Antimicrobial activity of newly synthesized Dehydrozingerone compound

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Running title: Antimicrobial activity of *Dehydrozingerone.*

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ABSTRACT:

Background: The continuous rise of antimicrobial resistance underscores the urgent need to discover and develop novel antimicrobial agents. **Objective;** This investigation aims to characterize the antimicrobial profile of *Dehydrozingerone* by systematically assessing its efficacy against selected bacterial and fungal pathogens. **Materials & Methods;** The antimicrobial potency of *Dehydrozingerone* was evaluated by determining both the Minimum Inhibitory Concentration via the broth serial dilution technique and the Zone of Inhibition using the agar well diffusion assay. Testing was conducted against bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) as well as fungal cultures (*Aspergillus niger* and *Candida albicans*). Organisms were sub cultured and incubated in Mueller-Hinton Broth under optimized conditions. Following incubation, culture dynamics were assessed to establish the lowest concentration required to suppress visible microbial growth, where a lower MIC value denotes superior therapeutic efficacy. Additionally, the ZOI was quantified by measuring the clear diameter of growth inhibition surrounding the diffusion wells. **Results;** *Dehydrozingerone* exhibited substantial inhibitory activity against all tested bacterial and fungal strains. The quantitative data gathered from both the MIC and ZOI assays highlight its prospective utility as a plant-derived antimicrobial candidate for future therapeutic interventions. **Conclusion;** The findings of this study demonstrate that *Dehydrozingerone* possesses potent antimicrobial attributes, establishing it as a promising lead compound for the design and development of next-generation therapeutic agents.

Keywords: *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Aspergillus niger*, Minimum Inhibitory Concentration.

List of Abbreviations

DZ Dehydrozingerone

DZG Dehydrozingerone 4-O-β-D-glucopyranoside

DMSO	Dimethyl Sulfoxide
MIC	Minimum Inhibitory Concentration
ZOI	Zone of Inhibition
MOA	Mechanism of Action
PDA	Potato Dextrose Agar
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
HBA	Hydrogen Bond Acceptors
HBD	Hydrogen Bond Donors
PPI	Protein–Protein Interaction
KEGG	Kyoto Encyclopedia of Genes and Genomes

Introduction

Antimicrobials are substances used to prevent or treat infections in humans and animals. They include a wide range of agents such as antiseptics, antibiotics, antivirals, antifungals, and antiparasitic drugs. These substances work by either killing microorganisms or stopping their growth [1]. They do this by targeting essential processes in microbial cells, such as the production of vital molecules, enzyme activity, or structures like the cell wall and cell membrane. Disinfectants are a type of antimicrobial, but they are specifically used on non-living surfaces to eliminate harmful microorganisms. Each year, thousands of tonnes of antimicrobials and their by-products enter the environment, especially aquatic ecosystems. This raises concerns about their impact on ecosystems and the development of antimicrobial resistance [2].

Interestingly, antimicrobials are not just human-made-they have existed in nature for millions of years. Microorganisms naturally produce them as part of their interactions with other organisms. Studying these natural interactions, whether among bacteria (prokaryotes) or more complex organisms (eukaryotes), plays a key role in discovering new antimicrobial compounds. Understanding the ecological roles, production conditions, and functions of antimicrobials in natural environments is essential. This knowledge helps scientists develop new treatments and ensures that antimicrobials are used safely and effectively.[3]

The application of antibiotics spans far beyond human healthcare, finding extensive utility in agronomy, animal husbandry, and aquaculture to manage bacterial outbreaks. These agents exert their therapeutic effects through precise biochemical pathways designed to either induce bacterial cell death or inhibit cellular proliferation, the dynamics of which are detailed in this review. However, over time, bacteria have learned how to defend themselves. This has led to antibiotic resistance, where medicines that once worked no longer have an effect. Bacteria can resist antibiotics by using different strategies, and this review explains those mechanisms. Another major concern is that resistance can spread from one bacterium to another, turning previously treatable infections into difficult ones. This problem is made worse by factors like the misuse and overuse of antibiotics. To tackle this growing issue, the review also discusses possible alternative approaches and solutions to slow down and control antibiotic resist. [4]

Many studies have looked at natural products like medicinal plants, plant chemicals (phenolics, alkaloids), essential oils, and propolis for their antifungal properties. These natural options are considered safer and more eco-friendly, so they are already being explored for use in food preservation, medicine, and agriculture. However, only a few studies have examined their use as disinfectants for indoor air and surfaces [5]. This review focuses on how these natural substances could be used in such environments. It explains that these natural antifungals work by damaging fungal cells in different ways-they can disrupt energy production (ATP), disturb ion balance (Ca^{2+} and K^{+}), damage cell walls and membranes, produce harmful reactive oxygen species, and block important fungal proteins like efflux pumps.

