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RESEARCH ARTICLE OPEN ACCESS

Natural Compounds from *Aureobasidium* sp. TD-062: A New Frontier in Targeting BlaR1 Protein

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Abstract

***Aureobasidium* sp. strain TD-062, a microcolonial fungus, was isolated from the Thar Desert, India, and it's aqueous and ethyl acetate extracts were evaluated for antimicrobial activity against clinical isolates of *Pseudomonas aeruginosa* (sputum), *Escherichia coli* (pus, blood, and urine), *Klebsiella pneumoniae* (urine), and *Proteus*. The ethyl acetate extract was analysed by gas chromatography-mass spectrometry (GC-MS) and examined for toxicity using ProTox-II software. Three compounds, selected based on toxicity and safety profiles, were analysed for molecular docking on BlaR1, a protein that induces antimicrobial resistance in bacteria. The ethyl acetate extract was shown to exhibit antimicrobial activity against clinical pathogens. GC-MS analysis showed that squalene, stigmasterol and delta-tocopherol had lower toxicity profiles. Molecular docking analyses demonstrated that stigmasterol exhibited the highest binding affinity (-8.9 Kcal/mol), compared to positive control clavulanic acid (-6.7 Kcal/mol), suggesting its potential as a potent BlaR1 inhibitor. These findings underscore the bioactive potential of *Aureobasidium* sp. TD-062 as a promising source of bioactive compounds to combat antimicrobial resistance. The identification of squalene, stigmasterol, and delta-tocopherol as lead compounds for drug development represents a significant advancement in the search for novel antimicrobial agents to address the growing global threat of antimicrobial resistance.**

Keywords: *Aureobasidium* sp., BlaR1 Protein, Squalene, Delta-tocopherol, Molecular Docking, Bioactive Compounds

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INTRODUCTION

Natural products are receiving increasing attention as promising reservoirs for novel drug candidates, given that more than 60% of approved medications trace their origins to natural compounds.[1-3] Due to the increasing prevalence of drug resistance, the identification of novel antimicrobial agents is becoming progressively challenging. For a considerable period, microorganisms have been valuable reservoirs of bioactive natural compounds. Through a bioprospecting initiative in the Thar Desert of India, a fungal strain, *Aureobasidium pullulans* TD-062, was isolated. *A. pullulans*, a yeast-like fungus, is known for its presence across diverse habitats and environmental conditions.[4] *Aureobasidum* is a yeast-like fungus that is a valuable resource for biotechnological applications that extend beyond geographical constraints.[5] Recent research in therapeutics has shifted towards exploring natural products as potential candidates, primarily due to their perceived low toxicity or enhanced bioactivity and tolerance.[6]

BlaR1 is an integral membrane protein that plays a crucial role in bacterial resistance to β-lactam antibiotics, including widely used drugs such as penicillin, cephalosporins, and carbapenems.[7] BlaR1 has been found in gram-positive bacteria, such as *Staphylococcus aureus*, and gram-negative bacteria, such as *Escherichia coli, Klebsiella, pneumonia,* and *Pseudomonas aeruginosa.*[8] BlaR1 functions as both a sensor and a signal transducer. It detects the presence of β-lactam antibiotics in the environment and triggers a molecular response that ultimately leads to antibiotic resistance.[9]

Targeting BlaR1 with bioactive compounds from *A. pullulans* TD-062 may induce apoptosis and combat drug resistance. In our previous report, antimicrobial and anticancer compounds were detected in ethyl acetate extract using GC-MS.[10] Molecular docking studies enable the exploration of protein-ligand interactions, providing insights into ligand-binding mechanisms and the stability of complexes. The objective of the study was: (i) To assess the antimicrobial activity of aqueous and organic solvents strain TD-062 against clinical pathogen and (ii) To evaluate the role of antimicrobial compounds identified through GC-MS analysis using *in silico* modeling against BlaR1 protein as a target.

