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Green synthesis of Zinc Oxide Nanoparticles (zno-nps) using leaf extract of *Cucumis sativus;* Characterization, *in vitro* properties and environmental applications

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The current study is an efficient, ecological friendly and simple method to synthesized ZnO nanoparticles using *Cucumis sativus* peel extract. The purpose was the enhancement of antibacterial activity, antifungal and environmental application of ZnO nanoparticles (NPs) and minimization of use of toxic chemicals in nanoparticle fabrication. The synthesized ZnO nanoparticles were identified with X-ray diffraction analysis (XRD), UV-Visible spectroscopy (UV-Vis), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy dispersive X-ray (EDX) and Thermo gravimetric analysis (TGA). The XRD results indicated that the particles are well crystalline having size of 21nm. SEM analysis indicated that the particles are in agglomerated form with irregular morphology, while elemental composition of Zn and O were confirmed via EDX, From the UV-visible spectra the lambda max observed at 360nm while, band gap calculated was 3.00eV respectively. TGA analysis revealed that the ZnO nanoparticles are much more stable. Furthermore the prepared ZnO NPs were also investigated for their therapeutic applications. Best antibacterial activity was shown against *Citrobacter, Staphylococcus aureus,Providencia,* and *Streptococus* bacteria, while best antifungal activity was shown against *A.flavus* and *A. niger* with inhibition of 48% and 72% respectively at 500 ppm, The ZnO nanoparticles were also used for the degradation of methyl violet dye, approximately 88.3% dye degradation was noticed in 180 minutes.

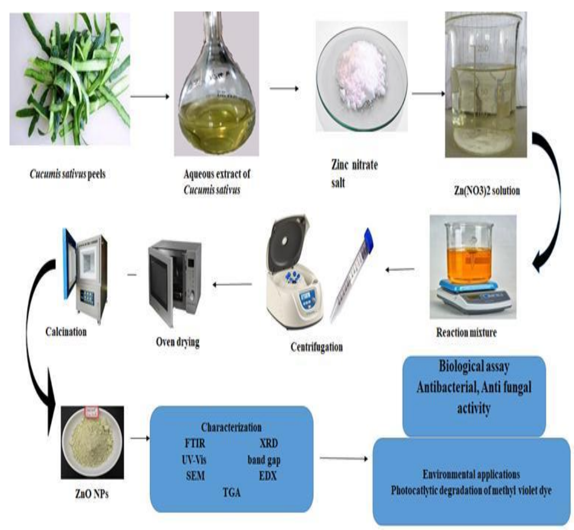
Keywords: Zinc Oxide, in vitro, environmental application

INTRODUCTION

Due to their diverse qualities, NPs are a wide spectrum of materials with diameters below 100 nm that can be employed in a variety of applications, including pharmaceutical, environmental, medical, electronics, energy gathering, mechanical industries, manufacturing and materials (Khan et al. 2019; Saleh et al. 2016; Guleria et al. 2020). Carbon nanotubes, quantum dots, nano-rods, nano-capsules, nano-emulsions, fullerenes, metallic NPs, ceramic NPs, and polymer NPs are all examples of NPs (Sudha et al. 2018; Khan et al. 2021).The science uses synthetic and material chemistry approaches to create nano-materials with particular sizes, shapes, and surface qualities. Cross-disciplinary nano science research involving physicists, chemists, and engineers is concerned with the need to produce environmentally benign and long-term nanomaterial synthesis processes (Raj et al. 2018; Pandiyarasan et al. 2014). Various chemical and physical approaches are now used for the green synthesis of NPs, but most of them have drawbacks such as cost and the use of toxic solvents (Bawazeer et al. 2021). Green synthesis nanoparticles have excellent qualities and are produced by all parts of a plant, including the base, leaf, stem, flower, and bark. The use of dried nano-particles in the synthesis of nano-particles. Every plant has terpenoids, alkaloids, flavonoids, and total phenolic content, all of which aid in the production of nanoparticles (Iravani et al. 2011). The approach of green biosynthesis of eco-friendly metal NPs procedures, in which bio-extracts are synthesized as NPs, is widely investigated (Makarov et al. 2014; Seddek et al. 2010). It entails the reduction of metal ions by extract phytochemicals in order to produce them as nanoparticles (Lestari et al. 2021). *Cucumis sativus* (cucumbers) belongs to the *Cucurbitaceae* family of gourds. It produces cylindrical fruits that are used in cooking. This plant mostly was the native of Southern Asia, but now a days found in almost every continent (Rajendran et al. 2010).

