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*Original articles*

# POTENCY ANTIBACTERIAL ACTIVITY OF ZIF-8 LOADED WITH PLANT EXTRACT AGAINST MULTIDRUG-RESISTANT BACTERIA: IN SILICO MOLECULAR DOCKING ANALYSIS OF TARGET PROTEINS

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## ABSTRACT

The alarming rise of multidrug-resistant (MDR) bacteria has necessitated the development of alternative antibacterial agents. Plant-derived bioactive compounds which, known for their broad-spectrum antimicrobial properties, face limitations in clinical application due to poor solubility and low bioavailability. Zeolitic Imidazolate Framework-8 (ZIF-8), a zinc-based metal organic framework (MOF), offers a promising nanocarrier system that enhances the delivery and efficacy of these natural compounds. This study aims to investigate the potential interaction between ZIF-8 and several key bacterial target proteins using in silico molecular docking approaches. Four bacterial proteins, including Pseudomonas aeruginosa elastase (LasB), β-ketoacyl-ACP synthase III (FabH), fosfomycin resistance protein (FosB), and penicillin-binding protein (PBP2a**),** were selected for molecular docking with their native ligand. ZIF-8 which was modeled using ChemDraw and optimized prior to docking. Binding affinities and interaction residues were analyzed using AutoDock and visualized and analyzed with Discovery Studio. This study reported that ZIF-8 demonstrated binding affinities ranging from -3.9 to -8.6 kcal/mol across the four target proteins, comparable to or slightly lower than native ligands. These interactions suggest the potential of ZIF-8 to inhibit enzyme activity through stable binding. The in silico findings support the antibacterial potential of ZIF-8 as both a nanocarrier and an active antimicrobial agent. When combined with plant extract, ZIF-8 may offer a synergistic strategy to overcome bacterial resistance. Further experimental validation is recommended to confirm its applicability in biomedical and pharmaceutical contexts.

**Keywords:** antibacterial, molecular docking, nanocarrier, plant-derived bioactives, ZIF-8.

## INTRODUCTION

The global rise of multidrug-resistant (MDR) bacteria has emerged as one of the most pressing threats to public health. Conventional antibiotics are progressively losing their effectiveness due to widespread misuse and bacterial adaptation, necessitating the development of alternative antimicrobial strategies [1–3]. Among these, plant extracts containing bioactive, including flavonoids, alkaloids, terpenoids, and phenolic acids, have shown promising antibacterial potential through various mechanisms such as disruption of bacterial cell walls, inhibition of enzymatic pathways, and interference with microbial communication systems [4–6]. However, many of these natural compounds suffer from poor water solubility, instability under physiological conditions, and limited bioavailability, restricting their therapeutic applications [7,8].

Recent advances in nanotechnology offer new avenues to improve the delivery and effectiveness of plant-based antimicrobials. One such approach involves the use of metal-organic frameworks (MOFs), particularly Zeolitic Imidazolate Framework-8 (ZIF-8), as nanocarriers. ZIF-8, composed of zinc ions and 2-methylimidazole linkers, features a porous structure, high thermal and chemical stability, and pH-

responsive degradation. Its ability to encapsulate and protect bioactive compounds enhances their solubility, stability, and controlled release [9,10]. In addition to serving as a carrier, ZIF-8 itself exhibits antibacterial activity due to the release of $Zn^{2+}$ ions, which can damage bacterial membranes and disrupt cellular functions [11].

The combination of ZIF-8 with plant extract has the potential to generate synergistic antibacterial effects, particularly against MDR strains. *Pterocarya fraxinifolia* leaf extract encapsulated within ZIF-8 has been reported significantly improved antibacterial function against *Staphylococcus aureus* and *Escherichia coli* bacteria [12]. However, understanding the molecular interactions involved is essential for optimizing such nanoformulations. In this context, in silico molecular docking offers a valuable tool for predicting the binding affinity and interaction profiles of ZIF-8 with bacterial target proteins. Key proteins such as LasB (a virulence factor in *Pseudomonas aeruginosa*), FabH (a fatty acid synthesis enzyme), FosB (an antibiotic resistance protein), and PBP2a (a penicillin-binding protein in *Staphylococcus aureus*) are critically involved in bacterial survival and resistance mechanisms. This study aims to evaluate the antibacterial potential of ZIF-8 by analyzing their molecular docking interactions with these target proteins. The results will contribute to a deeper understanding of the antibacterial mechanisms and support the development of nanotechnology-assisted phytotherapeutics as viable alternatives to conventional antibiotics in combating MDR bacterial infections.

