Retraction Notice

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Bioinspired Synthesis of Zinc Oxide Nanoparticle and its Combined Efficacy with Different Antibiotics against Multidrug Resistant Bacteria

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Abstract

Bioinspired synthesis of nanoparticles is a way to synthesize nanoparticles by using biological sources. It's gaining importance due to its ecofriendly, cost effective and large scale production properties. In this present study, the plant *Ficus carica* was taken to study its ability for synthesizing zinc oxide nanoparticle. The leaf extract of antimicrobial susceptibility showed that most of the antibiotics were resistant towards bacterial isolates of zinc sulphate hepta hydrate and sodium hydroxide, were used to synthesize the zinc oxide nanoparticles and were confirmed by their change of color to yellowish white due to the phenomenon of reduction. The characterization studies were done by UV-vis spectroscopy, Scanning electron microscopy (SEM), Energy dispersive Analysis of X-rays (EDAX), X-Ray diffraction (XRD), and Fourier Transmission infrared spectroscopy (FTIR). It was confirmed from the XRD pattern that the structure of ZnO nanoparticles (NPs) is crystalline and the average crystalline size of ZnO NPs is 66 nm. The morphology of the nanoparticle was confirmed through SEM and EDAX analysis which shows the hexagonal shape of zinc oxide nanoparticles respectively. FTIR analysis proved that the particle is of biological origin and identified that phenols played a role as a reducing agent. For antibacterial activities, selected antibiotics were impregnated with Zinc oxide nanoparticles synthesized from *Ficus carica* which showed good activities against *Staphylococcus aureus* (17.4 ± 1.81659), *Proteus* (24.4 ± 4.82701), *Acinetobacter* (31.2 ± 0.83666), *Pseudomonas aeruginosa* (28.8 ± 1.30384) *and Escherichia coli* (20.8 ± 0.44721). Antimicrobial susceptibility showed that most of the bacterial isolates were resistant towards antibiotics that became sensitive after nanoparticles application.

Keywords

Bioinspired Synthesis, UV, SEM, EDAX, FTIR, XRD

1. Introduction

New technologies often generate new challenges to science in accumulation to their assistances and raise concerns about health and numerous environmental harms. Current nanotechnology holds a promise and an extensive aspect towards wide-range of applications of nanoparticles in a multiple way of developing fields of science and technology. Over the last years, nanotechnology has established as the great modernization of science and technology. Nanotechnology is a science and engineering branch of well recognized technology referring at the nanoscale *i.e.* 1 to 100 nm. Commonly, metal oxide nanoparticles are inorganic. Several nanoparticles like Fe, Ni, Co, Mn, Zn etc. are known as the massively accepted magnetic materials for extensive range of applications like various electronic ignition systems, generators, vending machines, medical implants, wrist watches, inductor core, transformer circuits, magnetic sensors and recording equipment, telecommunications, magnetic fluids, microwave absorbers, etc. They are also valid in other high-frequency uses [1]. The vast applications of nanoparticles in medical sciences are drug supply, imaging and diagnosis. Nanoparticles possess high surface to volume ratio due to its small size, which gives very distinct features to nanoparticles. A lot of research has proven that zinc oxide nanoparticles have the antifungal and antibacterial activity.

The green synthesis technique proves himself to be versatile, low cost, less evaluation of toxic gases, bestows and economical route of high yield. For ZnO NPs synthesis, various synthesis techniques such as chemical vapor deposition, sol-gel, sputtering, pulsed laser deposition, oxidation of metallic zinc powder and hydrothermal were used previously. However, all these techniques use long time sophisticated equipment as compared to green's route due to which researchers recommended this technique more suitable as compared to others. The nanoparticles keep extraordinary optical, physicochemical and biological properties which can be used according to the desired applications. The nanoparticles contain externally small size and large surface to volume ratio and are unique characteristics compared to their bulk counter parts [2]. These changes in characteristics are due to quantum size effects. Metallic nanoparticles contain unique optical, thermal, chemical, and physical properties because of having surface atoms of high energy relative to the bulk solids indicating that the free electrons change their conductivity and mobility. It is realized from the research that temperature affects the size and uniformness of nanoparticles. Here by the growth of nanoparticles can be prevented by hold on the temperature [3].

Among nanometer size multifunctional materials zinc oxide is an inorganic compound having the formula ZnO with wurtzite hexagonal structure. It is nonsoluble in water and available in white powder which is extensively used in

plastics, ceramics, glass, cement, car tyres, lubricants, paints, ointments, adhesives, pigments, batteries, and as an additive. Zinc and oxygen are the members of 2nd and 6th groups of the periodic table respectively and so often called as II-VI. The zinc oxide (ZnO), is nontoxic and biocompatible semiconductor material in biological fields, it is having wide band gap (3.37 eV) and large exciton binding energy (60 meV). It is used for fabrication of nanoscaled electronic devices [4].

Ficus carica is commonly known as a good source of elements like Ca, Cr, Cu, Fe, K, Mg, Mn and Mo, so it can be considered that 5 kg of dried fig covers more than 15% of the Recommended Dietary Allowances (RDA) [5]. The *Ficus* contains a lot of antioxidants, a good source of polyphenols flavanoid glycosides, tannins, phenolic acids, steroids, saponins, alkaloids [6]. The nonenzymatic constituents are phenolic compounds (gallic acid and ellagic acid), flavanoids, vitamin C and enzymatic components present are ascorbate oxidase, ascorbate peroxidise, catalase, peroxidise [7]. *Ficus* contains maximum potassium content which is beneficial to hypertension patients and also prevents rapid thinning of bones by stopping the calcium loss in urine. Unique interrelations are present between potassium and copper, potassium and iron and copper and zinc [8]. Its fruit, root and leaves are using in a number of medicine for many disorders like colic, indigestion, diarrhea, sore throats, coughs, bronchial problems, inflammatory, cardiovascular disorders, ulcerative diseases, and cancers. The bioactive compounds present in *Ficus carica* have cytotoxic effects [9]. Recently, the ZnO NPs synthesized from Sol-gel technique and study the role of stirring on antimicrobial activities. They observed that when the agitation speed increases the aspect ratio decreases due to which the UV-Vis λ_{max} peak shift were observed. The ZnO NPs synthesized at 2000 rpm shows better thermal stability respectively. The ZnO NPs prepared at different stirring condition shows good antifungal as well as antibacterial properties [1].