Direct band gap of zinc Oxide ZnO semiconductor is $E_g = 3.3$ eV. Owing to their high electron–hole binding energy (60 meV) it is a highly explored n-type semiconductors (El-Belely et al. 2021).Due to its unique chemical and physical features, such as a wide spectrum of radiation absorption, piezoelectric, pyroelectric, and high catalytic activity, zinc oxide nanoparticles (ZnO-NPs) are considered the most significant among metal oxide nanoparticles (NPs)(Yusof et al. 2019; Moghaddam et al. 2017). ZnO NPs are widely used in biology as antibacterial, antifungal and anti-inflammatory agents in catheter coatings, medical device disinfection, dental hygiene, eye therapies, wound dressings and antimicrobial filters. As compared to conventional antibacterial and antifungal medicines, these nanoparticles may easily penetrate pathogenic germs' cell walls and cell membranes. This is an important feature that contributes to their high antibacterial capabilities (Saravanan et al. 2018; Talebian et al. 2013).It may be able to take the place of traditional antibiotics. Because of the aforesaid features, zinc oxide nanoparticles (ZnO NPs) are nontoxic, chemically stable, and biocompatible, and could be employed as drug carriers, cell imaging agents, anticancer agents, antimicrobials, biosensors, antidiabetics, and cosmetics (Haque et al. 2020; Abedini et al. 2018). ZnO-NPs have recently been proven as dietary additives to boost growth performance, antioxidant properties, and immunological response, as well as improve the quality of eggs and layer chicken production (Natera et al. 2007).ZnO is now being researched for use in plant protection products, fertilizers, soil enhancement, and water purification(Ahmad et al. 2016). The advantages of green synthesis over other conventional chemical methods were kept in mind and a study was planned to synthesize ZnO NPs through green route using *Cucumis sativus* peel extract. Exploring therapeutic, environmental applications against the degradation of Methyl violet dye in aqueous medium was among the objectives of the present study.

Graphical Abstract



MATERIALS AND METHODS

Chemicals

All of the chemicals were bought from Sigma Aldrich Company and were analytical grade. Nutrient Agar, Nutrient Broth, Zinc nitrate, acetone solvents, ethanol, and methyl green dye were among the chemicals, media, and consumables used in this investigation.

Plant collection and preparation of *Cucumissativus* peel extract

On the basis of cost effectiveness and convenience of availability, cucumber (*Cucumis sativus*) was chosen for biosynthesis of ZnO NPs. Cucumber peel was collected from local fields in Pakistan's Charsadda district. The peel was rinsed with distilled water to remove any unclean or undesired visible particles that had adhered to the peel's surface. The cucumber pee was dried in the shade for about 20 days before being washed with deionized water to eliminate dust particles. The dried peel was then crushed until it was powdered. The powder peel was then steeped for two days in 500ml distilled water. Normal cold extraction was employed until the plant components were depleted. The extract was next concentrated under decreased pressure at low temperature using a rotary evaporator (Bawazeer et al. 2021).

Preparation of ZnO NPs

In a standard synthesis, 50 ml of Zinc nitrate (2 mM) solution was mixed with 50 ml cucumber peel extract. A mechanical stirrer was used to homogenize the mixture for 2 hours at 500°C (50 rpm). Visual observation of color change to dark brown, which was indicative of the creation of ZnO NPs, was used to monitor the reduction utilizing the cucumber peel method (Novelles et al. 2021). The suspension was then put into 15ml polypropylene tubes and centrifuged for 15 minutes at 9000 rpm. The pellet was washed three times with sterile water and twice with ethanol to remove adherent plant components, discarding the supernatant layer. The pellets were cleaned and then oven dried (80°C for 1 hour) before being calcined. The well-dried ZnO NPs were kept at room temperature until they were analysed further.