MATERIALS AND METHODS

Growth dynamics and maintenance

As per our published methodology, *Aureobasidium* strain TD-062 was inoculated in 100 mL of potato dextrose broth (PDB) and incubated at 37 °C 180-200 rpm for 5 days. After incubation, the culture was centrifuged to separate the supernatant. The supernatant was used as an aqueous extract. In parallel experiments, ethyl acetate (EA), was extracted with equal volume thoroughly agitated for 1 hr. The organic phase dried, and the resulting residue was weighed and mixed with methanol at the concentration of 1 $mgmL^{-1}$.[11]

Antimicrobial activity

Agar plugs of the cultures were used to evaluate antimicrobial activity according to Bauer et al. Antimicrobial activity of both aqueous and EA extracts was assessed using the well puncture diffusion method as described previously in our publication,[11,12] against clinical pathogens obtained from Jaypee Hospital Noida, 50 µl of clinical pathogens - *Pseudomonas aeruginosa* -1728 from sputum, *Escherichia coli* - 1894, 1610, 2646, 2647 from pus, blood, urine samples, *Klebsiella pneumoniae* - 1862, from urine, and *Proteus* - 1903, were loaded into wells on Muller Hinton Agar (MHA) media plates, which were then chilled at 5 °C prior to incubation (30 °C, 24 hours). Tetracycline was used as the standard control to compare the antimicrobial activity. The antimicrobial activity was measured by the zones of inhibition. Each experiment was carried out in triplicates and repeated thrice.

Following our earlier published protocol,[10] EA extract fractions showing antimicrobial activity against the selected target pathogens were further subjected to GC-MS analysis.

ProTox-II Toxicity prediction and molecular docking modelling

The toxicity of compounds obtained from GC-MS was predicted using ProTox-II,[13] a machine learning-based online tool. The 2D structure of the compounds were searched via PubChem, and

uploaded in mol format and a toxicity profile was obtained.[14]

Based on toxicity analysis, three compounds were used: squalene, stigmasterol, and delta-tocopherol as ligands in molecular docking studies. Clavulanic acid was selected as the control which inhibits beta-lactamase and is commonly used in antibiotic formulations.[15] Their chemical structures were obtained from the PubChem database in SDF format (Figure 1 a-d).[16-19] Open Babel version 2.3.2 was used to convert the structure compatible with PDB format.[20] Molecular docking was performed using Auto dock Vina 1.5.6 software with selected protein BlaR1.[21] The Research Collaboratory for Structural Bioinformatics (RCSB),[22] provided the protein structures of BlaR1 (PDB ID: 8 EXP) Figure 2, which were then saved in the PDBQT file format, water, hetatm, side chains, and ligand molecules were then removed from the protein structure using the BIOVIA discovery Studio 2021 tool.[23] PyMOL 2.5.0 was then used to predict the

Table 1. Antimicrobial activity of aqueous and ethyl acetate extracts from *Aureobasidium* sp. TD-062 against clinical isolates

	Inhibition zone (mm) Mean ± SD						
Extract	*Pseudomonas aeruginosa* (1728)	*Klebsiella pneumoniae* (1862)	*Proteus* (1903)	*E. coli* (1894)	*E. coli* (1610)	*E. coli* (2646)	*E. coli* (2647)
Ethyl acetate TD-062	23 ± 0.5	0	0	22 ± 0.5	20 ± 0.5	21 ± 0.5	18 ± 0.5
Aqueous TDs-062	18 ± 0.5	0	0	19 ± 0.5	0	0	17 ± 0.5
Control	26 ± 0.4	24 ± 0.4	25 ± 0.8	21 ± 0.4	21 ± 0.4	24 ± 0.4	22 ± 0.12

