyl and phenolic groups, which may act as surfactant and restrict the cluster formations. Hence, these groups play an important role in synthesis of FL–ZnO and FL–TiO₂ nanocomposites by a biosynthesis method. These organic materials were coated on the nanomaterial during fabrication which creates defects to generate holes and electrons, which easily react with the cell membrane and DNA to result in cell death. Furthermore, the large surface area and special morphology of ZnO gives a more pronounced effect. In this way, both of these biosynthesized materials show better results in bactericidal activity compared to the pristine material.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2504-477X/3/3/90/s1>. Figure S1. FTIR of Fragmented Lignin, Figure S2. ¹H-NMR of Fragmented Lignin, Figure S3. HRMS of Fragmented Lignin.

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