Characterization of ZnO nanoparticles

SEM Model No. JEOL-Jsm-5910, JEOL Company, Japan, was used to perform the morphological and structural analysis. The XRD examination was done with a JEOL-300 XRD, while the EDX study was done with an Inea 200 EDX spectrometer from a UK firm in Oxford, and the functional group determination was done with a PerkinElmer FT-IR Spectrophotometer. UV-Visible Spectrophotometer was used to investigate UV and photo deterioration (Shimadzu 160 A, Japan). TGA was used to examine thermal stability (Perkin-Elmer-TG instrument).

Anti-Bacterial Activity

Against *Staphylococcus aureus*, *Streptococus, Citrobacter, Providencia, Acinetobacter*, and *E. coli* the antibacterial activity of the produced zinc oxide nanoparticles was tested. Nutrient agar was used to cultivate bacteria on agar plates. 20 gram of Nutrient agar dissolved in 1 liter of distilled water was used to make bacteria growth media in the laminar flow cabinet, agar was dispensed into sanitized Petri plates. Bacterial cells were dispersed on fresh agar nutrition plates and kept at 37°C for 24 hours to maintain the germs alive (Mahalakshmi et al. 2019). Wells were created in agar using a borer, and different concentrations of ZnO NPs dissolved in 1% DMSO, 25l, 50l, 75l, and 100l were put in each well. The diameter of inhibitory zones was measured with a ruler after 25 hours and reported in millimeters (Fatima et al. 2015).

3.12 Antifungal activity

Plate culture method was used to test antifungal activity of zinc oxide NPs*. Aspergillus niger* and *Aspergillus flavus* were used in this study. Using the plate culture technique two fungal species was cultivated in PDA (potato dextrose agar) medium in petri dishes. In 75mL of distilled water 3g of PDA was dissolved to make growing media for the fungus. Autoclaved PDA media with zinc oxide nanoparticles was put in the petri dishes. After the PDA media had solidified, the fungus was injected. By evaluating the diameter of fungal growth at 2, 4, 6, 9, and 12 days, the efficiency of zinc oxide nanoparticles was calculated. As a control, DMSO was utilized. The percentage inhibition was determined using equation-1 (Arciniegas-Grijalba et al. 2017).

% inhibition = 100 - [growth in sample (cm) / growth in control (cm)] × 100 (1)

Photo catalytic study

The methyl violet dye was obtained from Sigma-Aldrich. In 100mL double distilled water, a 100ppm stock solution was prepared. Then, using the dilution formula (equation-2), a 10ppm solution was made in 50ml water

$$M_1V_1=M_2V_2 \qquad (2)$$

Photo catalytic capabilities of ZnO nanoparticles were assessed in the degradation of Methyl violet dye under UV light. The 5ml were taken before the nanoparticles were added. Then, in round-bottomed photocatalytic cells, 0.035g of ZnO catalysts were introduced separately to 45ml of dye aqueous solutions. To achieve adsorption desorption equilibrium, the solution was initially kept in the dark. The cell glass allows radiation to flow through, causing photosensitization of ZnO nanoparticles. Samples were collected and examined using a UV-Visible spectrophotometer at each time interval of the experiment. The dye's lambda max was found to be 585nm, respectively. The percent degradation was calculated by using equation3

$$\text{Degradation (\%)} = \left(\frac{Ci-Cf}{Ci}\right)\times 100 \qquad (3)$$

Where C_i show initial dye concentration, C_f show the dye concentration after the UV-irradiation.

RESULTS AND DISCUSSION

Synthesis of ZnO NPs

As seen in figure 1, the color changed from pale white to yellow, indicating the synthesis of ZnO NPs. Increases in zinc ion interaction time with peel extract resulted in an increase in absorbance, with the best synthesis occurring after 3 hours of incubation.There were no NPs created at the start of the process, but after 30 minutes, production began and lasted for another 3 hours.