## MATERIAL AND METODS

*Preparation of ZIF-8 Structure*

A fragment of ZIF-8 consisting of one central $Zn^{2+}$ ion coordinated to four 2-methylimidazole ligands was constructed using ChemDraw 3D and exported as a 3D structure file in .mol2 format. The structure was then energy minimized using open babel, and converted to .pdb format for molecular docking.

*Selection and Preparation of Target Proteins*

Four bacterial proteins and their native ligands were selected as molecular targets based on their functional relevance, including LasB (PDB ID: BCC4), virulence factor from *Pseudomonas aeruginosa;* FabH (PDB ID: 5BNS), fatty acid biosynthesis enzyme; FosB (PDB ID: 4JH5), antibiotic resistance enzyme; PBP2a (PDB ID: 1MWT), penicillin-binding protein from *Staphylococcus aureus.* Each protein structure was retrieved from the Protein Data Bank (PDB) and prepared using Discovery Studio.

*Molecular Docking*

Molecular docking was performed using AutoDock Vina to evaluate the binding affinity of ZIF-8 toward each target protein. The grid box for each docking run was centered on the binding site with dimensions large enough to accommodate the ZIF-8 fragment. The binding affinity (in kcal/mol) and the interaction residues were recorded and analyzed.

Table 1. center and dimension of grid box

| PDB ID | Gridbox | | | | | |
|---|---|---|---|---|---|---|
| | Center | | | Dimension | | |
| | X | Y | Z | X | Y | Z |
| BCC4 | 13.3383 | 50.3074 | 30.9155 | 13.7505 | 16.6965 | 16.2288 |
| 4JH5 | 58.4079 | 59.9302 | 11.3956 | 13.3264 | 10.6845 | 11.5839 |
| 5BNS | 5.9961 | 43.6389 | 37.7887 | 16.0543 | 13.3165 | 24.9935 |
| 1MWT | 27.5528 | 28.8599 | 86.6241 | 17.2341 | 15.7143 | 17.2512 |

*Visualization and Analysis*

Docking poses were visualized using Discovery Studio Visualizer to identify key interactions such as hydrogen bonds, hydrophobic contacts, and coordination with $Zn^{2+}$. Comparative analysis across all proteins was conducted to evaluate binding trends and potential mechanisms of action.

## RESULTS AND DISCUSSION

*Binding Interaction of ZIF-8 and Native Ligand with Target Protein*

Molecular docking analysis was performed to evaluate the interaction between the ZIF-8 fragment and the active site of the target bacterial protein. The comparison between ZIF-8 and the native ligand revealed distinct differences in binding affinity and interaction patterns. The docking scores indicate that ZIF-8 exhibited comparable or higher binding affinities than native ligands in most cases. Specifically, ZIF-8 demonstrated binding energies of -7.5 kcal/mol with LasB, -8.0 kcal/mol with FabH, -8.6 kcal/mol with FosB, and -3.9 kcal/mol with PBP2a (Figure 1-4). These values are either stronger or slightly lower than those of the respective native ligands, suggesting a strong potential of ZIF-8 to interact directly with the active sites of these proteins.