In the synthesis of metal or metal oxide nanoparticles using plants, the biological constituents that are primary and secondary metabolites act as agents to make the reduction of a metal ion or metal oxide possible in nanoparticles making. These reducing agents molecules present in surrounding coat stabilizing layeron the nanoparticles surface, preventing to aggregate in an improper manner during their synthesis [10]. Other experimental conditions like temperature, pH, and concentration of reagents can affect the preparation and properties of metallic nanoparticles using green synthesis methods [11]. Zinc Oxide (ZnO) has strongly antimicrobial properties and because of its small size and large surface area, it goes inside into the body of microbe and destroys it. This property is due to its reaction with oxygen [3]. These particles are used not only alone but also in combinations with other organic compounds. These particles are capable of to deliver medical preparations to the targetted location of pathological process. Therefore their mechanism of action may be prolonged, which is important point to treat the diseases. If ZnO nanoparticles are used as an antifungal agent on plants it will not affect soil fertility and it have cytotoxic behavior for the bacteria and fungi [12].

Antibiotics are saving millions of lives all over the world. This over use of antibiotics give rise to multi drug resistant bacterial strains and represents a serious harm to health of public and economy also. According to the estimates of Centers for Disease Control and Prevention about two million diseases and 23,000 deaths are caused by antibiotic-resistant bacteriaannually in the United States. If the efficacy of drugs to kill or inhibit the growth of bacteria is lost, we will no longer be able to cure health care associated infections [13]. And as a result surgery, transplants, and chemotherapy may no longer be effective due to the threat of infection. A new recent nanotechnology has a potential to reduce multi drug resistance. Nanoparticles have a quality of targeted drug delivery and controlled drug release. It can increase the effectiveness of drugs and shows antimicrobial activity, heals the wounds and infectious disease.

2. Materials and Methods

Zinc sulphate hepta hydrate ($ZnSO_4 \cdot 7H_2O$), sodium hydroxide (NaOH), potassium bromide (KBr), Meuller Hinton Agar and Nutrient broth media were used in this research work.

All chemicals were used directly without any further purity.

2.1. Synthesis of Zinc Oxide Nanoparticle

For preparation of plant extract, 10 g of plant powder was taken and added in 400 ml distilled water and heated for 10 mints at 60˚C. After that, the plant extract was filtered with Whattman paper in order to remove the unwanted residues. Furthermore, 1 mM $ZnSO_4 \cdot 7H_2O$ solution was prepared and 30 ml plant extract was added to it. The pH was maintained at 12 using 1 M NaOH solution drop wise and stirred for 1 h. The precipitates were appeared in the solution which was then centrifuged for 5 mints at 3000 rpm in order to collect precipitates at the bottom of the centrifuge tube, after that, put in over at 65˚C overnight for drying. The powder collected for further characterization (**Figure 1**).

2.2. Antimicrobial Activity

2.2.1. Maintenance of Culture

All of the identified bacterial strains obtained from microbiology laboratory, Abasyn University, Peshawar were sub cultured on the prepared nutrient agar media and incubated for 24 hr. It was done to get the fresh culture of strains. The strains were further preserved at 4˚C for further processing.

2.2.2. Inocculum Preparation into Broth Media

First fresh broth was prepared into test tubes and autoclaved at 121˚C for 15 minutes then a single colony was taken from 24 hr old culture with a sterile wire loop and inoculated in a prepared broth and kept in an incubator at 37˚C to get the maximum growth.

2.2.3. Bacterial Lawn Preparation on MHA

The turbidity of 24 hr broth culture was maintained by normal saline to a Mc

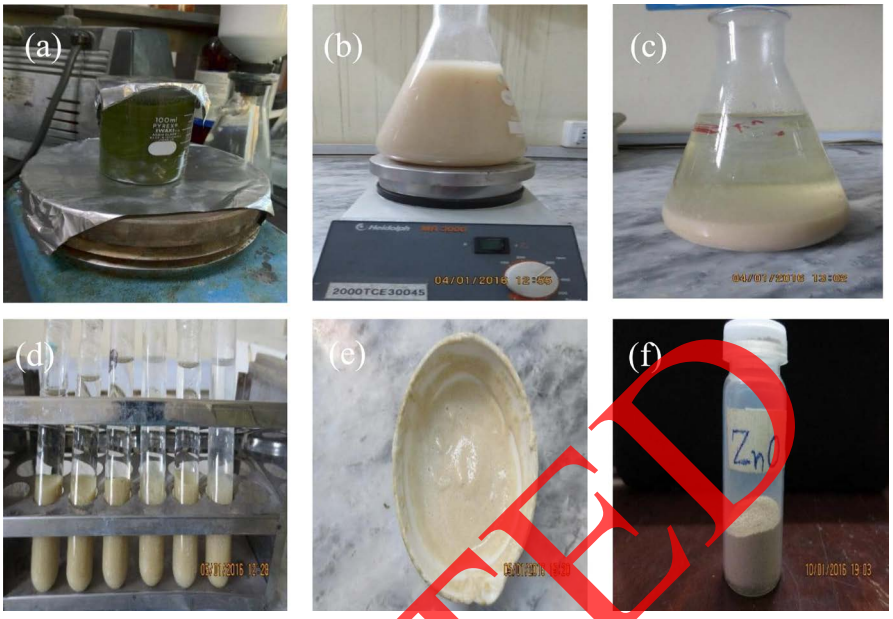

Figure 1. Synthesis process of ZnO NPs. (a) Ficus carica leaf extracts; (b) Stirring at hot-plate; (c) Washing with deionized water; (d) Washing after ethanol; (e) Transferred into china dish; (f) ZnO powder.

Table 1. List of antibiotics used against each bacterial species.

Species	*E. coli*	*P. aeroginosa*	*Staphaureus*	*Acinetobacter*	*Proteus*
Antibiotics	Gentamicin Erythromycin Fosfomycin	Gentamicin Amikacin Ciprofloxacin	Fusidic acid Oxacillin rifampicine	Tigecycline Amikacin rifampicine	Gentamicin Erythromycin fosfomycin

Farland standard 0.5. For making lawn of bacterial colonies on prepared Meuller Hinton agar petri plates, a sterile swab was taken and dipped in a broth culture and then the swab was gentally pressed with the wall of test tube to squeeze extra broth. The swab was gentally rubbed on the MHA plates to spread the colonies evenly in a four quarter.