Overall, these natural products show strong treatments. Potential as safer indoor antifungal disinfectants, especially as fungi are becoming more resistant to traditional [6].

Aim & Objectives

To contrast the comparative antimicrobial potential of *Dehydrozingerone* relative to established reference standards, specifically Ciprofloxacin (antibacterial control) and Fluconazole (antifungal control).

MATERIAL AND METHODS

Materials and Equipment:

Sterility was maintained using a laminar air flow chamber, autoclave, incubator, and refrigerator. Routine laboratory work was carried out using micropipettes, inoculating loops, sterile test tubes, and Petri dishes. DMSO and sterile water were used as solvents, while ciprofloxacin and fluconazole served as standard reference drugs. For microbial growth, Mueller-Hinton agar and broth were used for bacteria, and potato dextrose agar for fungi.

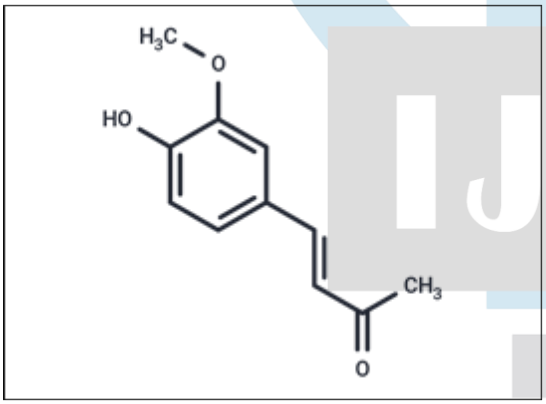
Pathogens and Approaches:

The samples were tested against *E. coli, S. aureus, C. albicans,* and *A. niger*. Antimicrobial activity was assessed using zone of inhibition (ZOI) and minimum inhibitory concentration (MIC) assays.

Network Pharmacology Software:

For bioinformatics analysis, tools such as Moli Soft, Swiss Target Prediction, and Gene Cards were used for target identification, Swiss ADME for pharmacokinetic profiling, and Venny 2.1.0 for visualizing data overlap.

Introduction to Dehydrozingerone:



Dehydrozingerone, which you might also see called feruloyl methane or vanillylidenacetone, is a bioactive phenolic compound found in ginger rhizomes (*Zingiber officinale*). Structurally, it is essentially a half-analogue of curcumin. In the lab, it appears as a yellow crystalline solid with a melting point of 128°C, a molecular weight of 192.21 g/mol, and the molecular formula : $C_{11}H_{12}0_3$ .Officially, its IUPAC name is (E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one, represented by the SMILES string CC(=O)/C=C/C1=CC(=C(C=C1)O)OC. When it comes to handling it, the compound dissolves easily in organic solvents like DMSO and ethanol.

Principle: Dehydrozingerone fights microbes by damaging their cell membranes, disrupting vital processes, and stopping biofilm formation. This prevents bacteria and fungi from growing and leads to their death. Its antimicrobial activity is mainly due to its phenolic structure.

Synthesis: To synthesize Dehydrozingerone, we started by dissolving 16 mM of vanillin into 50 mL of acetone (800 mM) under continuous stirring. Next, we slowly added 70 mL of a 10% w/v aqueous KOH solution dropwise and left the mixture to stir overnight at room temperature. The next day, we acidified the solution with 10% HCl to crash out a yellow powder precipitate. We collected this raw product via vacuum filtration using Whatman No. 1 filter paper, and then recrystallized it from hot aqueous ethanol to get our pure, light-yellow crystals [7].

MOA: Dehydrozingerone fights microbes in a few key ways. It first damages their cell membrane, making it leaky so essential nutrients and ions escape. It then interferes with important internal processes, including enzymes, DNA replication, and protein production, which stops the microbes from growing and multiplying. It also prevents them from forming protective biofilms, making them more vulnerable and easier to eliminate.

Methodology:

Network pharmacology:

Drug-likeness analysis of Dehydrozingerone showed a positive score, suggesting that it could be a promising bioactive drug candidate. It also showed good pharmacokinetic properties, including oral bioavailability and a suitable half-life. Target prediction studies suggest that Dehydrozingerone may interact with several proteins involved in disease-related pathways. These potential targets were identified using databases such as ttd, gene cards, and kegg, helping to better understand how Dehydrozingerone might work in the body and its possible therapeutic benefits.