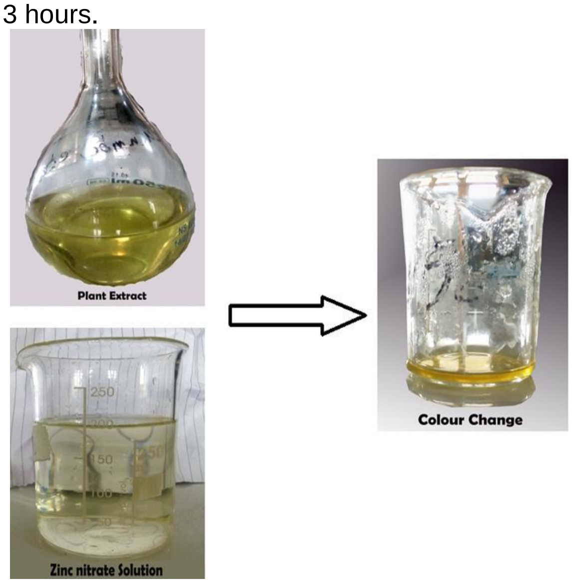


Figure 1: Confirmation pictures of *Cucumis sativus*assisted synthesis of ZnO-NPs

XRD Analysis

Cucumis sativus peel extract with various functionalities acts as a reducing agent in the green approach to ZnO nanoparticles. The inclusion of size-reducing chemicals in the extract could generate dwarf size ZnO nano-materials, according to the XRD data. XRD outline of ZnO NPs depicted a structure which is hexagonal wurtzite that corresponding to the intense diffraction peaks at (100), (002), (101), (102), (110), (103), (112), and (202) reflections. Using JCPDS card No. 36–1451 the XRD pattern of ZnO NPs synthesized using *Cucumis sativus* peel extract was matched as shown in Figure **2**. The peaks from the XRD measurements had noticeable line broadening, indicating that the produced particles were in the nano-scale range. Using the Debye–Scherrer formula, the average crystallite size of the particles was determined to be around 21nm. These XRD data matched the previous literature very well(Rahaiee et al. 2020; Zaree et al. 2019).

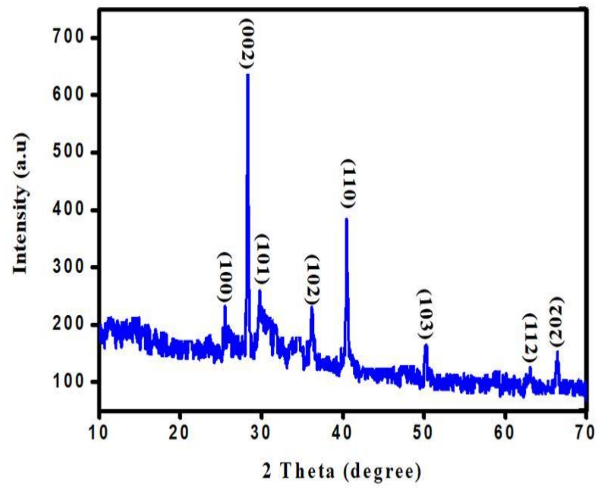


Figure 2: XRD spectrograph of *Cucumis sativus* synthesized ZnO-NPs

FTIR

Using FTIR analysis functional groups in the plant extract and nano-materials that contribute to the mechanism of bonding with ZnO NPs were identified. *Cucumis sativus* peel extract includes cyanidin-3-glucoside, thocyanins and flavonoids like polyphenolic derivatives which are considered promising bioactive chemicals for medicinal treatments and as reducing agents, as previously indicated(Mazhdi et al. 2018; Abdolhoseinzadeh et al. 2020; Awwad et al. 2020). Biosynthesized ZnO NPs and the plant extract FTIR spectrum is shown in Figure 3. The peaks at 880–1380, 1527–1700 and 3429 cm^{-1} corresponds to C-O or RCOO, N-H and O-H vibrations related to phenolic compounds, flavonoids and alkaloids respectively (Efafi et al. 2014; Sundrarajan et al. 2015).It should be noted that the extract's functional groups supply electrons, which can reduce zinc ions from Zn^{2+} to Zn^{+1}, and eventually to ZnO NPs. Furthermore, the extract's negative functional groups may have a stabilizing impact. The bending vibration of Zn-O bond is responsible for the new and intense peak at 526 cm^{-1} in the FTIR spectra of ZnO (Stan et al. 2016).

Furthermore, the extract's negative functional groups may have a stabilizing impact. The O-H of the phenol groups and $-NH_2$ bending vibrations are indicated by the major peak shifting from 3429 to 3426 cm^{-1} in ZnO using plant extract. While N-H binding is shown by another peak shifting from 1668 to 1579 cm^{-1}. C-H and C=C out-of-plane bending is shown by the appearance of new peaks at 700 and 2212–2230 cm^{-1} respectively (Alamdari et al. 2020).