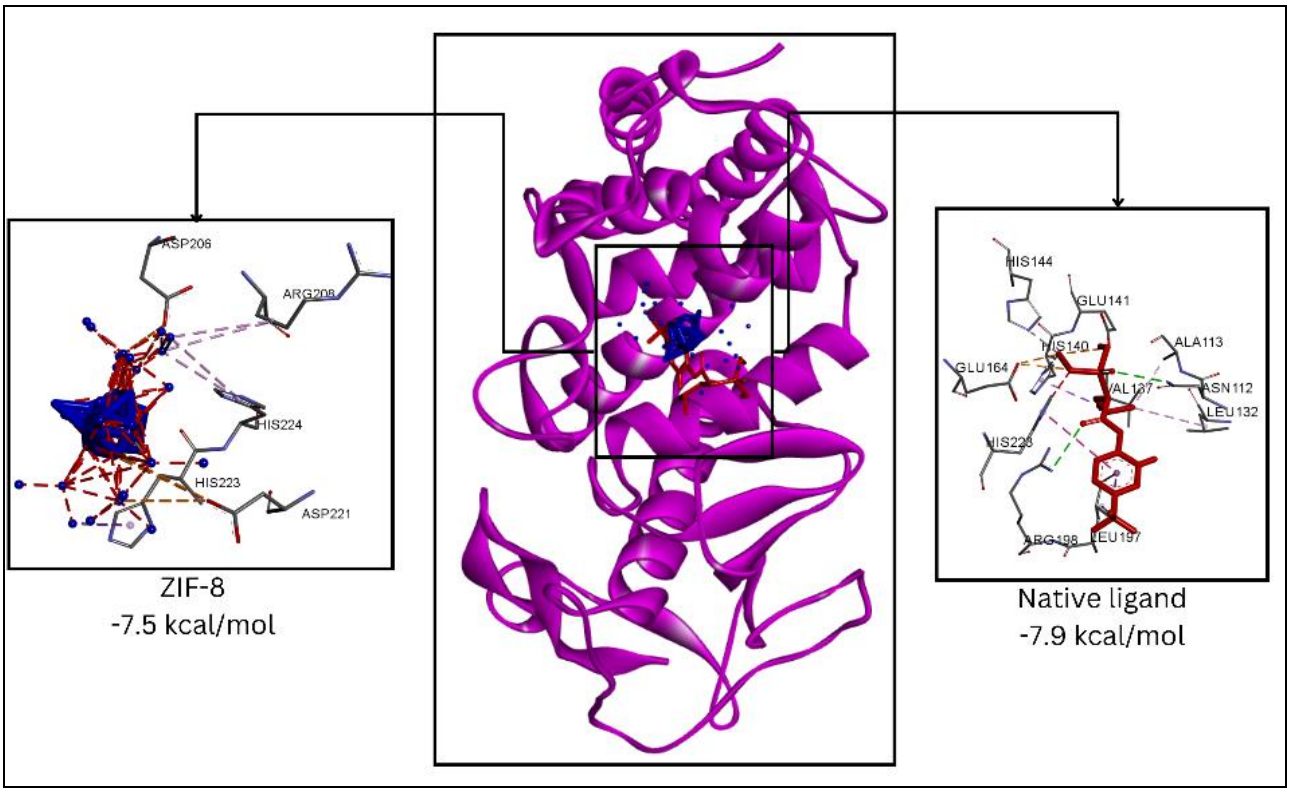


Figure 1. ZIF-8 and Native Ligand Docking with LasB. ZIF-8 showed a binding affinity of −7.5 kcal/mol, marginally better than the native ligand (−7.9 kcal/mol). ZIF-8 formed interactions with key residues like ASP206, ARG208, HIS224, HIS223, ASP221.

