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Antimicrobial Activity of Polyvinyl Alcohol Incorporating Bismuth-Zinc Oxide Nanocomposite against Escherichia Coli and Staphylococcus Aureus

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Abstract

More effective antimicrobial drugs are being sought for by researchers due to the rise of antibiotic-resistant microorganisms. This study presents the production and characterization of a nanocomposite of bismuth zinc oxide and polyvinyl alcohol that has antibacterial action against Staphylococcus aureus and Escherichia coli. Precision characterization using FTIR, XRD, EDX, and SEM was carried out in conjunction with the simple co-precipitation approach that was used to create the Bi-Zn oxide nanocomposite. The zone of inhibition was determined using the disks of the Kirby-Bauer diffusion method for both 5% and 10% of PVA (Bi-Zn) oxide. The outcomes revealed that 10% concentration displayed substantial sensitivity towards S. aureus with the zone of inhibition of 35 mm and moderate affectivity to E. coli. From these results, PVA blended with Bi-Zn oxide nanocomposites could be prospective candidate material for antimicrobial application, especially against gram-positive microorganisms.

Keywords: Nanocomposite synthesis, Antimicrobial efficacy, Biofilm Disruption, Bismuth Zinc-Oxide, Pathogen Resistance

INTRODUCTION

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A new generation of antimicrobials is urgently needed due to the alarming rise of bacteria that are resistant to existing antibiotics (Ventola & therapeutics, 2015). Some of the most researched bacteria include *E. coli* and *S. aureus* because to the significant impact they have on human health. According to Kaper, Nataro, and Mobley (2004), the gram-negative bacteria *Escherichia coli* is mostly present in the intestines of both humans and animals, where it assists in digestion and nutritional absorption. Some of the most serious symptoms that may be induced by pathogenic strains of *E. coli* are gastroenteritis and urinary tract infections (UTIs). Transmission often happens when people come into contact with contaminated food or water or when there is a lack of proper sanitation and hygiene in a specific area (Scallan et al., 2011). The most typical causes of *E. coli* infections include tainted meat, raw milk, and fresh vegetables. Urinary tract infections (UTIs) caused by *Escherichia coli* are prevalent in hospital settings and may spread to bloodstream infections, which can disproportionately affect the elderly and those with impaired immune systems (Russo, Johnson, & infection, 2003). The gram-positive bacteria *Staphylococcus aureus* is ubiquitous in the nasal passages and skin of healthy humans. Although *S. aureus* is a normal flora component in around 30% of the population, it can become harmful when it enters the body through wounds, cuts, or invasive devices (Lowy, 1998). The severity of infections caused by *Staphylococcus aureus* may vary from relatively minor skin infections like impetigo and boils to life-threatening illnesses such as pneumonia, endocarditis, and sepsis (Tong, Davis, Eichenberger, Holland, & Fowler Jr, 2015). According to Otto (2008), one major issue with *S. aureus* is that it may create biofilms and become resistant to treatment, which makes it harder to get rid of infections. As an example, the notorious methicillin-resistant *Staphylococcus aureus* (MRSA) strain has evolved resistance to many drug classes, posing serious problems in both clinical and community contexts (DeLeo, Otto, Kreiswirth, & Chambers, 2010). *S. aureus* is a prevalent pathogen that can infect humans through direct contact with those who are sick or contaminated surfaces. It is also a common culprit in hospital effluents, particularly from patients, surgical wounds, or embedded medical devices (Klevens et al., 2007). *E. coli* and *Staphylococcus aureus* are very dangerous to humans because they are pathogenic and may cause nosocomial infections. Better public health and a lower global incidence of bacterial illness may be achieved by increasing our understanding of these bacteria, including their virulence, method of transmission, and control strategies.