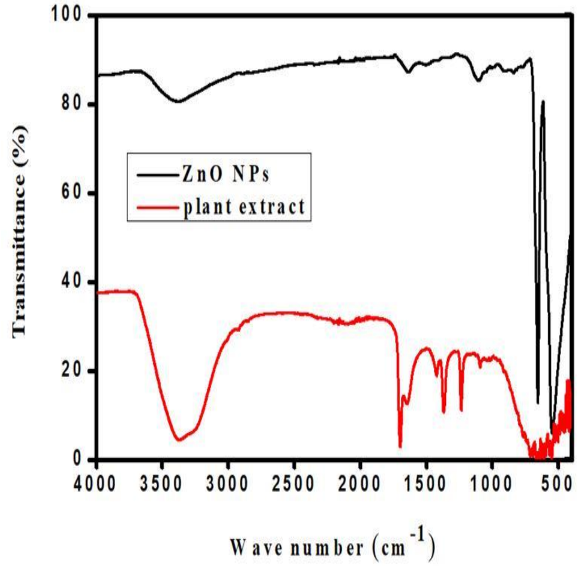


Figure 3: FTIR spectrograph of *Cucumis sativus* synthesized ZnO-NPs

UV visible spectroscopy and optical band gap of ZnO NPs

Figure 4 shows a UV-vis plot of green produced ZnO NPs (a). The auto combustion method confirms the generation of ZnO NPs by a prominent absorption peak at 360 nm. There was also a similar result in the earlier literature, where the UV-Vis spectra of green produced nanoparticles were at 364nm (Singhet al.2019). Furthermore, except from the characteristic peak, there was no other peak in the spectrum, indicating that ZnO NPs made from cucumber peel are of great purity. The blueshift in the excitation absorption clearly indicates the quantum confinement property of NPs. Furthermore the optical band gap were find out by using the formula $(Ah\upsilon)^n = B(h\upsilon - Eg)$ which was identified by using a tock plots (Ahυ)n versus hυ at (Ahυ) n = 0 for n = 2. As shown in Fig. 4(b) the band gap for the green synthesized ZnO-NPs was found to be 3.0 eV which is in accordance with results in the literature (Krupa et al. 2016).

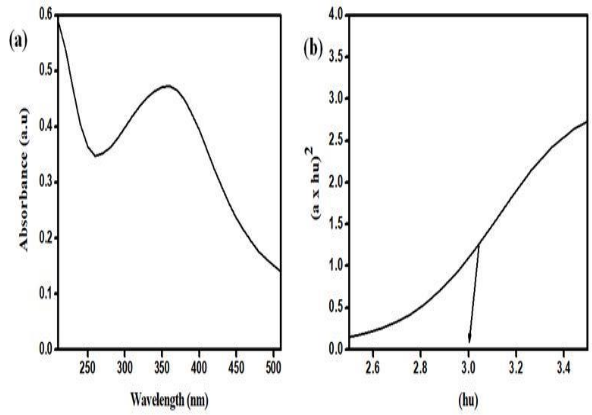


Figure 4(a) UV-visible spectrograph and 4(b) optical band gap of *Cucumis sativus*s assisted synthesized ZnO-NPs

SEM and EDX

The morphology of the surface was examined using SEM, and structural rectangle, radial hexagonal, triangular, spherical, and rod-like geometries were estimated. The SEM shapes of Zinc oxide nanoparticles were are spherical, rectangle, triangle, spheres and radial as exhibited in figure 5(a). The resulting zinc oxide nanoparticles morphology was quite similar to the previous literature (Nagarajan et al. 2013).

Figure 5(b) shows the EDX analysis of ZnO nanoparticles, confirming the synthesis of ZnO nanomaterials by the significant concentration of oxygen and zinc. However, the higher carbon content in the EDX spectrum is owing to the presence of a higher amount of carbon in the cucumber peel extract, while the other elements in the EDX are from the plant.