Molecular docking:

Molecular docking studies of Dehydrozingerone were carried out to understand how well it binds to selected microbial target proteins and how it interacts with them. The 3d structure of Dehydrozingerone was taken from the PubChem database, while the protein structures were obtained from the protein data bank (pdb). Computational docking tools were then used to simulate and predict the strength of binding and the types of molecular interactions between Dehydrozingerone and the target proteins.

MIC determination:

Procedure:

Start by autoclaving 12 cotton-plugged test tubes, pipette tips, and the nutrient broth at 121°C for 20 minutes. After letting everything cool, dispense 1 mL of the sterile broth into each tube. Label tubes 1 through 10 with their final concentrations—ranging from 250 down to 0.48 µg/mL (125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95, and 0.97 µg/mL)—and set up the final two as your Positive (+ve) and Negative (-ve) controls. To build the gradient, add 1 mL of your test sample to the 250 µg/mL tube, mix it thoroughly, and perform a 2-fold serial dilution by transferring 1 mL consecutively down to tube 10, ensuring a good mix between each step. Finally, add 10 µL of the microbial culture to every tube except for the Negative control, which stays inoculated to check for contamination [8].

ZOI determination:

Procedure: -

Start by pouring 30 mL of sterile Mueller Hinton Agar and Potato Dextrose Agar into your plates near a flame. Once they solidify, evenly swab your bacterial and fungal strains across the surface to get a nice, uniform microbial lawn. Next, take a sterile cork borer and punch 5 mm wells into the agar [9]. To prepare your treatment, make a stock solution by dissolving 10 mg of the drug in 10 mL of DMSO, then pipette 10 µL, 20 µL, and 30 µL doses into the designated wells. Leave the plates to incubate at room temperature for 18–24 hours so the sample can diffuse completely, and then just measure the zones of inhibition in millimetres to compare against your standards [10].

Fig.1. MIC of DZ on *E. coli* bacteria.

Fig.2. MIC of DZ on *S. aureus* bacteria

Fig.3. MIC of DZ on *Candida albicans*

Fig.4. MIC of DZ on *Aspergillus niger*

Fig.5. ZOI of DZ and Ciprofloxacin

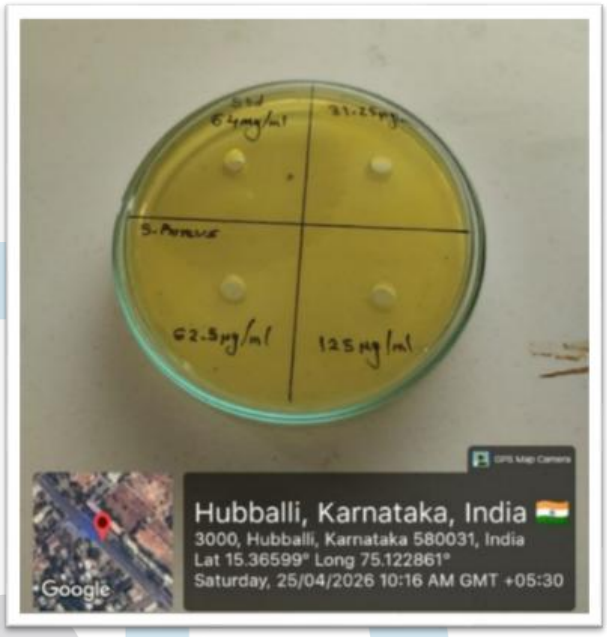


Fig.6. ZOI of DZ and Ciprofloxacin

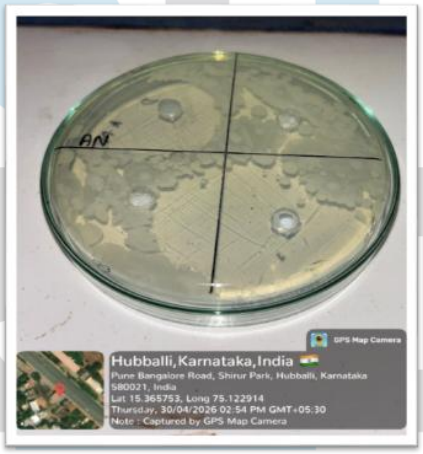


Fig.7. ZOI of DZ and Fluconazole against *A. niger*

RESULT

Table 1. Physicochemical properties of Dehydrozingerone:

Sl.No	Compound	Molecular formula	Molecular weight	HBA	HBD	Log P	DLS
01	Dehydrozingerone	C11H12O3	192.21	03	01	2.11	1

Venny analysis:

Anti-bacterial activity:

Venny analysis of compound targets and antibacterial proteins showed 1,431 total proteins, with 42 (2.8%) overlapping between the two groups. These common proteins may play an important role in antibacterial activity and could be potential therapeutic targets.