NEED FOR NOVEL ANTIMICROBIAL AGENTS

Due to the increasing cases of opportunistic and resistant disease-causing bacteria, it is critical to apply different approaches. In this respect, nanotechnology provides a viable solution. But metal-based nanocomposites received bunch of attentions because of its antimicrobial characteristics. Biocompatibility and film-forming capabilities make polyvinyl alcohol (PVA),

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a water-soluble polymer, useful in many biomedical and industrial contexts. (Oun et al., 2022). Recently, PVA based compounds have attracted considerable interest because they are reported to possess antibacterial activity especially when incorporated with other materials such as metal oxides. Several types of metal, one of which is a bismuth zinc nanocomposite which has been considered as having antibacterial effect. Bi-Zn Nano compounds are also recognized as effective in combating a wide range of microbes and bacteria. (Abdelhamid & Mathew, 2022; Huang et al., 2020; McDonnell & Russell, 1999; Stuart et al., 2020).

OBJECTIVE

Preparing and characterizing a PVA-capped Bi-Zn oxide nanocomposite and then testing its antibacterial properties on *Staphylococcus aureus* and *Escherichia coli* were the primary goals of this work. The antibacterial activity of a synthetic PVA-capped Bi-Zn oxide nanocomposite was examined at various nanocomposite concentrations in this work.

Materials and Methods

Chemicals

In this experiment all chemicals used were of analytical reagent quality.

- **Bismuth Nitrate Pentahydrate ($\text{Bi}_2\text{NO}_3 \cdot 5\text{H}_2\text{O}$):** Sigma-Aldrich, Germany
- **Zinc Sulfate Monohydrate ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$):** Sigma-Aldrich, Germany
- **Polyvinyl Alcohol (PVA):** Merck, Germany
- **Potassium Hydroxide (KOH):** Merck, Germany
- **Deionized Water (DI Water)**

SYNTHESIS OF PVA-CAPPED BISMUTH-ZINC OXIDE NANOCOMPOSITE

Methodology: These PVA-bismuth-zinc nanostructures were prepared using a basic co-precipitation technique.

Preparation of the Solutions

- 0.1 M solution of $\text{Bi}_2\text{NO}_3 \cdot 5\text{H}_2\text{O}$ was prepared in DI water.
- 0.1 M solution of $\text{ZnSO}_4 \cdot \text{H}_2\text{O}$ was prepared in DI water.
- 0.2 M solution of KOH was prepared in DI water.
- A 5% (w/v) PVA solution was prepared in DI water.

2. Synthesis Process:

3. A 100 mL beaker was used to mix the Bi_2NO_3 and ZnSO_4 solutions, which were then agitated for 15 minutes until fully combined. The precursor salt solution was continuously stirred while the KOH and PVA solutions were added dropwise. The solution was agitated at room temperature for 2 hours while covered with aluminium foil.

Deposits of bismuth-zinc oxides were created throughout the process, beginning in the liquid phase. Filtered and rinsed with distilled water, the precipitates were then collected. Lastly, the samples were oven-dried for 2 hours at 120 °C. To get pure crystalline PVA capped

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bismuth zinc oxide nanocomposite (PVA BiZn oxide NPs), the next step was to heat the product to 500 °C for four hours in a muffle furnace.

To get pure crystalline PVA-capped bismuth-zinc oxide nanocomposite (PVA-BiZn oxide NPs), the product was calcined at 500 °C for four hours in a muffle furnace.

Methods for Characterisation

Analysis via Fourier-Transform Infrared Spectroscopy (FTIR): The made PVA-Bi-Zn Oxide nanocomposite's functional groups were evaluated by FTIR. The FTIR spectra of both the pure PVA and the PVA-Bi-Zn Oxide nanocomposite were examined in order to validate the process of production and to determine the changes that took place in the nanostructures (Alswat et al., 2016; Mehmood et al., 2019; Ranjithkumar et al., 2023; Sudhamani, Prasad, & Sankar, 2003).

Figure 1 shows the Fourier transform infrared spectra of a nanocomposite of pure PVA and PVA-Bi₂O₃-Zn Oxide, which guarantees both the preparation and the structural modification. Previous research by Alswat et al. (2016) and Mehmood et al. (2019) has confirmed this.