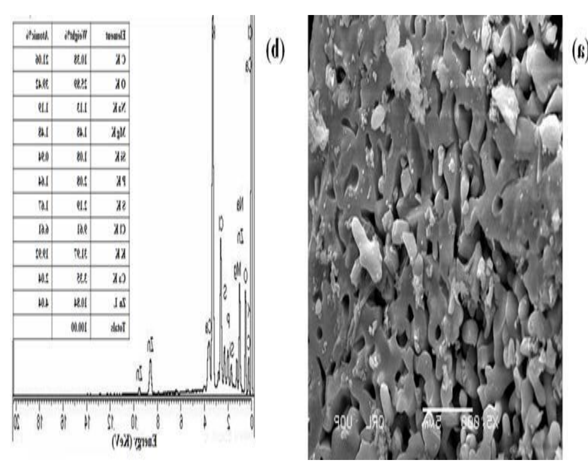


Figure 5: (a) SEM and 5(b) EDX spectrograph of *Cucumis sativus* synthesized ZnO-NPs

TGA

The TGA spectra of the biosynthesized ZnO-NPs state that the sample decays heavily as the temperature increase. The sample (4.8 mg) was taken totally from the range of 40 °C up to 600 °C temperature, the greater loss occurred it was due to the presence of different volatile components present in the ZnO nano-materials from the peel extract, as shown in Figure 6. The combination of ethanol and water in the sample caused the sample to initially lose its integrity. Previous research employing aqueous extracts of Myristica fragrant leaves to manufacture ZnO-NPs observed similar weight reduction (Faisal et al. 2021).

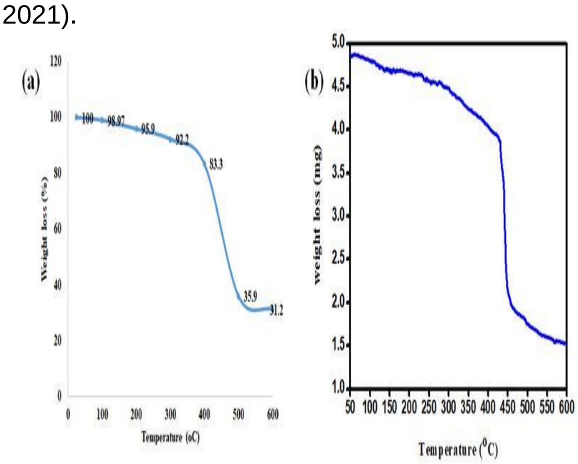


Figure 6 :(a) TGA percent loss of the sample and 6(b) mass loss in mg spectrograph of *Cucumis sativus* assisted synthesized ZnO-NPs

Anti-bacterial activity

Four Gram-negative bacteria species including Citrobacter*, Providencia, Acinetobacter*, and *E. coli* and two Gram-positive bacterial species *S. aureus* and *Strepcocuccus* were used for the assessment of antibacterial activity of the zinc oxide. Against the investigated bacterial species, different zones of inhibition were identified. The results revealed that the ZnO nanoparticles had a positive effect against the bacteria that were examined. The data also showed that when the concentration of biosynthesized ZnO NPs grew, so did the zone of inhibition. According to the literature, the antibacterial activity of nanoparticles increases as the number of nanoparticles increases (Khalil et al. 2020). The antibacterial activity was tested at different concentrations (25uL, 50uL, 75uL, and 100uL) as indicated in figure 7. Zinc oxide nanoparticles were shown to be the most effective against the microorganisms studied. *E.coli* and *Acinetobacter* were more sensitive to the produced ZnO NPs than *Staphylococcus aureus, Citrobacter, Providencia, and Streptococus.* As the number of nanoparticles grows, increase the activity.Figure 8 depicts the antibacterial activity of produced ZnO nanoparticles at various doses*.* According to earlier research, *Azadirachtaindica* zinc oxide NPs have high antibacterial activity against *S. aureus, S. pyogenes*, and *E. coli* and ZnO NPs have a stronger antibacterial effect against *S. aureus* and *E. coli* (Bhuyan et al. 2015).