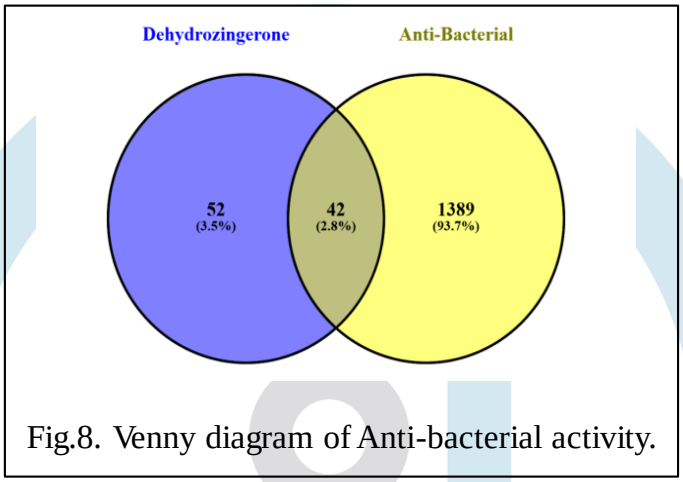


Fig.8. Venny diagram of Anti-bacterial activity.

Anti-fungal activity:

Venny analysis showed a total of 1,502 proteins when compound targets were compared with antifungal-related proteins. Out of these, 42 proteins (2.7%) were common to both groups. These overlapping proteins may be important for antifungal activity and could be useful targets for future treatment.

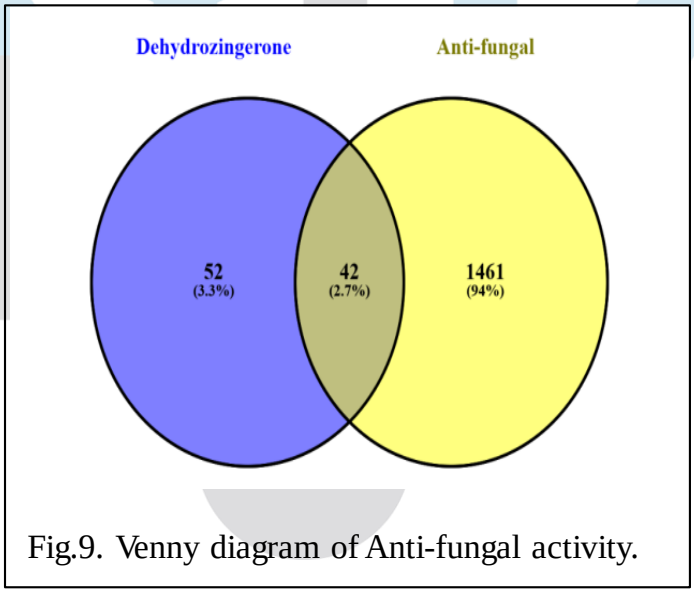


Fig.9. Venny diagram of Anti-fungal activity.

Network analysis:

Anti-bacterial activity: The network shows how Dehydrozingerone may work against bacteria by interacting with different proteins and biological pathways. In the diagram, the compound, its target proteins, and related pathways are shown as different coloured nodes, with connections showing how they influence each other. Important pathways involved include TNF, JAK-STAT, NF-κB, and IL-17, along with disease-related pathways such as shigellosis, toxoplasmosis, and leishmaniasis. Key proteins like MMP13, MMP1, MMP9, EGFR, STAT3, NOS2, PTGS2, BCL2, and RELA were also identified. Overall, this suggests that Dehydrozingerone may act on multiple targets at once, contributing to its potential antibacterial activity.