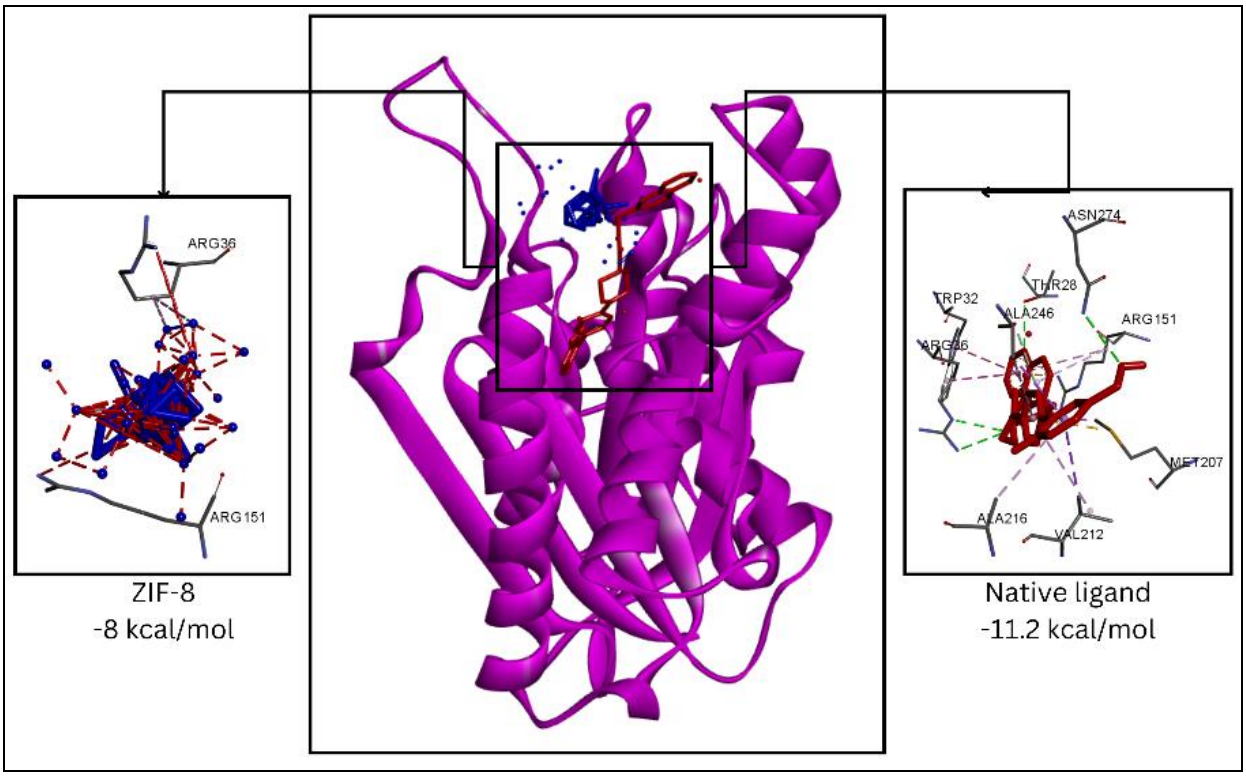


Figure 2. ZIF-8 and Native Ligand Docking with FabH. ZIF-8 showed a binding affinity of −8.0 kcal/mol, marginally better than the native ligand (−11.2 kcal/mol). ZIF-8 formed interactions with key residues like ARG36, ARG151.

*ZIF-8 Interaction Profile*

ZIF-8's interaction profile revealed its ability to engage in multiple hydrogen bonds, electrostatic interactions, and hydrophobic contacts within the active or catalytic sites of the target proteins. For LasB, ZIF-8 formed interactions with ASP206, ARG208, HIS224, HIS223, and ASP221 (Figure 1). In the case of FabH, interaction with ARG36 and ARG15 (Figure 2). For FosB, critical residues such as LEU121, ARG124, and GLU115 (Figure 3). With PBP2a, ZIF-8 interacted with TYR446, GLU602, and GLN613 (Figure 4). The presence of a central $Zn^{2+}$ ion in ZIF-8 likely contributes to stable coordination with charged amino acids, enhancing its binding strength. The ability of ZIF-8 to bind with charged amino acids has implications for various applications, including drug delivery, where ZIF-8 can be used to encapsulate and protect therapeutic proteins or peptides [13–15].