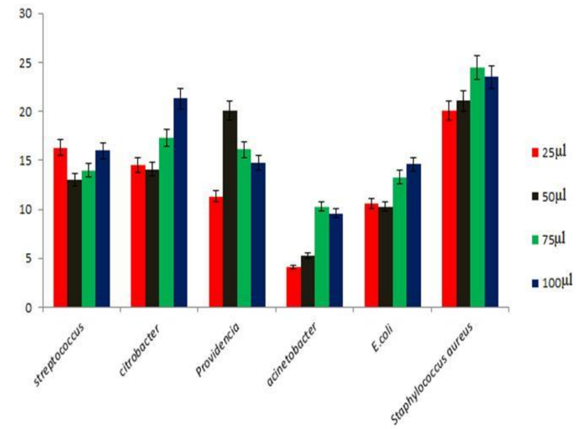


Figure 7: Antibacterial activity of ZnO NPs against *Streptococus, Citrobacter, Providencia, Acinetobacter, E. coli* and*S. aureus.*

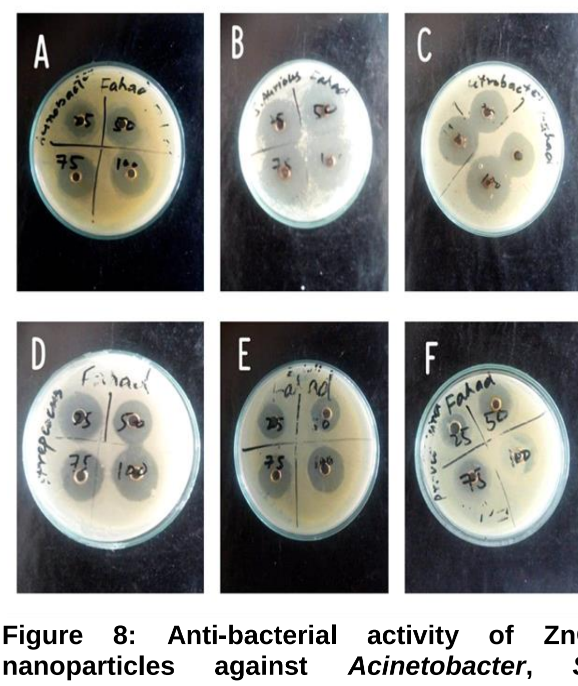


Figure 8: Anti-bacterial activity of ZnO nanoparticles against *Acinetobacter*, *S. aureus*, *Citrobacter*, *Streptococus*, *E. coli* and *Providencia*.

Antifungal Activity

The fungal pathogens *Aspergillus niger* and *Aspergillus flavus* were utilized to test the biosynthesized zinc oxide NPs. Figure 9 shows that the ZnO NPs had outstanding efficacy against *A. niger* and *A. flavus*, with inhibition of 72 and 48 percent at 500 ppm, respectively. As a negative control, DMSO was utilized, resulting in a zero percent inhibition zone. The findings were compared to previous research that identified these diseases to damage certain fruits and vegetables such as carrots, tobacco, tomatoes, wheat, ornamental plants and onions (Malaikozhundan et al. 2017).

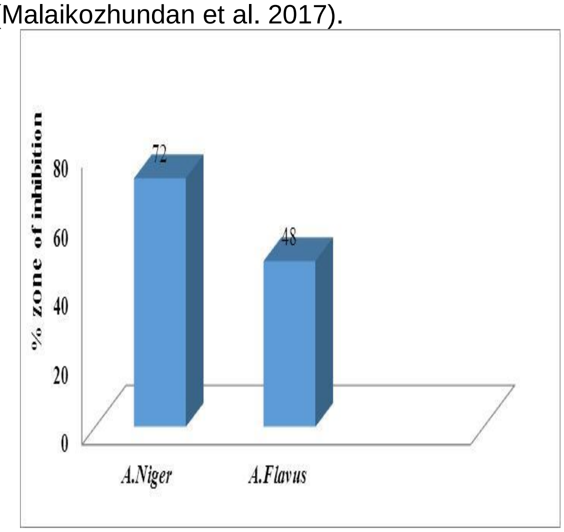


Figure 9: Antifungal activity ZnO nanoparticles against *A. niger* and *A. flavus*.

Photocatlytic Activity

Effect of time on degradation of Methyl violet dye

In the first 30 minutes of illumination, the degradation of methyl violet dye was 44.4 percent, but as the time went on, the degradation of dye increased, and the greatest degradation of methyl violet dye was recorded at 180 minutes, which was 88.3 percent, as shown in figure 10.MgO NPs' high rate of deterioration may be owing to their large surface area to volume ratio. For the absorption of reactant molecules more photocatalytic reaction centers can provided larger surface area. Furthermore, a bigger surface area is more effective for UV light absorption, resulting in more electrons and holes being generated(Rahmat et al. 2019; Salama et al. 2018).