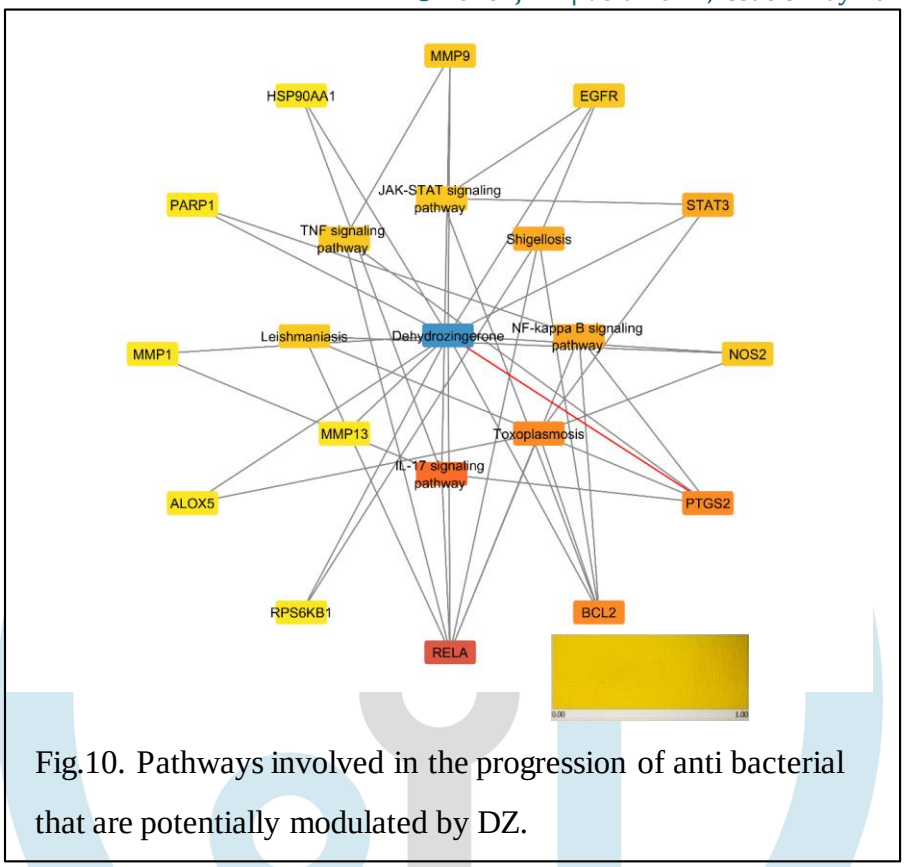


Fig.10. Pathways involved in the progression of anti bacterial that are potentially modulated by DZ.

Anti-fungal activity:

The network explains how Dehydrozingerone may work against fungal infections by interacting with different proteins and biological pathways. In the diagram, the compound, its target proteins, and the pathways are shown as coloured nodes, with connections indicating how they are linked. Important pathways involved include NF-kB, JAK-STAT, PI3K-Akt, IL-17, and apoptosis, which all play key roles in immune response and cell regulation. The study also highlights proteins such as CTSV, PIK3CG, STAT3, PIM1, RPS6KB1, EP300, MMP9, EGFR, PTGS2, GSK3B, HSP90AA1, PARP1, and BCL2. Overall, this suggests that Dehydrozingerone may work through multiple targets and pathways, supporting its potential as an effective antifungal agent. .

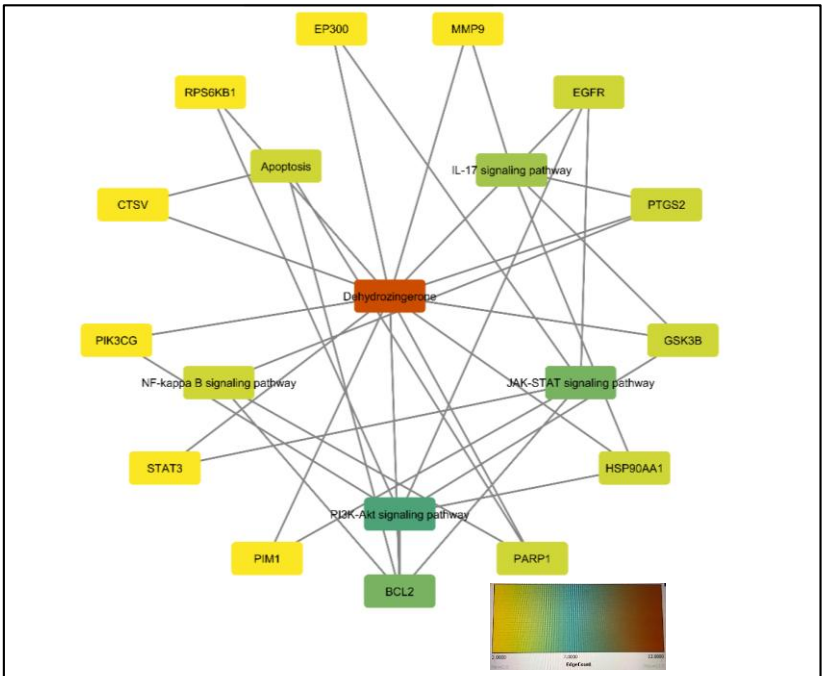


Fig.11. Pathways involved in the progression of anti fungal that are potentially modulated by DZ.