2.2.4. Preparation of Antibiotic Discs with Zinc Oxide Nano Particles Coating

Antibiotic Discs having Zinc Oxide Nanoparticles coating were prepared in such a way that 20 mg Zinc Oxide Nanopowder was dissolved in 1 ml of sterile distilled water. Nanopowder suspension was prepared in a concentration of 20 µg/µl. Then selected antibiotic discs were taken separately on a sterile, dry petri plate under sterile condition inside the laminar air flow hood. After that about 5 µl nanopowder suspension was pipette out with a micro-pipette and coated each disc with the prepared suspension. In this way each antibiotic disc was having 100 µg Zinc oxide nanoparticles. Then the petri plates having the impregnated or coated discs were covered with the lid and kept in an oven at about 80˚C for at least 15 minutes to dry. Stock suspension of zinc oxide was prepared just one time, but the coating method of discs was repeated each time for selected antibiotics for each bacterial strain (Table 1).

2.2.5. Disc diffusion Assay for Combined Effects of Antibiotics and Nano Particles

The antibiotics susceptibility tests were performed by Kirby Bauer disk diffusion method as mentioned in CLSI (2014). Both nanocoated and non-coated antibiotics were applied on each bacterial lawn prepared on Mullerltinton agar. The discs were placed with sterile forcep and pressed gentally to allow contact. The plates were incubated at 37˚C for 24 h. The inhibition zones were measured in millimeter.

3. Characterization

PerkinElmer UV-VISIBLE Spectroscopy lambda 25 was used to check the absorbance of synthesized ZnO nanoparticles. JEOL JSM-5910 JAPAN scaning electron microscope is used for the overall appearance of the sample. The accelerating voltage 1 kV and 10 kV were used. The EDX shows the quantity of the individual element present on the sample. Shimadzu IR Prestige21 was used for Fourier Transform Infrared Spectroscopy (FTIR). JEOL JDX 3532 JAPAN X-ray diffractometer was used to study the size, shape and internal spacing between the layers of atoms present in a singal crystal.

4. Results

4.1. UV-Visible Spectroscopy

PerkinElmer UV-VISIBLE Spectroscopy lambda 25 with wavelength range from 300 - 800 nm was used to measure the absorbance of synthesized ZnO nanoparticles. This equipment is used to study the absorbance of sample in the ultra violet region and visible regions of electromagnetic spectrum. Figure 2 shows that the sample has absorbed energy at 360 nm which is characteristic peak value of zinc oxide nanoparticles. The UV-Vis spectrum of ZnO NPs show strong absorption

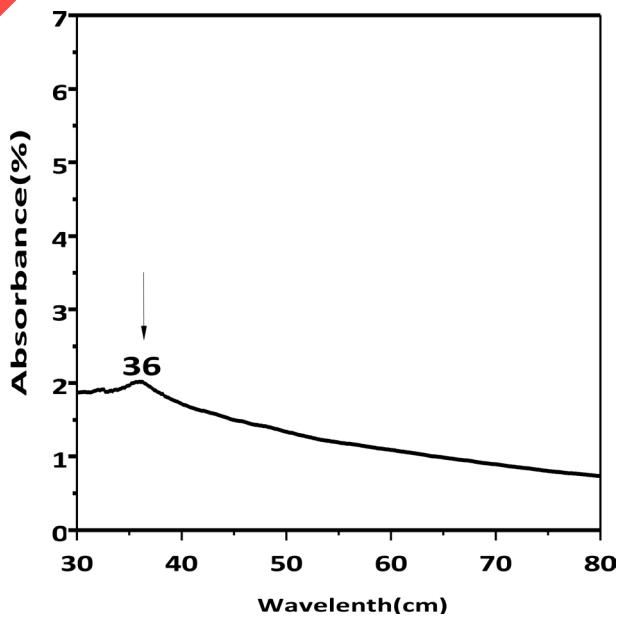

Figure 2. UV-Vis spectrum of ZnO NPs synthesized from green route.

at 360 nm with no other peaks shows high purity of the synthesized nanoparticles.

4.2. Scanning Electron Microscopy (SEM)

The SEM images of ZnO NPs show that the shape of the synthesized zinc oxide nanoparticles is hexagonal. Figure 3(a) & Figure 3(b) show the overall appearance of the sample which indicates that the successful formation of the ZnO NPs. The high resolution images of the sample Figure 3(c) & Figure 3(d) represent the hexagonal morphology of the ZnO NPs.

4.3. Energy Dispersive Analysis of X-Rays (EDAX)

The spectra in Figure 4 shows peaks of zinc and oxygen elements 45.27% and 45.42% proves ZnONPs prepared is essentially free from impurities. The EDS analysis of ZnO nanoparticles confirms the elemental composition of ZnO nanoparticles. EDX analysis determined the extent of oxygen and zinc in ZnO nanoparticles separately.

4.4. Fourier Transform Infrared Spectroscopy (FTIR)

In the FTIR spectrum of *Ficus carica*, the peak at 3464.15 cm^{-1} and 3394.72 cm^{-1} is due to vibration of stretch O–H in water, alcohol and phenols and the peak value of 3356.14 cm^{-1} 2067.69 cm^{-1} is the result of N–H stretching vibrations of primary and secondary amines in proteins. 1635.64 cm^{-1} is a peak of –C=O– in carboxylic acid. The C–N stretch vibrations of amide group in protein and the C–O–C functional groups in polysaccharides gave a band value of 1427.32 cm^{-1}. Similarly a band at 1095.57 cm^{-1} is due to the C–O groups in amino acid. From