Differential X-Ray Diffraction Analysis: Analysing the synthesised nanocomposite using XRD revealed its crystalline nature and structural shape. $D = K\lambda / \beta \cos\theta$ is the Debye-Scherrer equation that was used to determine the crystalline size. It's like a never-ending loop In equation 1, D is the size of the crystal, K is the Scherrer constant (0.9), λ is the wavelength of the X-rays, β is the breadth of the peak at half maximum, and θ is the Bragg angle. RD) The crystalline constitution and structural morphology of the synthesised nanocomposite were determined using XRD examination. The Debye-Scherrer equation, which states that the crystalline size is $D = K\lambda / \beta \cos\theta$, was used to determine this. It's quite a mouthful. In equation 1, D is the size of the

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crystal, K is the Scherrer constant (usually 0.9), λ is the wavelength of the X-rays, β is the extent of the peak's width at half maximum (FWHM), and θ is the Bragg angle.

(Abral et al., 2020; Mallahi, Shokuhfar, Vaezi, Esmaeilirad, & Mazinani, 2014; Mohan & Renjanadevi, 2016).

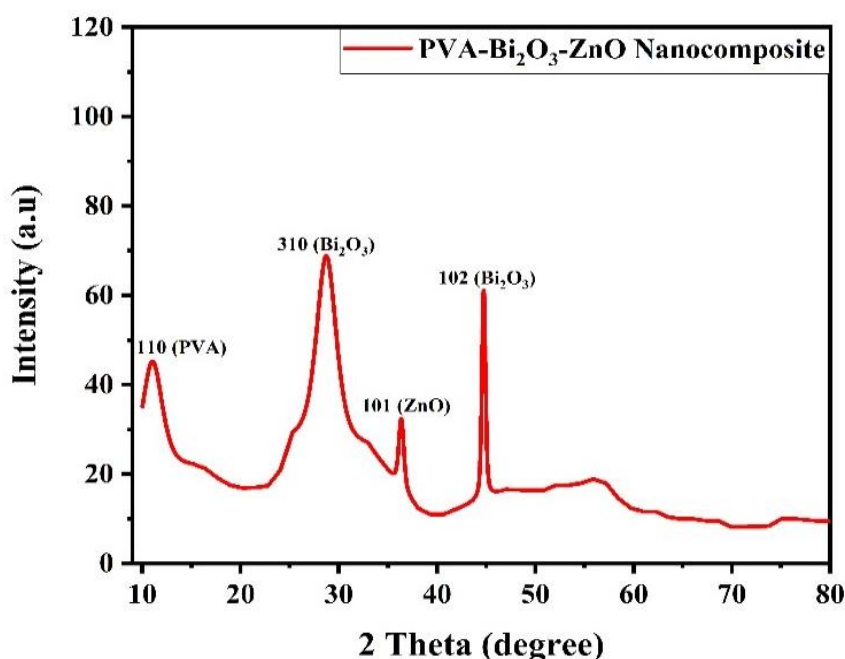


Figure 02. Identification of the nanocomposites crystallinity and the crystallite size through characterization by XRD and Debye-Scherrer formula applied to the diffraction patterns. Such sources include: (Abral et al., 2020; Mallahi et al., 2014)

ENERGY-DISPERSIVE X-RAY (EDX) ANALYSIS

Energy-dispersive X-ray (EDX) analysis is a common method of determining the actual elemental content of a material. As displayed in the figure, EDS spectra of the PVA-capped bismuth zinc oxide nanocomposite is presented. The results of the EDS analysis of PVA-Bi-Zn meant that the probe bismuth (Bi) is determined to be the most suitable measure in the percent of about 99%, testifying to the fact that Bi_2O_3 is dominant in the nanocomposite. The content of Zinc (Zn) is approximately about 9%, which is evidence that Zn has been integrated into the structure. Here it was observed that in the composition containing 10% O, both Bi_2O_3 and ZnO nanocomposite could be formed. Furthermore, as expected, the signifies the presence of carbon (C) at 5% proves that PVA is well capped in this synthesis. The measured outcomes presented here evidence that the PVA-capped bismuth zinc oxide nanocomposite was successfully synthesized and the synthesized composition was found to be quite closer to the theoretically expected value.