Molecular Docking

Table No.02: Result for target: BCL2 E. coli.

Pose	Affinity (kcal/mol)	Estimated ki	Ki unites	Ligand efficiency
8	-5.4	0.11	mM	-0.39
9	-5.4	0.11	mM	-0.39
1	-5.0	0.22	mM	-0.36
4	-5.0	0.22	mM	-0.36
7	-5.0	0.22	mM	-0.36
3	-4.9	0.26	mM	-0.35
2	-4.7	0.36	mM	-0.34
5	-4.7	0.36	mM	-0.34
6	-4.6	0.42	mM	-0.33

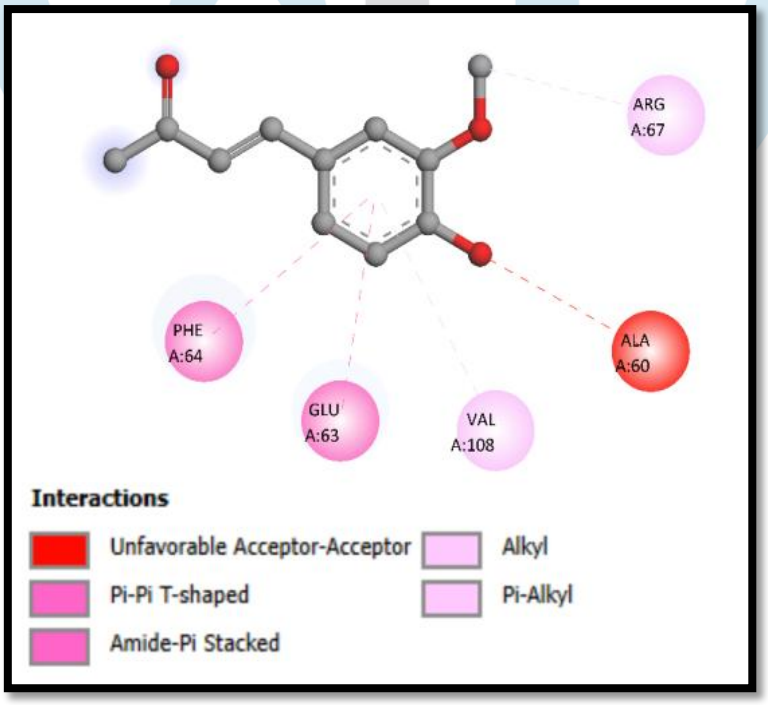


Fig.12. 2D illustration of the binding of DHZ with Bcl2 protein.