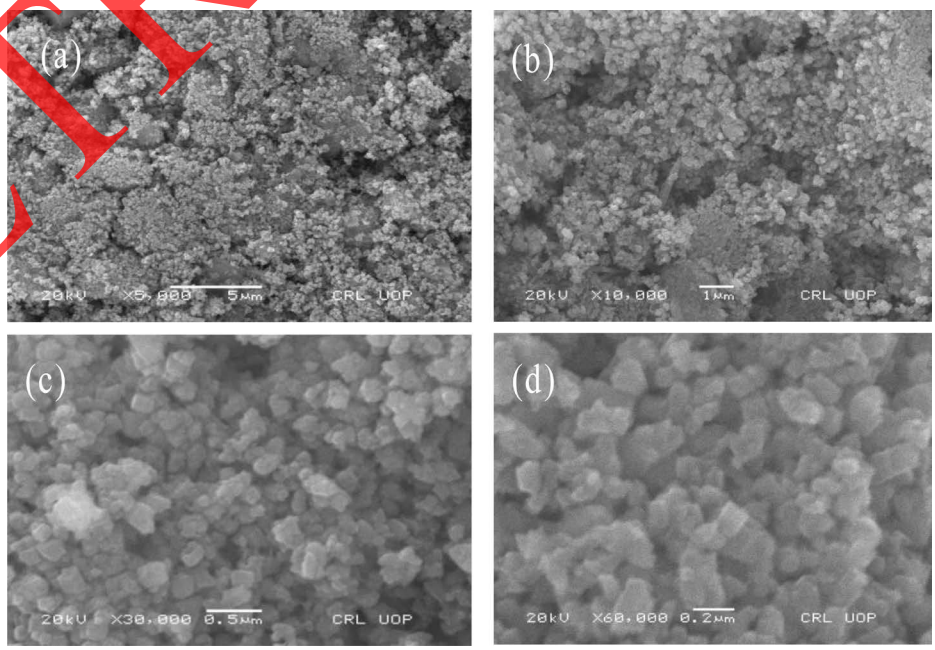

Figure 3. SEM images of the zinc oxide nanoparticles at scale bar (a) 5 μm (b) 2 μm (c) 0.5 μm and (d) 0.2 μm.

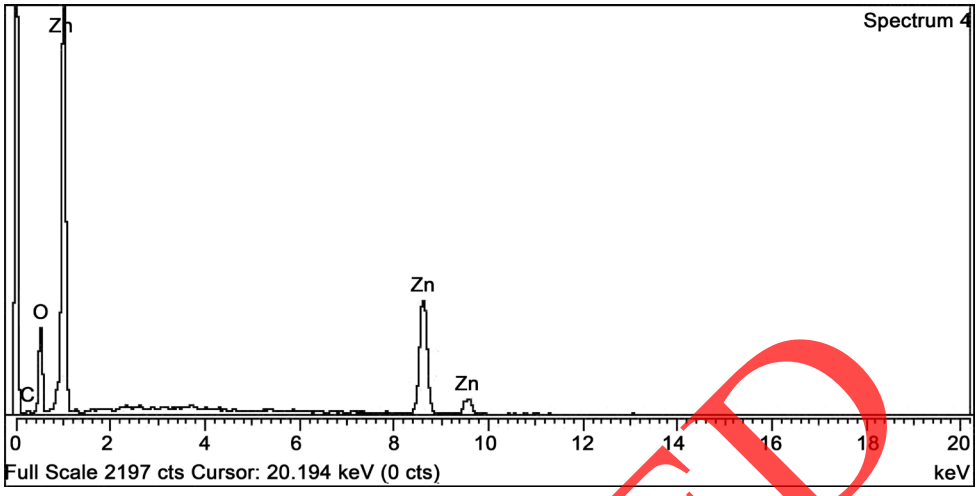

Figure 4. EDAX analysis of zinc oxide nanoparticles.

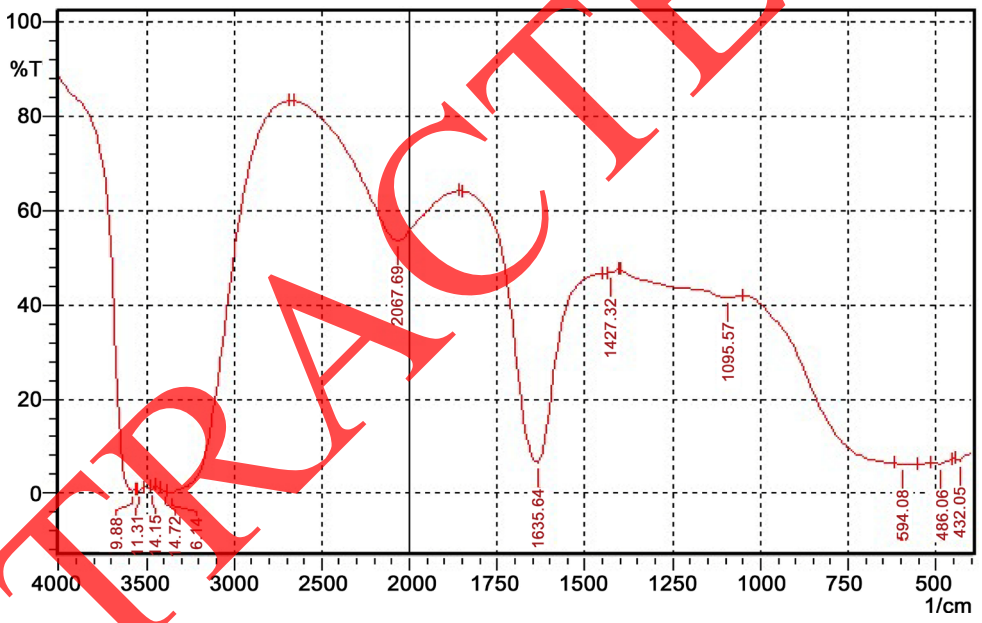

Figure 5. FTIR spectrum of zinc oxide nanoparticles.

Figure 5 it can be easily concluded that these phytochemicals are involved in capping, stabilization and reduction of the ZnONPs. The peaks other than the transmittance bands of these phytochemicals two peaks occurring at 594.08 and 486.06 cm^{-1} in the FTIR spectrum of the ZnONPs are the characteristic peaks of ZnO molecules.