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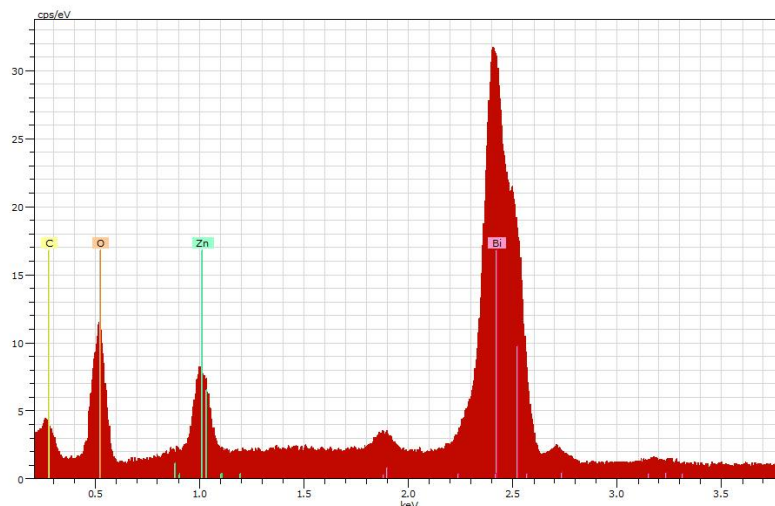
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Figure 03: EDX analysis of PVA-Bi₂O₃-ZnO nanocomposite showing Bi (99%), Zn (9%), O (10%), and C (5%), confirming successful synthesis.

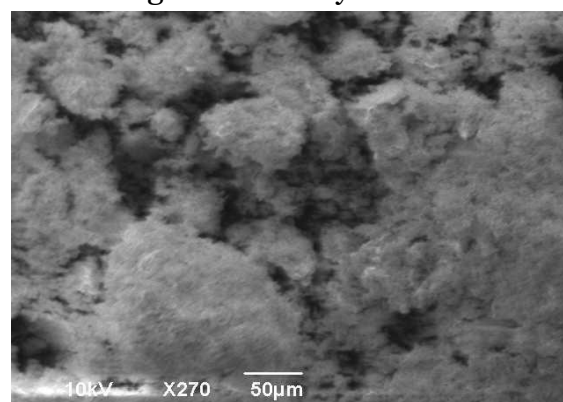
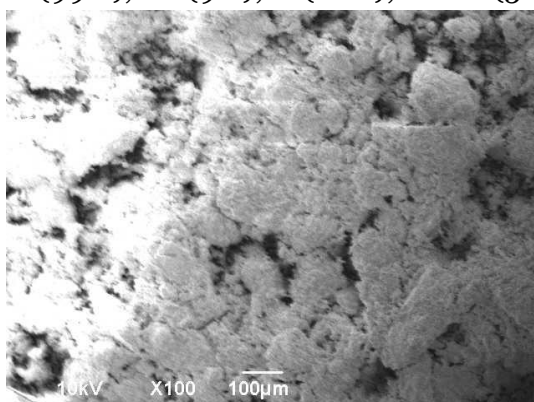


Figure 04: Scanning Electron Microscopy (SEM) Provided detailed insights into the surface morphology and structural characteristics of the nanomaterials.

ANTIMICROBIAL ACTIVITY ASSESSMENT

BACTERIAL CULTURE PREPARATION

These strains include the gram-negative *Escherichia coli* and the gram-positive *Staphylococcus aureus* bacteria. 5% and 10% PVA (Bi-Zn oxide) nanocomposites.

