

MODIFICATION NANO-SIZE OF ZINC OXIDE (ZnO-NS) WITH POLYETHYLENE GLYCOL AND APPLICATION AS ANTIMICROBIAL

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ABSTRACT

Sol-gel is a simple method used to synthesize nano-size ZnO (ZnO-NS) in many applications. It acts as an anti-microbial agent for *Escherichia coli* and *Aspergillus niger* infections. The nano-size and patterned nanorods structure of ZnO enhances anti-microbial properties. This study used Polyethylene Glycol (PEG-6000) polymer compounds with variations in their 3, 5, and 10% compositions as templates for the formation of the nanorods. The morphology of the ZnO-NS produced determined the increase in anti-microbial activity. Furthermore, those generated from 5% PEG composition (ZnO/PEG-5) have the smallest size with the greatest anti-microbial activity on inhibition zones against *E. coli* and *A. niger*, each of 8.5-10.5 mm. There are morphological differences in each variable in the homogeneity distribution level of the spherical and rod dimension patterns of ZnO-NS. This is based on characterization with UV-VIS-DRS and Fourier-Transform infrared (FT-IR) for the analysis of Zn-O interactions at wave number 500-550 cm⁻¹. Field Emission Scanning Electron Microscopy-Dispersive Scanning Electron Microscope (FE-SEM) and Energy dispersive X-ray spectroscopy (EDX) was used for dimensional pattern analysis, while X-ray diffraction (XRD) was applied for hexagonal wurtzite ZnO structures following the ICSD-65122 standard.

Keywords: Modification, ZnO-Nano Size, Polyethylene Glycol (PEG-6000), Textile, *Aspergillus niger*.

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INTRODUCTION

Nanotechnology refers to synthesizing and using substances up to 100 nm in size. Modern advances in this field, especially in synthesizing nanoparticles of various sizes and shapes, are directed toward producing new biocidal agents. Several studies show that ZnO can be used as a bactericidal tool.¹⁻² The antibacterial activity of ZnO-NS against various bacterial pathogens has been investigated. Therefore, it was discovered that ZnO could inhibit their growth at low concentrations.³⁻⁵ Nanosize ZnO shows several advantages in physical, chemical, and biological properties when compared to ZnO on a macro (micro-meter) scale. The application of basic nanotechnology concepts to design ZnO-NS can be accomplished by controlling the bottom-up stage of a synthesis process to obtain the desired morphology (size, shape, and surface area).³ This field's development, especially in synthesizing ZnO-NS, is conducted with several ZnO-NS synthesis techniques, such as biological and chemical methods. However, using environmentally friendly biological synthesis as a substitute for its chemical counterpart can help control toxicity. ZnO-NS is a multifunctional inorganic compound with effective anti-microbial, self-cleaning, anti-flame, and anti-UV activities. It has also been applied in several fields, such as medicine,⁸ agriculture,^{9,10} and biomedical.¹¹ Its distribution and absorption depend primarily on the particle size, dimensional pattern, and surface area.¹⁰ Because of the increased need for ZnO-NS, the supply in large quantities requires a simple, economical, effective, and environmentally friendly synthesis method. Sol-