4.5. X-ray Diffraction (XRD)

JEOL JDX 3532 JAPAN X-ray diffractometer with Cu k_α (λ = 1.54 Å) was used for the X-ray diffraction to determine the size of the ZnO nanoparticles synthesized from zinc sulfate hepta hydrate and sodium hydroxide in the presence of *Ficus carica* leaf extract at room temperature. The peak position with 2θ values of 31.6˚, 34.46˚, 36.26˚, 47.46˚, 56.54˚, 62.82˚, 68.01˚, 69.10˚ are indexed as (100), (002), (101), (102), (110), (103), (112) and (201) planes, which agreed with

the International Center of Diffraction Data card (JCPDS-36-1451) which confirmed the synthesis of a crystalline hexagonal structure. There was no detection of extra diffracted peaks of other phases which indicated the phase purity of ZnO nanopowder. The average crystalline size of the synthesized zinc oxide nanoparticles was calculated to be 66 nm using Debye-Scherrer formula (Figure 6).

5. Discussions

In this research work, ZnO NPs were prepared from *Ficuscarica* leaf extract from green synthesis approach. Various characterization tools were used to investigate different properties of the sample such as SEM, XRD, FTIR, UV-Vis and EDX. The XRD of the sample were taken in the range of (0˚ - 70˚). The XRD diffractogram show peaks at 2θ = 31.6˚, 34.46˚, 36.26˚, 47.46˚, 56.54˚, 62.82˚, 68.01˚, 69.10˚ with crystal planes (100), (002), (101), (102), (110), (103), (112) and (201) respectively. The crystal structure and planes of the sample were hexagonal which match well with the work of [14] [15] respectively. The XRD pattern shows no extra impurity of any other material, indicating high purity of the sample. However, the intense peak shows the crystalline nature of the sample. The average crystalline size is calculated from Debye-scherrer formula which is 66 nm.

The ZnO NPs synthesized from ficus carica plant extracts at temperature of 60˚C for 1 hr. respectively. When the temperature is increased above 60˚C then the size of the NPs going to increased and vice versa. In addition, when the time reaches at 30 minutes, the color of the solution will be changed from green pale to half white precipitate will be appeared indicating formation of ZnO NPs. Below this temperature; there is no formation of the NPs at all. Recently, the flower shape ZnO NPs synthesized from sol-gel approach with different temperature 75˚C, 25˚C, 35˚C and 55˚C. They observed that the NPs synthesized at room temperature shows greater activity as compared to higher temperature [1].

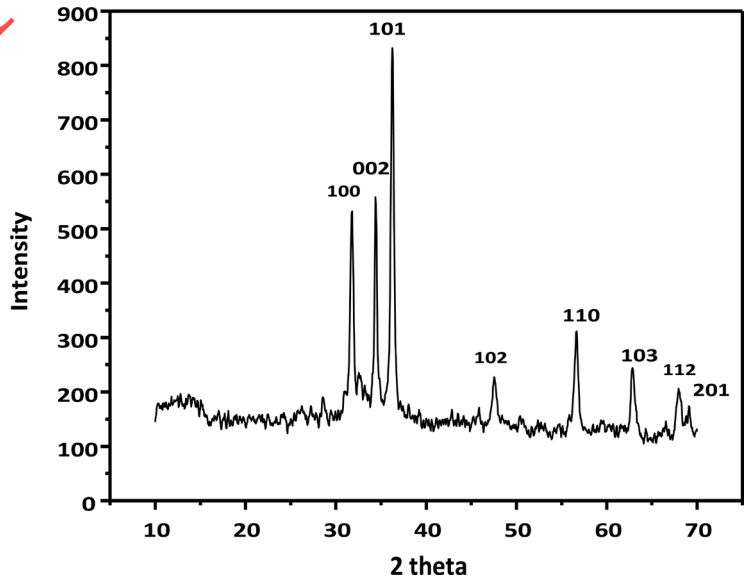

Figure 6. XRD spectrum of zinc oxide nanoparticles synthesized from leaf extract.

Herein, this research work strong absorption was observed at 360 nm which is due to the increase in particle size. Horrision *et al.* synthesized ZnO NPs with the hydroxyl-footed methylresorcinarene (HFMR) and zinc acetate as the starting material for the formation of ZnO NPs. They reached at conclusion that, as the nanoparticle size and the binding agent increases the UV-Vis absorption spectra shifted towards red respectively. Thus, by our synthesized sample the maximum absorption were observed at 360 nm which is a good agreement with [15].

The FTIR spectrum obtained from *Ficus carica* plant extracts showed peaks at 594.08 and 486.06 cm^{-1} is the stretching vibration peaks of ZnO NPs which is similar to [16]. The band occurred between 3464.15 cm due to O-H of water molecules, were N-H bands which phenolandalcohols while the peak at and the peak at 3394.72 cm represent the primary and secondary groups present in proteins. The band at 1635.64 cm^{-1} is the absorption band of the –C=O-(carboxylic acid) stretching. In addition, the same results were also obtained from stem bark extract of *Boswelliaovalifoliolate* using green's method [17]. They found the same bands position which is good agreement with our FTIR results. So, the bands at 1095.57 cm^{-1} reveal the functional group of C-O due to amino acids.

As from **Figure 4**, EDX spectrum of ZnO NPs were recorded which shows that there is only prominent peaks of zinc and oxygen present on the spectrum with no other materials peaks indicating high purity of the sample. In addition, the reflection of zinc has higher intensity which shows the co-existence of the desired materials. The EDX spectrum also shows oxygen peak which confirm that the sample synthesized were pure ZnO NPs. From **Figure 4**, it is also evident that there is 45.47% of zinc and 45.42% of oxygen present on the sample.

Nagarajan *et al.* used green synthesis method to synthesize ZnO NPs from seaweeds such as, red Hypnea, Caulerpa peltata Sargassum myriocystum and Valencia and brown respectively. In addition, the size of ZnO NPs was measured 36 nm using characterization techniques DLS, SEM, EDX, TEM, AFM, XRD, FTIR and UV-Vis. The EDX spectrum showed peaks which are indexed only oxygen and zinc. The elemental composition of zinc and oxygen shows 52% and 48%. The EDX spectrum originated due to surface plasma resonance of ZnO NPs. On the other hand, the elemental ratio of the zinc and oxygen in present work was 45% but no peak of other materials was observed which is in good agreement with [18]. In the light of these results, it agrees the successful formation of ZnO NPs.