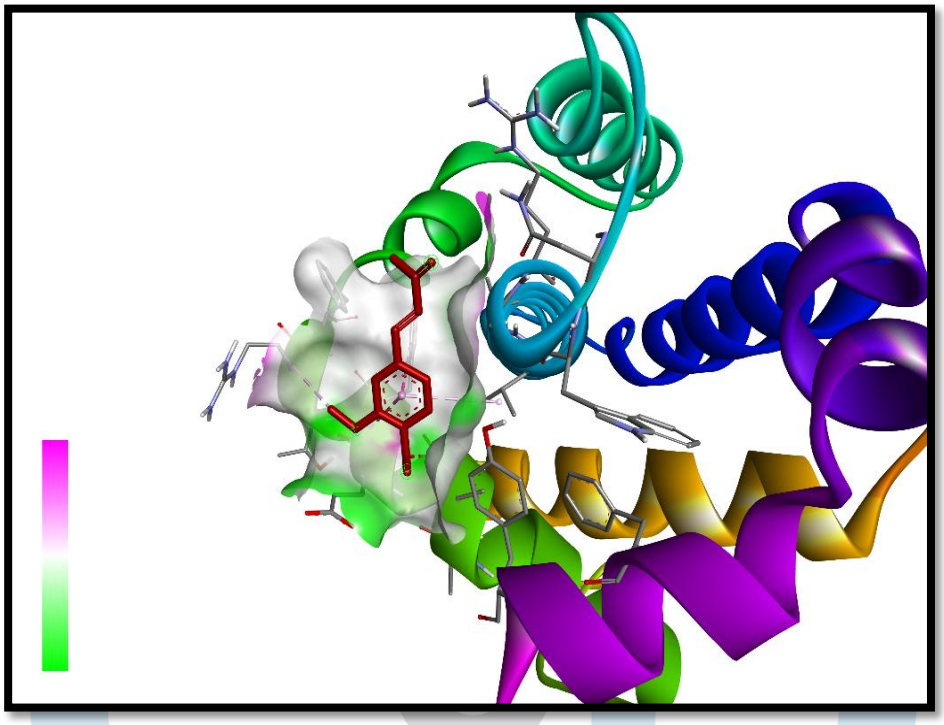


Fig.13. 3D illustration of the binding of DHZ with Bcl2 protein

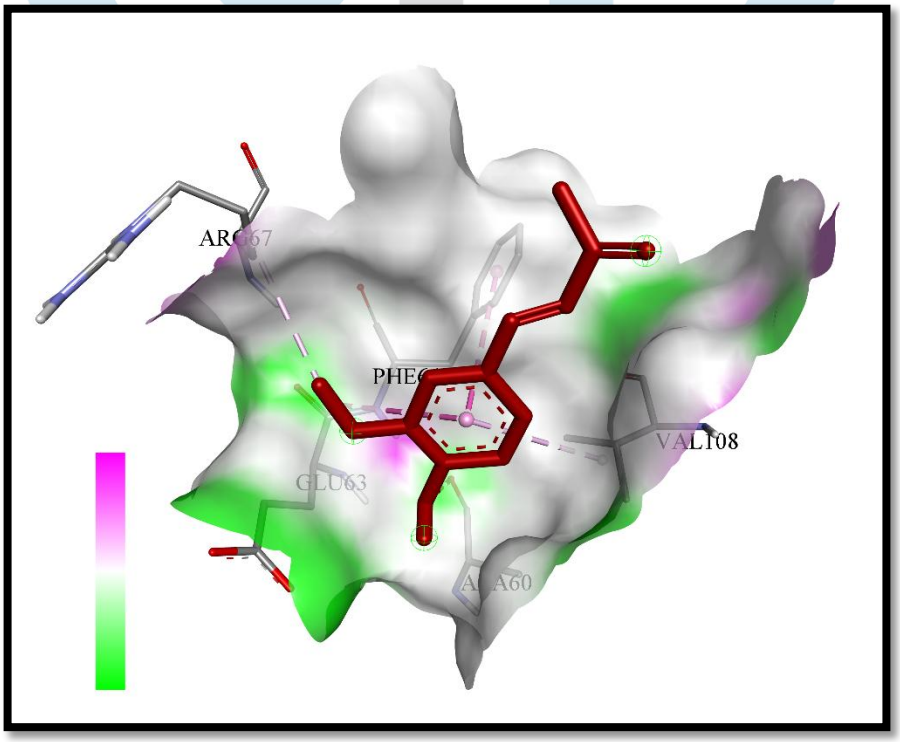


Fig.14. 3D illustration of the binding of DHZ with Bcl2 protein

MIC and ZOI results:

When evaluating the Minimum Inhibitory Concentration (MIC), Dehydrozingerone demonstrated clear efficacy against all four tested pathogens, showing particularly strong potency against *Escherichia coli* with a remarkably low MIC of 15.62 µg/mL It also successfully inhibited the growth of the mold *Aspergillus niger* at 31.25 µg/mL, while requiring a slightly higher concentration of 62.5 µg/mL to hold off both *Candida albicans* and *Staphylococcus aureus*. However, the Zone of Inhibition (ZOI**)** agar diffusion assays revealed a distinct contrast between the compound's bacterial and fungal performance.

Against *A. niger*, Dehydrozingerone (30 µg/mL) showed moderate, clear antifungal activity with a 2.6 cm diameter zone, while the positive control Fluconazole achieved the highest clearance at 4.4 cm. Conversely, in the bacterial assays, while the standard Ciprofloxacin created massive clearance zones (4.6 cm for *S. aureus* and 4.0 cm for *E. coli*), Dehydrozingerone failed to produce any visible zone of inhibition (0.0 cm).

This complete lack of a bacterial ZOI does not mean the compound is inactive against bacteria—especially since the liquid broth MIC data confirms it is highly effective. Instead, this discrepancy most likely stems from a matrix-specific diffusion limitation, where the hydrophobic nature of Dehydrozingerone prevented it from migrating effectively through the water-heavy bacterial agar gel, whereas it was able to diffuse successfully across the fungal media plates to show its true potential.