gel is one of the approaches with several advantages, including simplicity, the uniform size distribution of the final product, and a more homogeneous dimensional pattern. This process occurs in several stages, namely hydrolysis, condensation, aging, drying, and calcination. The formation of ZnO-NS in the sol-gel process begins with the crystal nucleus's development, which leads to the growth of crystals and nanosize ZnO particles. Furthermore, using biological compounds in the form of plant extracts and microorganism cells provides alternative opportunities in the large-scale production of ZnO-NS.¹² Microorganism cells consisting of fungi, bacteria, actinomycetes, and viruses have been reported as a method for producing nanoparticles in a biologically efficient manner compared to chemical techniques.⁴ Microbes scattered in nature are diverse and widely distributed in various soil, water, and air habitats.³ The *Aspergillus niger* fungal was selected as a template for ZnO-NS biosynthesis and a source of stabilizer or capping agent because of its ease of culture and handling to obtain biomass quickly, abundantly, simply, and economically. The mechanisms and conditions of the biosynthetic process should be controlled to optimize the modification of nano size with a homogeneous level of size uniformity. Meanwhile, the capping agent needs to be synergized with surfactant or polymers as a template of nanorods formation to design the dimensional pattern. Zheng *et al.* (2017) have successfully synthesized ZnO using the PEG-10000 additive.¹³ Polyethylene Glycol (PEG) was used as a template to form the nanorods' morphology.⁶ Furthermore, the interaction between hydrogen bonds with the O atoms in its chain results in an interfacial microemulsion effect such that the growth of ZnO-NS crystals is distributed along the monomer chain and then combines to produce a collection of nanorods.^{7,8} Along with industrial development, it has a negative impact by increasing environmental pollution globally. This includes the emergence of several infectious diseases with the development of pathogenic microorganism cells. However, control to inhibit microorganism cells has encountered obstacles due to antibiotic resistance. This has become a worldwide issue as it raises the concern of health research analysts.^{12,13} *Escherichia coli* and *Aspergillus niger* are significant pathogens that cause various human diseases. The anti-microbial mechanism of action of ZnO-NS has implications for hydrogen peroxide production. ZnO-NS produces reactive oxygen species, such as $\bullet\text{OH}$ and $\bullet\text{O}_2$, which are created on its surface, penetrate microbial cells, and effectively inhibit cell growth.¹²⁻¹⁵ This study synthesized it as a textile coating by the biogenic method using *Aspergillus niger*. Additionally, it was tested against *Escherichia coli* and *Aspergillus niger* in vitro for anti-microbial textile fibers, increasing fiber strength, textile preservation during storage, convenience, and hygienic properties.¹³⁻¹⁵

EXPERIMENTAL

Material

The materials used in this study were Textile Cotton, Nutrient Agar (NA) Media, Nutrient Broth (NB) Media, Potato Dextrose Agar (PDA), Potato Dextrose Broth Media (PDB), Zinc Oxide (ZnO) (Merck), Zinc (II) Nitrate Tetrahydrate ($\text{Zn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) (Merck), Hydrochloric Acid (HCl) (Aldrich 37%), Ammonium Hydroxide (NH_4OH) (Merck 25%), Sodium Hydroxide (NaOH) (Merck), Hexamethylenetetramine ($\text{C}_6\text{H}_{12}\text{N}_4$) (Merck), Ethanol ($\text{C}_2\text{H}_5\text{OH}$) (Merck), Aquadest, Polyethylene Glycol (PEG-6000) (Merck), Sodium Dihydrogen Phosphate (NaH_2PO_2), Sodium Carbonate (Na_2CO_3), Media Nutrient Agar, and Biomass *Aspergillus niger*.

ZnO Nanoparticle Biosynthesis

ZnO of 1.626 g was dissolved in 200 mL of distilled water. The solution was homogenized for 2 hours. The mixture was then left for 24 hours at room temperature and heated at 110°C until it became a gel. Furthermore, the gel was coated on a glass substrate. A 200 mL solution consisting of 5.446 g $\text{Zn}(\text{NO}_3)_2$ and 3.2 g NaOH was dissolved in distilled water and homogenized for 4 hours. 5.6 g of Hexamethylenetetramine (HMT) was added and homogenized for 4 hours. PEG-6000 was added with various compositions of 3%, 5%, and 10%, after which 1.2 grams of *Aspergillus niger* mushroom biomass was added. The mixture was adjusted to pH 13.0 and homogenized for 2 hours. The solution dried at 90°C for 3 hours to form a gel and coated on a glass substrate with seed ZnO. Subsequently, the glass substrate was heated at 250°C for 4 hours, and then calcination was performed at 600°C for 4 hours. ZnO-NS characterization was determined using the FESEM-EDX (Field Emission Scanning Electron Microscopy-Dispersive) instrument, X-ray Spectroscopy, X-ray Diffraction (XRD), Fourier Transform

Infrared (FT-IR) with wave number of 400-4000 cm^{-1} , and Ultraviolet-Visible- Diffuse reflectance spectroscopy (UV-VIS-DRS).¹²