In the present work, Fosfomycin, gentamicin and erythromycin were applied against *E. coli* without nanoparticles that showed maximum mean difference of 17.2 ± 1.30384 for gentamicin but when the same antibiotic was applied in incorporation with ZnONPs then the maximum mean difference of 20.8 ± 0.44721 was observed (**Figure 7**). The *E. coli* showed resistance to erythromycin but showed sensitivity of 19.8 ± 0.83666 in conjugation with the nanoparticles in a concentration of 100 $\mu g \cdot mL^{-1}$ This study in turn indicates the better antibacterial activity of the ZnO NPs in combination with antibiotics.

Here ceftriaxone, amikacin and clindamycin were used against Proteus. The maximum mean difference was recorded for ceftriaxone with nanoparticles as 24.4 ± 4.82701 which was only 17.2 ± 1.30384 without nanoparticles. Proteus also showed resistance to clindamycin alone but the combination of nanoparticles made the Proteus sensitive to clindamycin with a mean difference of 24 ± 2.91548. Proteus was sensitive to amikacin when it was applied without nanoparticles. According to the Harshiny *et al.* the significant increased occurred in the antibacterial activity of broad spectrum antibiotics in combination with the silver nanoparticles AgNPs [19]. They synthesized AgNPs from garlic *(Allium sativum)* and evaluate their activity in combination with antibiotics and alone also. Enhancement was seen in the antibacterial activity of Amoxyclav when applied with biogenic AgNPs at a concentration of 20 µg/mL against Proteus mirabilis and Pseudomonas aeruginosa respectively.

In case of *S. aureus,* the mean difference of zone of inhibition of ZnO nanoparticles incorporated with fusidic acid, oxacillin and rifampicine was 17.4 ± 1.81659, 17.2 ± 1.30384 and 16.8 ± 0.83666 respectively with 100 µg concentrations of nanoparticles. Without nanoparticles, the mean difference wasrecorded as 14.4 ± 1.14018, 14.4 ± 0.89443 and 13.8 ± 0.83666 respectively for the above mentioned antibiotics. *S. aureus* showed more sensitivity toantibiotics with nanoparticles. In the work of Singh *et al.* nanoparticle also enhanced the antibacterial of the Ampicillin, Rifampicin, Cefalexin, Cefotaxime, Ceftazidime, Gentamycin, Clarithromycin, Nalidixic Acid, Cloxacillin, Cotrimoxazole, and Chloramphenicol against S. aureus [12]. While the activity of Penicillin against S. aureus was remained same *i.e.* with and without nanoparticles but the activity of cloxacillin was enhanced through nanoparticle upto 13 mm from 9 mm.

The three antibiotics Amikacin, Gentamicin and ciprofloxacin were used against *P. aeruginosa,* the mean differences of zone of inhibition in combination with ZnO nanoparticles were recorded as 28.8 ± 1.30384, 27.4 ± 1.140175 and 24.6 ± 1.140175 respectively. So maximum enhanced was noted for amikacin while it was only 24 ± 2.48998 by the drug alone. Acinetobacter was also tested against Amikacin, tigecyclin and rifampicin. The antibiotics showed slight difference in their activity against Acinetobacter. In combination of drugs with ZnO nanoparticles, highest mean difference was observed for Rifampicin *i.e.* 31.2 ± 0.83666 and the maximum increase in the mean difference of antimicrobial activity was observed for Tigecyclin *i.e.* from 25.2 ± 0.83666 to 28 ± 1.00000. However, Gnanasangeetha *et al.* worked on the following five nanoparticles such as MgO, CeO_2, Fe_3O_4, ZrO_2 and Al_2O_3. Fe_3O_4 showed maximum activity of 15 ± 0.32 mm against *Pseudomonas aeruginosa* but did not show antibacterial activity against Acinetobacter species [14].

6. Antimicrobial Activity

I. E. coli

Table 2 shows the mean antimicrobial activity of antibiotics against *E. coli* with and without nanoparticles. Fosfomycin, gentamicine and erythromycin

showed the mean differences of 17.8 ± 2.28035, 20.8 ± 0.44721, 19.8 ± 0.83666 with nanoparticles incorporation and 14 ± 3.39116, 17.2 ± 1.30384 and 0.0 without nanoparticles respectively.

Proteus

Table 3 shows the mean antimicrobial activity of antibiotics against *Proteus* with and without nanoparticles. Ceftriaxone, amikacin and clindamycin showed the mean differences of 24.4 ± 4.82701, 20.6 ± 1.14018 and 24 ± 2.91548 with nanoparticles incorporation and 17.2 ± 1.30384, 11.2 ± 0.83666 and 0.0 without nanoparticles respectively (Figure 8).

Staphylococcus aureus

Table 4 shows the mean antimicrobial activity of antibiotics against *Staphylococcus aureus* with and without nanoparticles. Fusidic acid, oxacillin and rifampicine showed the mean differences of 17.4 ± 1.81659, 17.2 ± 1.30384 and 16.8 ± 0.83666 with nanoparticles incorporation and 14.4 ± 1.14018, 14.4 ± 0.89443 and 13.8 ± 0.83666 without nanoparticles respectively (Figure 9).

Table 2. Mean antibacterial activity of antibiotics against *E. coli* with and without nanoparticle.

Antibiotics		Zone of inhibition in mm					Mean/S.D
Fosfomycin	Zone of inhibition with nanoparticles	20	20	18	15	16	17.8 ± 2.28035
	Zone of inhibition without nanoparticles	18	16	15	10	11	14 ± 3.39116
Gentamicin	Zone of inhibition with nanoparticles	21	21	20	21	21	20.8 ± 0.44721
	Zone of inhibition without nanoparticles	18	16	16	17	19	17.2 ± 1.30384
Erythromycin	Zone of inhibition with nanoparticles	20	19	21	19	20	19.8 ± 0.83666
	Zone of inhibition without nanoparticles	0	0	0	0	0	0

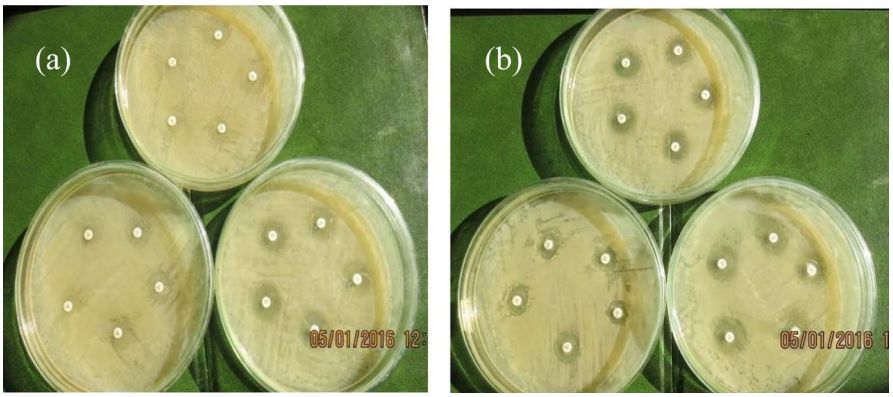

Figure 7. (a) Antibacterial activity of antibiotics without ZnO NPs. (b) Antibacterial activity of antibiotics with ZnO NPs.