Testing Procedure for Kirby-Bauer Disc Diffusion Susceptibility

Methods for Making Bacterial Suspensions: Mueller-Hinton agar was used to cultivate bacteria overnight. To make bacterial suspensions equal to 0.5 McFarland standard ($\sim 1.5 \times 10^8$ CFU/mL), direct colony transfer was carried out. The organisms under examination were streaked over the surface of the plate using a sterile swab in a back-and-forth motion. The infected agar

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surface was covered with sterile discs that had been impregnated with nanocomposite samples. Four samples were added to each plate. The plates were kept in an incubator set at a temperature of $35 \pm 2^\circ\text{C}$ for a period of 18 to 24 hours. We assessed the zones of inhibition after 24 hours.

Discussion and Findings

Analysis using Fourier-Transform Infrared Spectroscopy (FTIR)

The PVA-Bi₂O₃-ZnO nanocomposite was confirmed to have formed and its functional groups identified using FTIR spectroscopy.

- **Pure PVA Spectrum:**

- The characteristic absorption peaks at
 - 3316 cm^{-1} (O—H stretching)
 - 2922 cm^{-1} (asymmetric stretching of CH₂)
 - 2906 cm^{-1} (symmetric stretching of CH₂)
 - 1430 cm^{-1} (CH₂ bending)
 - 1727 cm^{-1} (C=O stretching)
 - 1220 cm^{-1} (C—O stretching corresponding to the crystalline sequence of PVA)
 - 1094 cm^{-1} (C—O stretching and OH bending associated with the amorphous sequence of PVA) (Vanitha, Kanchana, Basavaraj, & Watage, 2023).

- **PVA-Bi₂O₃-ZnO Nanocomposite Spectrum:**

- New peaks appeared at:
- The symmetric and asymmetric stretching vibrations of O—Bi—O are 443 and 897 cm^{-1} , respectively (Zulkifli, Razak, & Rahman, 2018). According to Selim, Azb, Ragab, and HM Abd El-Azim (2020), the symmetric and asymmetric stretching of Zn—O and O—Zn—O bonds are represented by 586 and 981 cm^{-1} , respectively.

The C—O stretching of PVA is seen by the band at 1128 cm^{-1} .

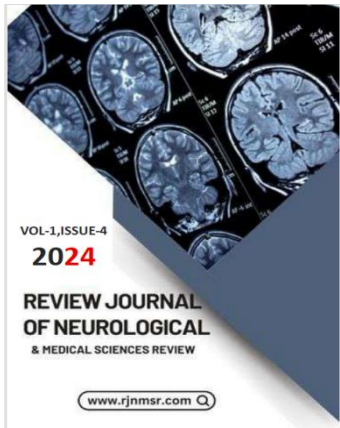
The effective creation of the nanocomposite is indicated by the observed shifts and lower intensities of the PVA peaks (Sudhamani et al., 2003).

X-RAY DIFFRACTION (XRD) ANALYSIS

XRD analysis was performed to determine the crystalline structure of the PVA-Bi₂O₃-ZnO nanocomposite.

- **Observations:**

- Distinct sharp peaks at angles of approximately 26.3° , 44.8° , and 56.4° correspond to the (101), (103), and (220) planes, respectively (Irmawati, Nasriah, Taufiq-Yap, & Hamid, 2004) and (Kumar, Venkateswarlu, Rao, & Rao, 2013).
- The broad peaks at 2θ angles of $\sim 11.08^\circ$ and 28.6° are attributed to the capping effect of PVA.
- The crystallite size, calculated using the Debye-Scherrer formula, was approximately 30 nm.



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- The diffraction peaks align well with the reference data (JCPDS file No. 01-071-0467), confirming successful synthesis.

ENERGY-DISPERSIVE X-RAY (EDX) ANALYSIS

The nanocomposites' elemental composition was determined by EDX analysis.

- **Results:**

- **Bismuth (Bi):** Detected as a major element, indicating the predominant presence of Bi₂O₃.
- **Zinc (Zn):** This confirms the incorporation of ZnO into the structure.
- **Oxygen (O):** Supporting the formation of both Bi₂O₃ and ZnO.
- **Carbon (C):** PVA capping material.
- The elemental composition closely matched the expected values, demonstrating the successful synthesis.