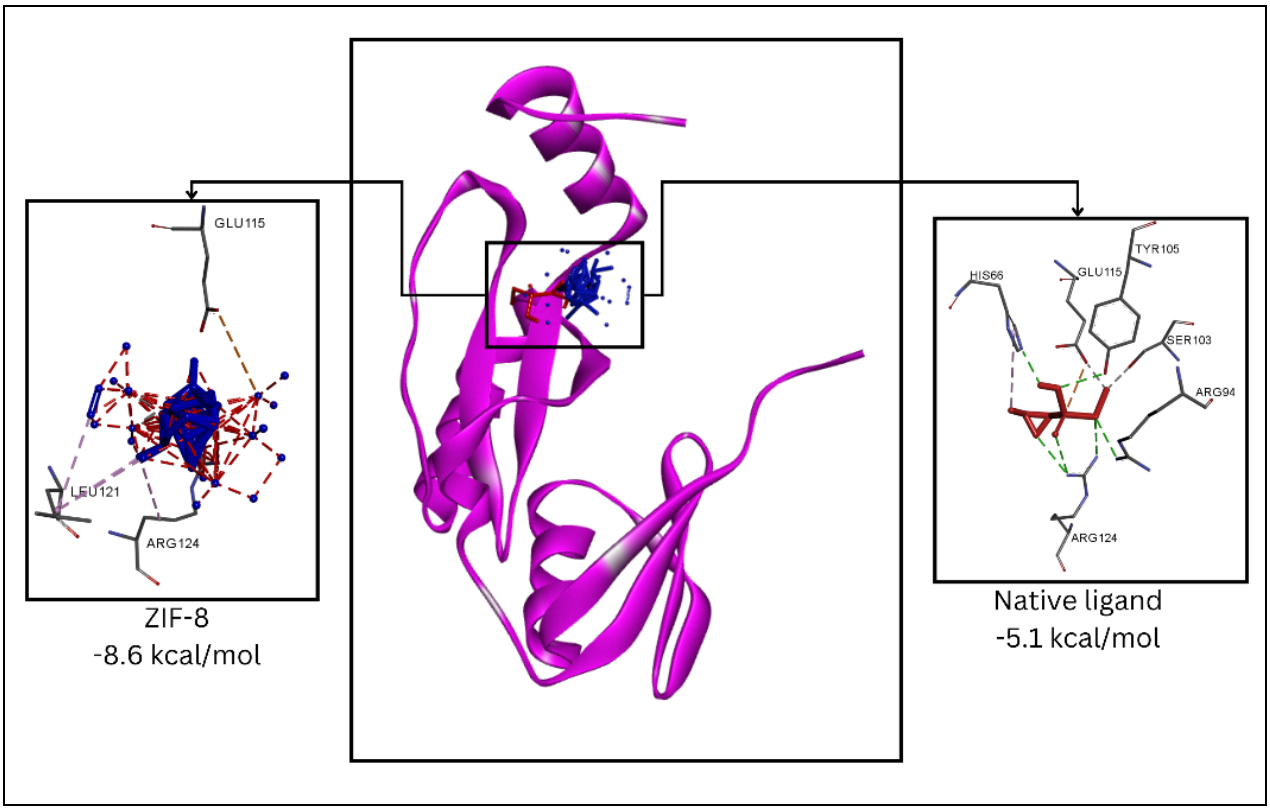


Figure 3. ZIF-8 and Native Ligand Docking with FosB. ZIF-8 showed a binding affinity of −8.6 kcal/mol, marginally better than the native ligand (−5.1 kcal/mol). ZIF-8 formed interactions with key residues like LEU121, ARG124, GLU115.