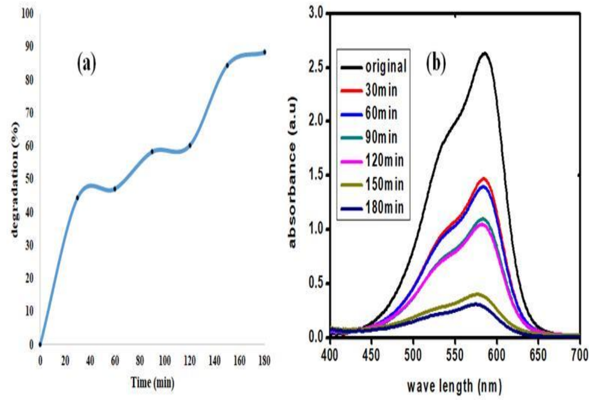


Figure10: (a) percent degradation of methyl violet dye with MgO NPs and 10(b) and in spectra of UV-Visible Spectrophotometer

Mechanism of photocatalytic degradation of dye

The possible photocatalytic degradation of Methyl violet is as follows: first on the surface of the photocatalyst the molecules of Methyl violet dye are adsorbed, which on exposure to UV light causing excitation of electrons from valence band to the conduction band, leaving holes (h+) in the valance band. Hydroxyl (•OH) and superoxide ($O_2^{\bullet-}$) radicals are generated when electrons and holes at the surface of photo catalyst react with adsorbed water molecules, dissolved oxygen, and the surface's hydroxyl groups (equation 4-8) also shown in figure 11.The dye molecules would be degraded by these photo-generated radicals, resulting in intermediates that completely break down into CO_2 and H_2O (Rajeshwar et al. 2008; Casbeer et al. 2011).

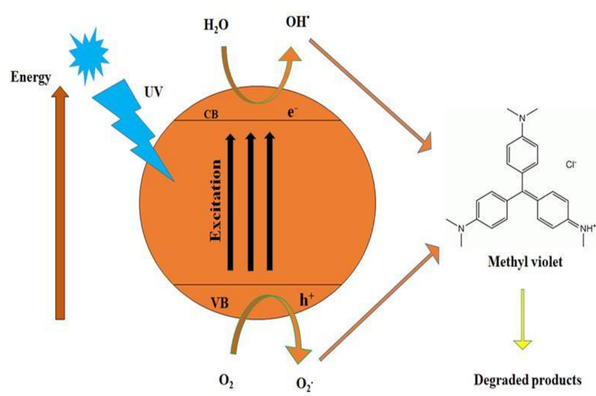


Figure 11: Mechanism for the degradation of methyl violet dye.

ZnO (nps) +hv → e^- + h^+

(4)

$H_2O + h^+ \rightarrow {}^{\cdot}OH^{\bullet} + H^+$ (5)

$O_2 + e^- \rightarrow {}^{\cdot} O_2^{\bullet -}$ (6)

${}^{\cdot}OH$ + dye→ degradable product (7)

O_2^- + dye→ degradable product (8)

CONCLUSION

Using *Cucumis sativus*, versatile plant peel extract one-pot eco-friendly biomedical important ZnO-NPs were successfully synthesized. The green synthesized ZnO-NPs was found to have a band gap of 3.0 eV. ZnO-NPs had crystalline structure as shown by XRD analysis, FTIR analysis confirmed the existence of different phytochemicals involved in the transfer of ions to ZnO NPs. Morphological study revealed that the particles are spherical, rectangle, triangle, spheres and radial. TGA analysis shown that about 69% weight loss occurred at the heating range 40 to 600 ^oC. The prepared bio-fabricated zinc oxide nanoparticles shown best antibacterial and antifungal activity for example E.coliand *Acinetobacter* were more sensitive to these nanoparticles. These ZnO nanoparticles were also effective against the photo degradation of Methyl violet dye and about 88.3% dye degradation was found at 180 minutes time interval. To explore their applications in biomedicine at both in vivo and in vitro levels more research on zinc oxide nanoparticles is needed.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

F. A. Jan and A. Khan**:** proposed, supervised and the entire work and revised the manuscript.

S. Fahad, Wajidullah and R. Ullah: performed the experiments and also wrote the manuscript.

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