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

The SEM images provided detailed insights into the surface morphologies of the nanocomposites.

- **Observations:**

- A unique nano-flower-like morphology is observed.
- This arrangement significantly enhances the surface area, offering more active sites for interaction with bacterial cells.
- PVA capping stabilized the nanocomposite, prevented particle agglomeration, and maintained its uniformity.

ANTIMICROBIAL ACTIVITY ZONE OF INHIBITION MEASUREMENTS:

Sample	Concentration	Zone of Inhibition (mm)	Interpretation
		<i>E. coli</i>	<i>S. aureus</i>
PVA (Bi-Zn) Nanocomposite	5%	15	25
Susceptible (<i>S. aureus</i>)			
PVA (Bi-Zn) Nanocomposite	10%	22	35
Susceptible (<i>S. aureus</i>)			

Table 1. Antimicrobial Activity of PVA-BiZnO Nanocomposite Against *E. coli* and *S. aureus*

- **Against *E. coli*:**

- The 5% concentration resulted in an inhibition zone of 15 mm, indicating intermediate antibacterial activity.

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- A 10% concentration resulted in a larger inhibition zone of 22 mm, suggesting improved, but still intermediate, effectiveness.
- **Against *S. aureus*:**
 - A concentration of 5% produced an inhibition zone of 25 mm, indicating full susceptibility.
 - A concentration of 10% increased the inhibition zone to 35 mm, indicating a stronger antibacterial effect.

ANTIMICROBIAL EFFICACY

Increased Propensity for *Staphylococcus aureus*. The unique structural characteristics of the cell walls of *S. aureus* and *E. coli* are responsible for their different susceptibilities. According to Silhavy, Kahne, and Walker (2010), gram-positive bacteria, including *S. aureus*, are more susceptible to antimicrobial drugs because they have a thicker peptidoglycan layer but no outer membrane. According to Stuart et al. (2020), cells may get damaged when bismuth and zinc ions bind strongly to peptidoglycan layers.

Moderate Effectiveness Against *Escherichia coli*: Some bacteria, like *E. coli*, have an outer layer that prevents antimicrobial drugs from penetrating (Li, Plésiat, & Nikaido, 2015). This moderate activity suggests that, although PVA (Bi-Zn) holds promise, further optimization may be necessary to enhance its efficacy.

- **Dose-Dependent Effect:** The enhanced antibacterial activity at 10% concentration indicated a dose-dependent effect. Higher concentrations of Bi-Zn led to more significant microbial inhibition, which was consistent with our findings.

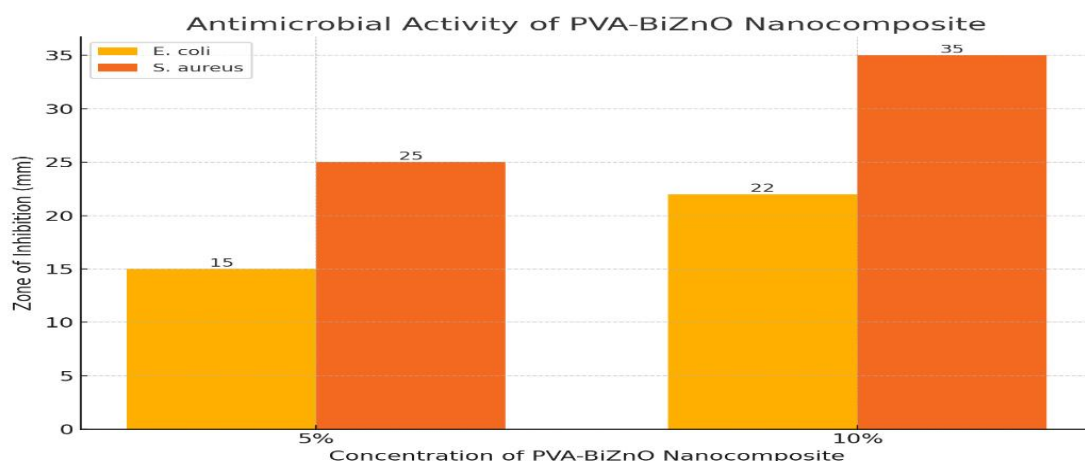


Figure:05 At 5% and 10% concentrations, the grouped bar chart above shows that the PVA-BiZnO nanocomposites are antibacterial against *E. coli* and *S. aureus*, respectively.