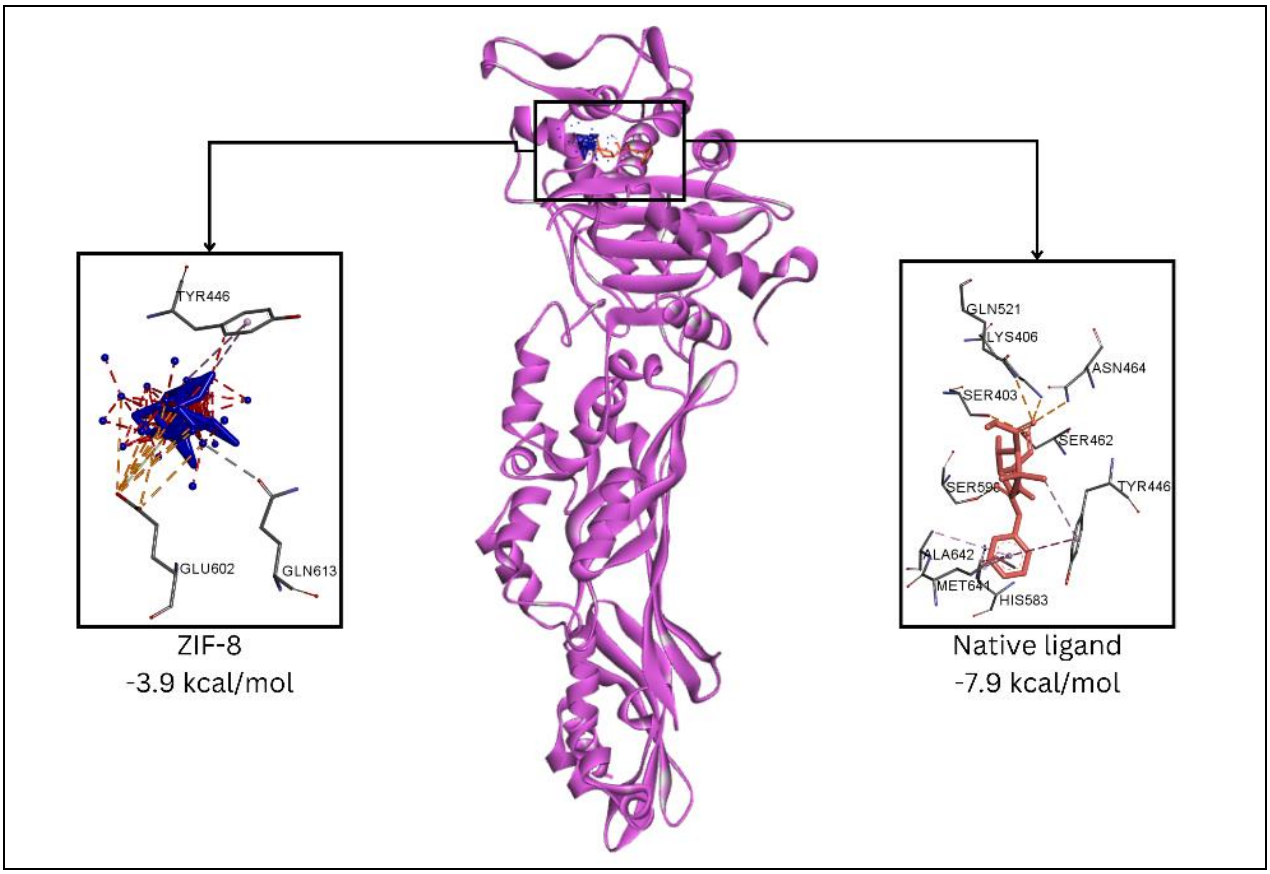


Figure 4. ZIF-8 and Native Ligand Docking with PBP2a. ZIF-8 showed a binding affinity of −3.9 kcal/mol, marginally better than the native ligand (−7.9 kcal/mol). ZIF-8 formed interactions with key residues like TYR446, GLU602, GLN613.

Although the docking results indicate a lower affinity of ZIF-8 compared to the native ligand, the ability of ZIF-8 to interact with the active site suggests its potential as a non-competitive modulator or as part of a synergistic antimicrobial system. ZIF-8 may exert antibacterial effects not only through direct inhibition of enzymatic activity but also via zinc ion release, generation of reactive oxygen species (ROS), and membrane disruption mechanisms that are not captured in static docking simulations [11,16]. Its ability to stably bind and potentially interfere with essential enzymatic functions of LasB, FabH, FosB, and PBP2a supports its potential as a dual-action antimicrobial agent. Additionally, the porous structure of ZIF-8 may facilitate the loading and targeted delivery of antibiotics, enabling a dual function as both antimicrobial agent and drug carrier.

While the docking studies provide valuable insights, they are inherently static and do not capture the full dynamic behavior of protein-ligand interactions. Further studies using molecular dynamics simulations are essential to validate the stability and conformational adaptability of ZIF-8 complexes under physiological conditions. Moreover, experimental validation, such as in vitro enzyme inhibition assays and bacterial growth studies, will be necessary to confirm the computational predictions. Future work should also explore ZIF-8 functionalization strategies to enhance selectivity and reduce potential cytotoxicity in biomedical applications.

## CONCLUSION

The in silico findings support the antibacterial potential of ZIF-8 as both a nanocarrier and an active antimicrobial agent. When combined with plant-derived bioactives, ZIF-8 may offer a synergistic strategy to overcome bacterial resistance. Further experimental validation is recommended to confirm its applicability in biomedical and pharmaceutical contexts.

## CONFLICT OF INTERESTS

The authors declare no conflict of interests related to the publication of this paper.