Table 3. Mean antibacterial activity of antibiotics against *Proteus* with and without nanoparticle.

Antibiotics		Zone of inhibition in mm					Mean/S.D
Ceftriaxone	Zone of inhibition with nanoparticles	20	20	18	15	16	24.4 ± 4.82701
	Zone of inhibition without nanoparticles	18	16	15	10	11	17.2 ± 1.30384
Amikacin	Zone of inhibition with nanoparticles	21	21	20	21	21	20.6 ± 1.14018
	Zone of inhibition without nanoparticles	18	16	16	17	19	11.2 ± 0.83666
Clindamycin	Zone of inhibition with nanoparticles	20	19	21	19	20	24 ± 2.91548
	Zone of inhibition without nanoparticles	0	0	0	0	0	0

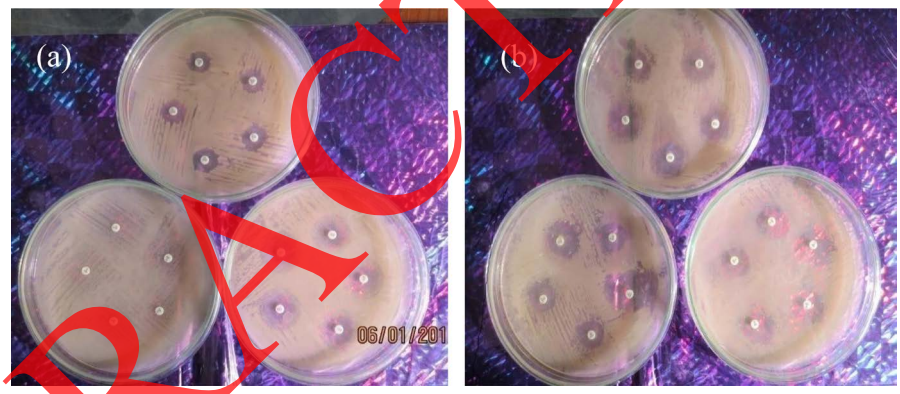

Figure 8. (a) Antibacterial activity of antibiotics without ZnO NPs. (b) Antibacterial activity of antibiotics with ZnO NPs.

Table 4. Mean antibacterial activity of antibiotics against *S. Aureus* with and without nanoparticle.

Antibiotics		Zone of inhibition in mm					Mean/S.D
Fucidic acid	Zone of inhibition with nanoparticles	20	20	18	15	16	17.4 ± 1.81659
	Zone of inhibition without nanoparticles	18	16	15	10	11	14.4 ± 1.14018
Oxacillin	Zone of inhibition with nanoparticles	21	21	20	21	21	17.2 ± 1.30384
	Zone of inhibition without nanoparticles	18	16	16	17	19	14.4 ± 0.89443
Rifampicin	Zone of inhibition with nanoparticles	20	19	21	19	20	16.8 ± 0.83666
	Zone of inhibition without nanoparticles	0	0	0	0	0	13.8 ± 0.83666

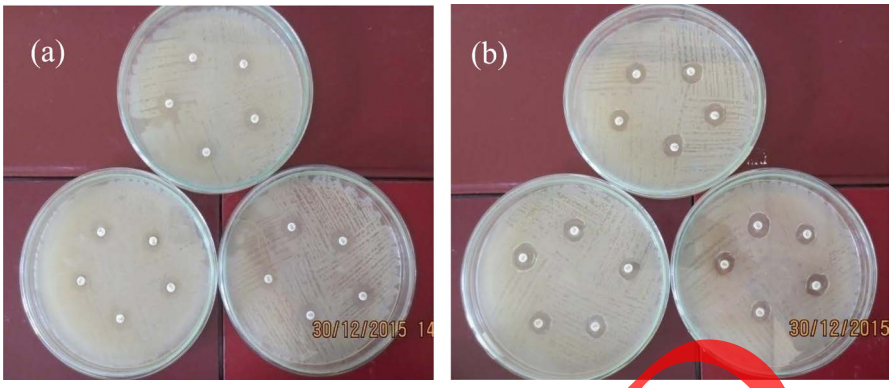

Figure 9. (a) Antibacterial activity of antibiotics without ZnO NPs. (b) Antibacterial activity of antibiotics with ZnO NPs.

Pseudomonas aeroginosa

Table 5 shows the mean antimicrobial activity of antibiotics against *Pseudomonas aeroginosa* with and without nanoparticles. Amikacin, gentamicine, and ciprofloxacin showed the mean differences of 28.8 ± 1.30384, 27.4 ± 1.140175 and 24.6 ± 1.140175 with nanoparticles incorporation and 24 ± 2.48998, 25.4 ± 0.547723 and 23.2 ± 0.83666 without nanoparticles respectively (Figure 10).

Acinetobacter

Table 6 shows the mean antimicrobial activity of antibiotics against *Acinetobacter* with and without nanoparticles. Amikacin, tigecycline and rifampicine showed the mean differences of 31 ± 0.70711, 28 ± 1.00000 and 31.2 ± 0.83666 with nanoparticles incorporation and 29 ± 0.70711, 25.2 ± 0.83666 and 29.4 ± 2.07364 without nanoparticles respectively (Figure 11).