OVERALL FINDINGS

- PVA mixed with Bi-Zn demonstrated stronger antibacterial potential, especially against gram-positive bacteria, such as *S. aureus*.

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- These results indicated that PVA (Bi-Zn) may be a successful option for managing bacterial infections.
- The significance of the formulation and concentration on antimicrobial activity was highlighted.

CONCLUSIONS

These findings demonstrate that the addition of Bi-Zn oxide to PVA enhances its antimicrobial efficacy, particularly against *S. aureus*, as indicated by its full susceptibility at both 5% and 10% concentrations. However, *E. coli* exhibited intermediate susceptibility, highlighting the need for further optimization. The differences in bacterial responses highlight the importance of targeting specific microbial structures, especially when dealing with gram-negative bacteria, which possess more complex defenses, such as an outer membrane. The observed antimicrobial effect of PVA (Bi-Zn) oxide is in line with previous findings that establish the PVA impart synergistic action in the antimicrobial activity (Zhang et al., 2022). However, to ensure that it applies to as many effects and sectors as possible, and against as many different types of bacteria as possible, including the relatively resistant *E. coli*, more trials have to be conducted to determine the right concentration and preparation. antibacterial action (Zhang et al., 2022). Nevertheless, more research is needed to refine the concentration and formulation for broader efficacy, especially against resistant bacterial strains such as *E. coli*. In addition, analysing potential resistance mechanisms and other additives, the present antimicrobial potency of PVA-based formulations could be enhanced. In conclusion, although PVA (Bi-Zn) oxide presents a promising material – above all against Gram-positive bacteria like, for instance, *S. aureus* – its effectiveness against Gram-negative bacteria could be raised. Future studies should therefore aim at improving these formulations for better efficacy because of the known emerging antibiotic resistance.

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WORK CONTRIBUTED BY THE AUTHOR

The study's design, methodology, analysis, and paper writing were all done by the same group of writers.

FUNDING

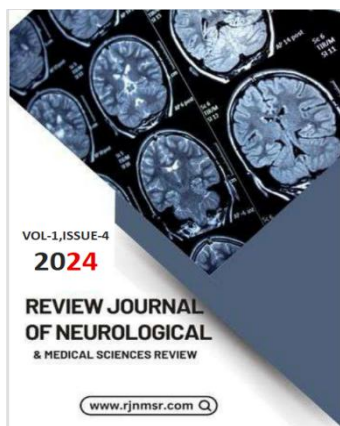
Therefore, no external source provided support for the study.

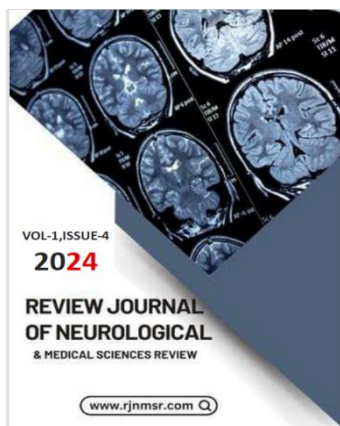
PUBLIC PROCLAMATIONS

There are no declared conflicts of interest by the author.

Nobody has a vested interest in the results of this study.

Moral Clearance: Moral factors to think about: None necessary since this study does not constitute the typical kind that would need ethical approval. "Not applicable" participant consent since no humans were engaged in the





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research.

Consent: for Publication As per Journal Standards and Norms.

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