Antimicrobial Test

Anti-microbial tests were conducted on textiles as a coating medium for ZnO. At the beginning of the process, the 8 x 8 cm textile was washed with 2 g/L detergents. Furthermore, it was rinsed with distilled water and dried at 70°C. The dried fabrics were then soaked as a dewaxing process with Na_2CO_3 3.7×10^{-3} M at 100°C for 5 minutes. It was rinsed with distilled water and dried at 70°C. The textile was immersed in 0.3 M citric acid crosslink in 0.5 M NaH_2PO_2 for 12 hours at room temperature. Subsequently, it was dried at 70°C for 15 minutes. The ZnO-NS coating process was conducted using the dip-spin coating method. The textile was dipped in ZnO-NS suspension for 10 minutes and then dried at 70°C for 15 minutes. Additionally, the coating was repeated 3 times to obtain more even distribution so that spin-coating is performed. Textiles coated with ZnO-NS were cut into discs with a diameter of 0.6 cm. It was positioned on sterile NA and PDA media in a Petri dish inoculated with *Escherichia coli* bacteria and *A. niger* fungi. Petri dish plates were incubated with UV irradiation for 24-96 hours. Finally, the inhibition zone was measured at 24-hour intervals.¹²

RESULTS AND DISCUSSION

Fourier Transform Infra-Red (FT-IR) Analysis

FT-IR analysis was conducted to determine the interactions between precursor molecules and the additives used by identifying organic or inorganic functional groups at wave numbers from 4000-400 cm^{-1} . According to Fig.-1, wave number 500-550 cm^{-1} shows the Zn-O interaction, where $\sim 867 \text{ cm}^{-1}$ is Zn-OH formed from the hydrolysis reaction between Zn and NaOH. At 1350-1500 cm^{-1} , there is a strain vibration absorption of C-N aromatic and aliphatic amines.^{17,18}

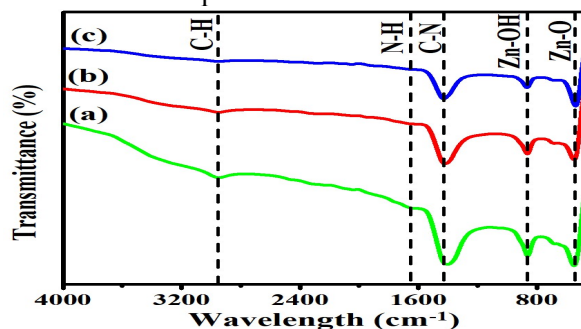


Fig.-1: FT-IR Spectrum of ZnO-NS with PEG-6000 Composition, (a) ZnO/PEG-3, (b) ZnO/PEG-5, (c) ZnO/PEG-10

X-Ray Diffraction (XRD) Analysis

Figure-2 shows the ZnO diffractogram at 2θ 31.76°; 34.43°; 36.22°; 47.56°; 56.63°; 62.89°; 67.98°; 69.13°, which reveals the ZnO wurtzite structure. The XRD pattern exhibits the hkl fields (100), (002), (101), (102), (110), (103), (200), (112), and (201), hence, ZnO-NS is categorized as having a hexagonal unit cell according to the ICSD-65122 standard. The size of ZnO-NS can be determined based on the peak area in the spectrum.

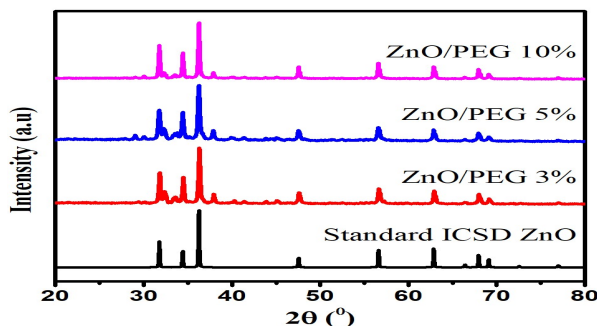


Fig.-2: XRD Pattern of ZnO-NS with PEG-6000 Composition, (a) ZnO/PEG-3, (b) ZnO/PEG-5, (c) ZnO/PEG-10, pH = 13, at calcination temperature of 600°C.

According to Table-1, the crystal size was determined based on the composition of the PEG used in the ZnO-NS biosynthesis process. The size obtained was 26.35-33.53 nm, and the smallest crystal size was discovered in ZnO/PEG-5.