Table No. 03: MIC of Dehydrozingerone

Sample	*Staphylococcus aureus*	*Escherichia coli*	*Candida albicans*	*Aspergillus niger*
Dehydrozingerone	62.5µg/mL	15.62µg/mL	62.5µg/mL	31.25µg/mL

Zone of Inhibition (ZOI)

Table 4. ZOI of Dehydrozingerone against Bacteria.

Sample	Staphylococcus aureus		Escherichia coli	
	Radius	Diameter	Radius	Diameter
Ciprofloxacin	2.3cm	4.6cm	2cm	4cm
Dehydrozingerone	0.0cm	0.0cm	0.0cm	0.0cm

Table 5. ZOI of Dehydrozingerone against Fungi.

Sample	Aspergillus niger	
	Radius	Diameter
Fluconazole	2.2cm	4.4cm
Dehydrozingerone (30 µg/mL)	1.3cm	2.6cm

The drug shows the MIC for both bacterial and fungi. Fluconazole and Dehydrozingerone drug compounds have the highest and moderate zone of inhibition respectively. Ciprofloxacin compound has the highest zone of inhibition whereas the drug Dehydrozingerone did not show the zone of inhibition.

Discussion:

Network pharmacology and molecular docking studies showed a difference between the predicted compound targets and the actual antibacterial and antifungal proteins, suggesting that further research is needed. In antifungal testing, fluconazole showed the strongest activity against *Aspergillus niger* with the largest zone of inhibition, while Dehydrozingerone showed moderate activity. In antibacterial tests, ciprofloxacin showed strong inhibition against both *Escherichia coli* and *Staphylococcus aureus*, whereas Dehydrozingerone showed no antibacterial activity. Overall, fluconazole and ciprofloxacin were the most effective antifungal and antibacterial agents, respectively, in this study [11].

One of the main advantages of Dehydrozingerone is that it comes from a natural source, so it is often seen as safer and may be less toxic than many synthetic drugs. This makes it a good starting point for developing new medicines. Another benefit is that its simple structure makes it easier for scientists to

modify and improve, which could help increase its antimicrobial strength, stability, and overall effectiveness [12]. Dehydrozingerone is worth studying further because it is a natural compound with a biologically active phenolic structure that shows some antimicrobial potential. Even though it is not as strong as standard drugs like fluconazole or ciprofloxacin, natural compounds like this are important because they can be modified to improve their activity [13].

In the current situation of rising antimicrobial resistance, there is a constant need to explore new molecules. Dehydrozingerone can act as a starting point for developing new and improved antimicrobial agents that may become more effective after structural changes or when used in combination with other drugs. It has become very important to explore new chemical structures that could lead to the development of better drugs. Even compounds that show only moderate or weak antimicrobial activity at the early stage of research are still valuable, because they help scientists understand new possibilities and guide the design of improved drug molecules in the future [14]. Dehydrozingerone fits into this category as a promising starting point for further investigation. Although it may not be highly effective on its own, it can still provide useful insights for drug development. Another important aspect is that it could be tested alongside existing antibiotics or antifungal drugs to see whether it enhances their activity. Such combined effects, known as synergistic action, may help improve overall effectiveness and potentially reduce the required dose of stronger drugs [15].

Overall, studying compounds like Dehydrozingerone is important not just for their direct antimicrobial activity, but also for how they can contribute to developing new strategies and more effective treatment approaches in the fight against resistant microorganisms.

In simple terms, it may not be a powerful drug on its own, but it has good potential as a building block for future antimicrobial research [16].

CONCLUSION:

Network pharmacology and Molecular docking studies indicate that Dehydrozingerone may have promising therapeutic potential by interacting with several target proteins and showing strong binding ability, making it a possible candidate for future drug development and further research. Due to its intrinsically active biological properties, Dehydrozingerone exhibits notable antimicrobial efficacy against a diverse spectrum of microorganisms, encompassing both bacterial and fungal lineages. Specifically, our investigations reveal that this compound demonstrates significant inhibitory activity against key pathogenic bacteria, including *Escherichia coli* and *Staphylococcus aureus*. Even though its antimicrobial effect may not be as strong as commonly used antibiotics in the market, since it is a natural compound with added antioxidant and anti-inflammatory benefits. Therefore, Dehydrozingerone has potential for further pharmaceutical research and may be useful in the future, either on its own or along with existing antibiotics, to help manage antimicrobial resistance

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Conflict of interest:

Authors not have any conflict of interest

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