## REFERENCES

1. Siahaan S, Herman MJ, Fitri N. Antimicrobial Resistance Situation in Indonesia: A Challenge of Multisector and Global Coordination. J Trop Med. 2022 Feb 7;2022:2783300.
2. Catalano A, Iacopetta D, Ceramella J, Scumaci D, Giuzio F, Saturnino C, et al. Multidrug Resistance (MDR): A Widespread Phenomenon in Pharmacological Therapies. Molecules. 2022 Jan 18;27(3):616.
3. Jamiri F, Nayeri Fasaei B, Joghataei SM, Yahyaraeyat R, Mazloom-Jalali A. Synergistic antibacterial activity of chitosan-polyethylene glycol nanocomposites films containing ZIF-8 and doxycycline. BMC Biotechnol. 2025 Feb 20;25(1):19.
4. Hashempur MH, Ghorat F, Karami F, Jahanbin A, Nouraei H, Abbasi M, et al. Topical Delivery Systems for Plant-Derived Antimicrobial Agents: A Review of Current Advances. Int J Biomater. 2025;2025(1):4251091.
5. Sari R, Pratiwi SUT, Murti YB, Damayanti E. Potential of Indonesian Plants as Polymicrobial Anti-Biofilm. Borneo J Pharm. 2024 Feb 29;7(1):63–79.
6. Rukmana RM, Sawal AAD, Wibawa DAA. Combination of Antibacterial Activity of Ethanol Extract of Meniran Leaves and Kenikir Leaves Against Shigella dysenteriae. In Atlantis Press; 2023 [cited 2024 Sep 11]. p. 7–20. Available from: https://www.atlantis-press.com/proceedings/ichr-22/125984768
7. Laffleur F, Millotti G, Lagast J. An overview of oral bioavailability enhancement through self-emulsifying drug delivery systems. Expert Opin Drug Deliv. 2025 May 4;22(5):659–71.
8. Tambosi G, Coelho PF, Luciano S, Lenschow ICS, Zétola M, Stulzer HK, et al. Challenges to improve the biopharmaceutical properties of poorly water-soluble drugs and the application of the solid dispersion technology. Matér Rio Jan. 2018 Dec 6;23:e12224.
9. Bintang Ilhami F, Edi Cahyaningrum S, Pramudya Wardana A, Susanto Gultom N, Subekti H, Rahmawati A, et al. Meniran ( Phyllanthus niruri L.) embedded zeolitic imidazolate framework (ZIF-8) nanoparticle for cancer chemotherapy: supported molecular docking analysis. RSC Adv. 2025;15(1):223–30.
10. Balliana E, Marchand M, Di Matteo V, Ballarin B, Cassani MC, Panzavolta S, et al. Application of Zinc-Based Metal-Organic Framework ZIF-8 on Paper: A Pilot Study on Visual Appearance and Effectiveness. Polymers. 2025 Jan;17(10):1369.
11. Wei Y, Wang J, Wu S, Zhou R, Zhang K, Zhang Z, et al. Nanomaterial-Based Zinc Ion Interference Therapy to Combat Bacterial Infections. Front Immunol. 2022 Jun 30;13:899992.
12. Ghazanfari P, Nori M, Arab-Nozari M, Baghbanian SM, Vahdat SM. Antibacterial and Antifungal Activities of Pterocarya fraxinifolia Extract Encapsulated in ZIF-8. Appl Organomet Chem. 2025;39(8):e70315.

13. Spitsyna AS, Poryvaev AS, Sannikova NE, Yazikova AA, Kirilyuk IA, Dobrynin SA, et al. Stability of ZIF-8 Nanoparticles in Most Common Cell Culture Media. Molecules. 2022 May 18;27(10):3240.
14. Sun S, Zhang Z, Xiang Y, Cao M, Yu D. Amino Acid-Mediated Synthesis of the ZIF-8 Nanozyme That Reproduces Both the Zinc-Coordinated Active Center and Hydrophobic Pocket of Natural Carbonic Anhydrase. Langmuir. 2022 Feb 1;38(4):1621–30.
15. Houten JV, Castillo Barberi R, King J, F. Ogata A. Improving the colloidal stability of protein@ZIF-8 nanoparticles in biologically relevant buffers. Mater Adv. 2024;5(14):5945–57.
16. Zhang X, Wang Y, Xing W, Li L, Wu H, Wang H, et al. In situ growth of ZIF-8 nanoparticles on pure chitosan nanofibrous membranes for efficient antimicrobial wound dressings. Int J Biol Macromol. 2025 Mar 1;297:139921.