Table-1: ZnO-NS Crystal Size Based on Difference in PEG-6000 Composition

Composition PEG-6000	Crystal Size (nm)
ZnO/PEG - 3	32,88
ZnO/PEG - 5	26,85
ZnO/PEG - 10	33,53

FESEM-EDX Analysis

Field Emission Scanning Electron Microscopy (FE-SEM) analysis was used to determine the surface morphology and particle size distribution of ZnO-NS. Meanwhile, Energy Dispersive X-Ray (EDX) analysis is used to identify the chemical elements in the sample semi-qualitatively. Figure-3 shows a ZnO-NS powder with a spherical and rod-like structure. The FE-SEM pattern discovered that ZnO/PEG-3, ZnO/PEG-5, and ZnO/PEG-10 has a spherical pattern, rod pattern, and agglomerated pattern rods, respectively. Based on the FESEM-EDX results in Fig-4a, the particle distribution of the ZnO/PEG-5 sample was measured to determine the rods' size with the nanorods' homogeneity level at 35.31 nm. Meanwhile, EDX analysis was used to determine the composition and distribution of elements contained in ZnO powder, as shown in Fig.-4b. The results show the percentage of elements in a semi-qualitative manner, with each being O at 10.47%, Na at 14,70 %, and Zn at 74.82%.

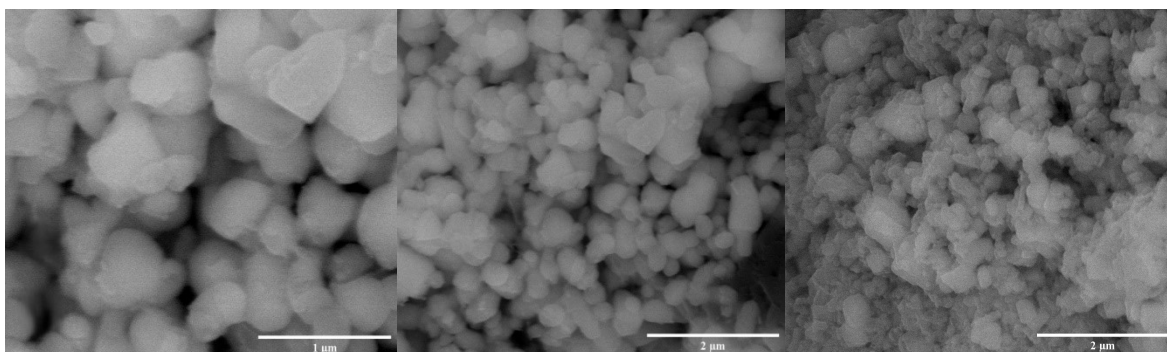


Fig.-3: FE-SEM Pattern of ZnO-NS With Difference Composition of PEG-6000 (a) ZnO/PEG-3, (b) ZnO/PEG-5, and (c) ZnO/PEG-10

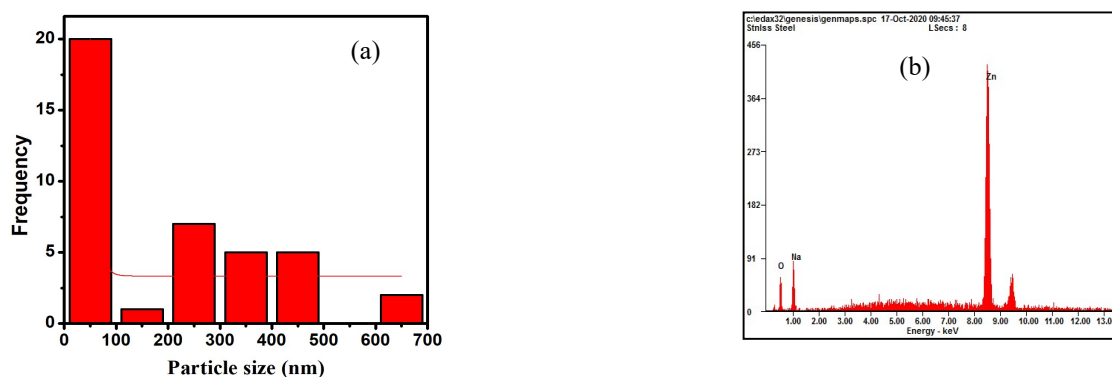


Fig.-4: (a) Particle Size Distribution of ZnO/PEG-5, (b) ZnO/PEG-5 EDX Analysis

UV-DRS Analysis

The UV-DRS analysis was conducted on ZnO/PEG-5 and ZnO/PEG-10, as shown in Fig.-5. Changes in the energy gap of ZnO-NS were predicted due to modifications with the addition of PEG-6000 polymer, which could give different dimensional patterns at varying compositions. Furthermore, the PEG-6000-10 are 3.01 eV and 3.05 eV, respectively. Energy changes in the ZnO-NS gap can be used as a model for the modification of its structure. Dibanding dengan tanpa PEG 3.2.