The antibacterial activities of all antibiotics were increased by Zinc oxide nanoparticles against bacterial species. The maximum enhancement in the antibacterial activities by Zinc oxide nanoparticles were observed for clindamycin and erythromycin. Similarly less increase was observed in case of tigecycline, rifampicin, gentamicin and ciprofloxacin. Ceftriaxone, amikacin, oxacillin, fusidic acid and fosfomycin showed moderate enhancement. The ZnO NPs used in medications in excess amount it will causes toxicity and accumulate in our body cells and damaged it. The activity against microbes and disease causing agents are called antimicrobial while in the lab it is called invitro activity. The purpose of these activities was to identify which antibiotics will be suitable for specific microbe.

7. Conclusion

Green synthesis of zinc oxide nanoparticles is a very cost effective, safe, harmless, environment friendly way of synthesis. It is a route to synthesize nanoparticles at maximum scale. *Ficus carica* showed its large capacity to manufacture zinc oxide nanoparticles at room temperature. The XRD clearly shows high purity and crystallinity of the sample. The resonance peak, absorption peak appeared at 360 nm mean that ZnO NPs absorb maximum in the blue region of electromagnetic spectrum. Also, the FTIR analysis gave confirmation about cap-

Table 5. Mean antibacterial activity of antibiotics against *P. aeroginosa* with and without nanoparticle.

Antibiotics		Zone of inhibition in mm					Mean/S.D
Amikacin	Zone of inhibition with nanoparticles	20	20	18	15	16	28.8 ± 1.30384
	Zone of inhibition without nanoparticles	18	16	15	10	11	24 ± 2.48998
Gentamicin	Zone of inhibition with nanoparticles	21	21	20	21	21	27.4 ± 1.140175
	Zone of inhibition without nanoparticles	18	16	16	17	19	25.4 ± 0.547723
Ciprofloxacin	Zone of inhibition with nanoparticles	20	19	21	19	20	24.6 ± 1.140175
	Zone of inhibition without nanoparticles	0	0	0	0	0	23.2 ± 0.83666

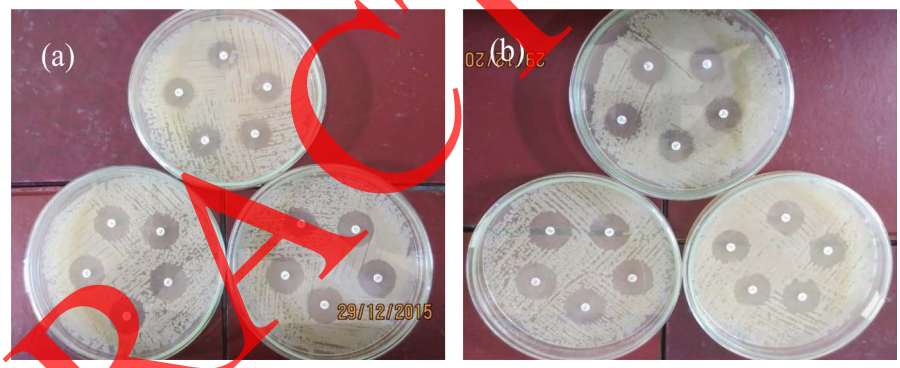

Figure 10. (a) Antibacterial activity of antibiotics without ZnO NPs. (b) Antibacterial activity of antibiotics with ZnO NPs.

Table 6. Mean antibacterial activity of antibiotics against *Acinetobacter* with and without nanoparticle.

Antibiotics		Zone of inhibition in mm					Mean/S.D
Amikacin	Zone of inhibition with nanoparticles	31	31	30	32	31	31 ± 0.70711
	Zone of inhibition without nanoparticles	29	28	29	30	29	29 ± 0.70711
Tigecyclin	Zone of inhibition with nanoparticles	29	28	29	27	27	28 ± 1.00000
	Zone of inhibition without nanoparticles	25	24	26	25	22	25.2 ± 0.83666
rifampicin	Zone of inhibition with nanoparticles	31	32	31	32	30	31.2 ± 0.83666
	Zone of inhibition without nanoparticles	33	29	28	29	28	29.4 ± 2.07364

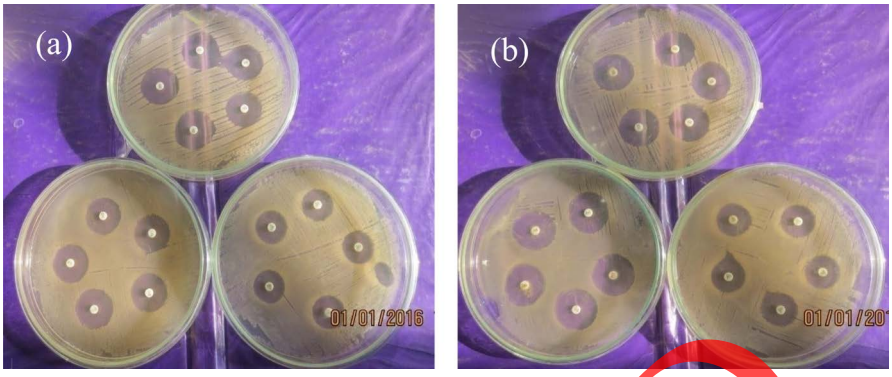

Figure 11. (a) Antibacterial activity of antibiotics without ZnO NPs. (b) Antibacterial activity of antibiotics with ZnO NPs.

ping of ZnO through biochemicals or phytochemicals by the action of their functional groups present in the *Ficus carica* leaf extract. It can be say that reduction done by phenolic groups and stabilization components of ZnO are amide linkage and amino acid. The SEM images confirm the hexagonal structure of ZnO NPs with an average size comparable to the XRD. The ZnO NPs show high antimicrobial activity; it enhanced the antimicrobial activities of antibiotics against *S. aureus*, Proteus, *P. aeroginosa*, Acinetobacter and *E. coli* respectively. It was confirmed by comparing the zones created by antibiotics with the zones created by the combination of antibiotics and nanoparticles. Nanoparticles of this plant could be of great importance in medical field for their antibacterial function. The combination of nanoparticles and antibiotics signifies their importance in medicine research respectively. These findings introduce a simple, inexpensive process to synthesize ZnO-NPs using conventional methods without the use of sophisticated equipment and its application as a potent nano-antibiotic. The application lies on textile industries, water purification, medical field, foods products, paints, nano-generators, field-emission transistors, highly effective solar cells, UV-detection, gas sensors and biomedicines.

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