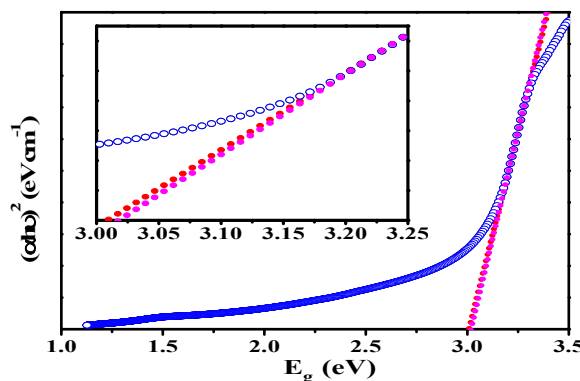
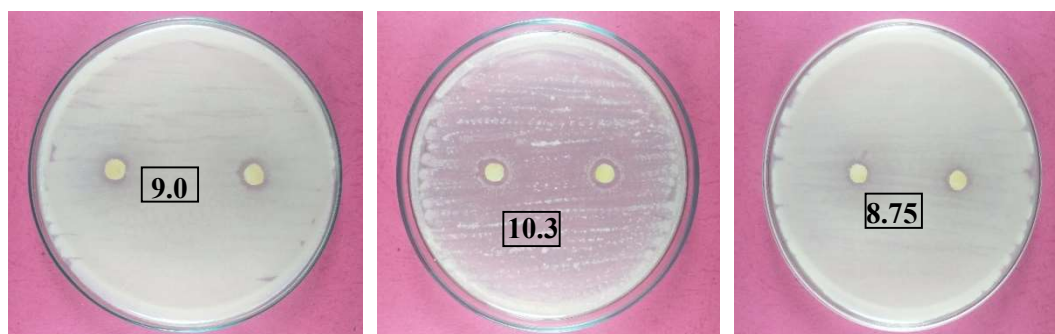


Fig.-5: UV-DRS Plots of ZnO/PEG-5

Anti-microbial Textile Test

Escherichia coli bacteria and *Aspergillus niger* fungal were tested for anti-microbial activity using the diffusion method and the inhibition zone measurement to compare the inhibitory ability of ZnO-NS on textile fiber media. *Escherichia coli* are pathogenic bacteria that can cause various diseases, such as diarrhea, vomiting, and nausea.¹⁵⁻²⁵ They are widely discovered around the human intestine. Meanwhile, *A. niger* is a pathogenic fungus that causes aspergillosis and attacks the lungs. They can also grow on textile fibers, especially on those with high humidity, and can damage the quality. Figure-7 shows the antibacterial activity test using *Escherichia coli*. The inhibition zone is indicated by the presence of ZnO-NS activity against test bacteria. Table-3 presents the size of the clear zone in each ZnO-NS with variations in PEG-6000. It was correlated that ZnO/PEG-5 has the smallest crystal size, hence, a larger surface area is obtained. The contact time also affects the size of the inhibition zone. It is directly proportional to the antibacterial activity. Additionally, the contact time is based on the duration of irradiation. In this process, the valence band electrons will continue to experience excitation towards the conduction band. They will react with O_2 to form radicals. Meanwhile, the band left by electrons leaves holes, which also react with H_2O and form $\cdot OH$ radicals. These radicals degrade the organic compounds and cause the bacterial cell wall to lyse and die.¹⁷⁻²² The antifungal activity test can be seen in Fig.-8. The clear zone indicates that ZnO-NS can also play a role in inhibiting the growth of *A. niger*. Table-4 shows the size of the clear zone in each ZnO powder with different compositions of PEG-6000 and inhibited fungal growth at 24 hours. The inhibition zone after 24 hours can be contaminated in the clear zone that ZnO-NS previously inhibited. This is due to easy fungal spores and cell walls that are difficult to degrade.

Fig.-7: Inhibition Zone of Textile Fibers in *Escherichia coli* Bacteria (a) ZnO/PEG-3, (b) ZnO/PEG -5, and (c) ZnO/PEG-10

The anti-microbial activity through the attachment of ZnO-NS on the cell surface damages the structure of the membrane, reducing the activity of various membrane enzymes. Physical damage, such as disruption of the phospholipid and protein bilayer of the cell membrane by ZnO nanoparticles, causes lysis of the cell membrane. Furthermore, ZnO-NS initiates lipid peroxidation reactions, which will cause DNA damage, depletion of glutathione, disruption of membrane morphology, and electron transport chain. DNA strand breaks are caused by prolonged contact of ZnO-NS with the bacterial cell membrane. The toxic effect on bacterial cell death is due to the different positive zeta potentials of the biological

sources used to synthesize nanoparticles. Additionally, penetration of ZnO-NS into the bacterial membrane will inactivate the enzyme and, ultimately, cell lysis.²⁰ Figure-9 can be described as a cycling mechanism for the anti-microbial activity of ZnO-NS.

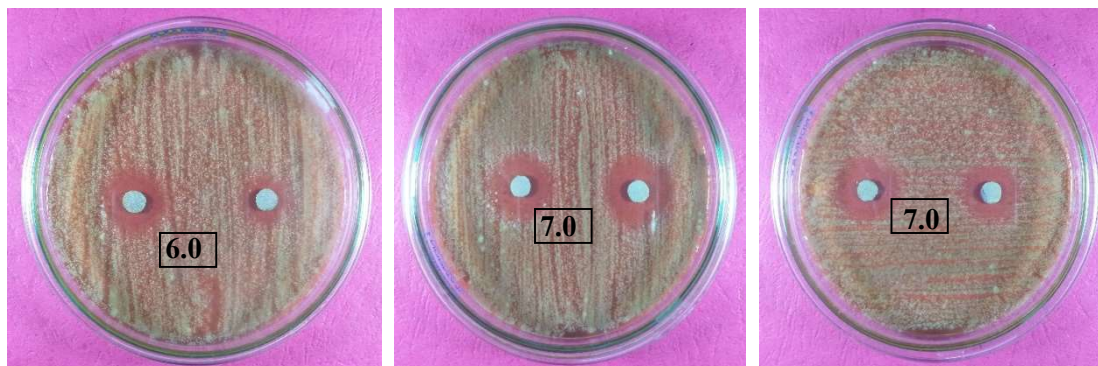


Fig.-8: Inhibition Zone of Textile Fibers in *A. niger* (a) ZnO/PEG-3, (b) ZnO/PEG-5, and (c) ZnO/PEG-10

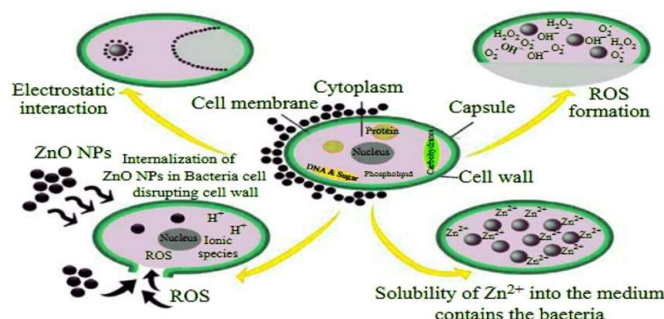


Fig.-9: Cycle Mechanism on Activity of Antimicrobial ZnO Nanoparticles

CONCLUSION

ZnO-NS biosynthesis was successfully conducted by the Sol-gel method using the biological agent of *Aspergillus niger* at pH = 13.0. Adding PEG-10,000 can modify the morphology of ZnO, and the resulting structure is PEG-5% nanorods with crystal and nanoparticle sizes of 26.85 nm and 35.31 nm. The effectiveness of ZnO as an anti-microbial agent on textile fiber media can be related to crystal size. Furthermore, its nanorods on ZnO/PEG-5 provided the most potent antibacterial properties against *Escherichia coli* and *Aspergillus niger* with inhibition zones of 9.0 - 10.5 mm and 16.5-190 mm, respectively.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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