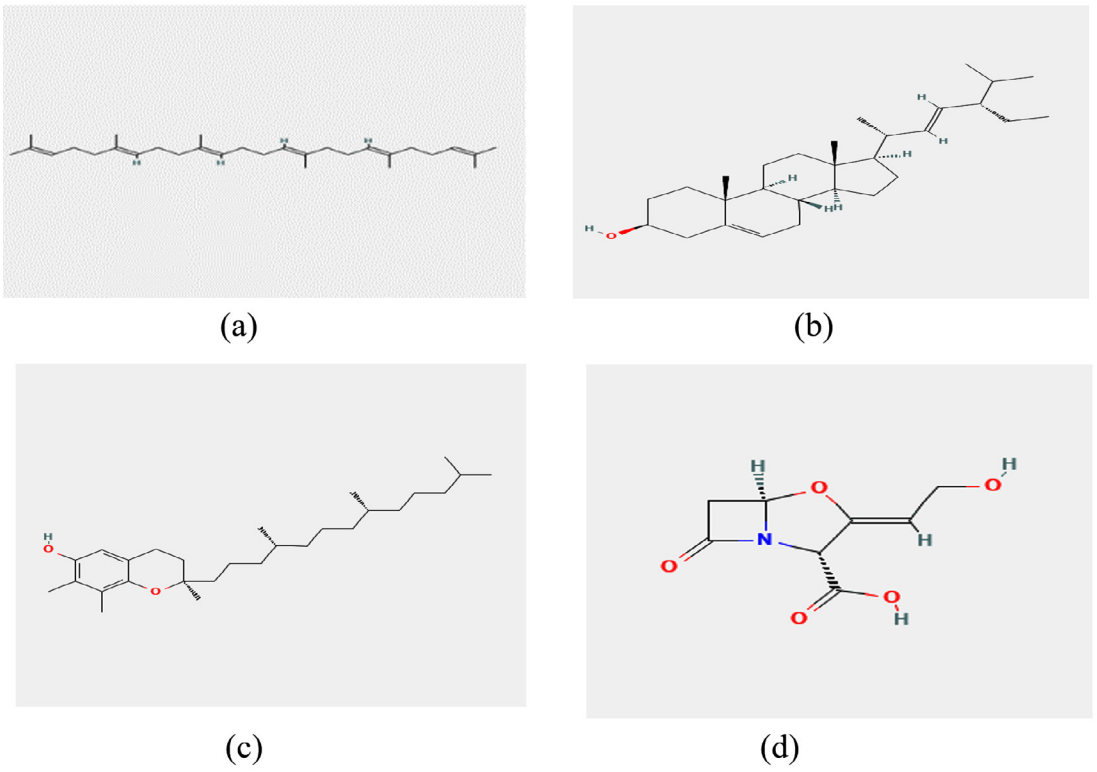

Figure 1. Chemical structures obtained from PubChem: (a) Squalene, (b) Stigmasterol, (c) Delta-tocopherol, and (d) Clavulanic acid[16-19]

Table 2. Toxicity prediction of compounds obtained from *Aureobasidium* sp. TD-062 based on Protox-II analysis

Target	Squalene	Stigmasterol	Delta-tocopherol	Clavulanic acid
Molecular weight	410	412	416	199
Number of hydrogen bond acceptors	0	1	2	6
Number of hydrogen bond donors	0	1	1	2
Hepatotoxicity	Not-active	Active	Active	Not-active
Neurotoxicity	Not-active	Not-active	Not-active	Not-active
Nephrotoxicity	Not-active	Not-active	Not-active	Active
Respiratory toxicity	Not-active	Active	Active	Not-active
Cardiotoxicity	Not-active	Not-active	Not-active	Not-active
Carcinogenicity	Not-active	Not-active	Not-active	Not-active
Immunotoxicity	Not-active	Not-active	Not-active	Not-active
Mutagenicity	Not-active	Not-active	Not-active	Not-active
Cytotoxicity	Not-active	Not-active	Not-active	Not-active
Bbb-barrier	Active	Active	Active	Not-active
Nutritional toxicity	Not-active	Not-active	Not-active	Active
Clinical toxicity	Not-active	Not-active	Not-active	Active
Cytochrome CYP1A2	Not-active	Not-active	Not-active	Not-active
Cytochrome CYP2C19	Not-active	Not-active	Not-active	Not-active
Cytochrome CYP2C9	Active	Active	Active	Not-active
Cytochrome CYP2D6	Not-active	Not-active	Not-active	Not-active
Cytochrome CYP3A4	Not-active	Not-active	Not-active	Not-active
Cytochrome CYP2E1	Not-active	Not-active	Not-active	Not-active

protein's tertiary structure.[24] Active site prediction for the targeted blar1 proteins was carried out using CASTp 3.0.[25]

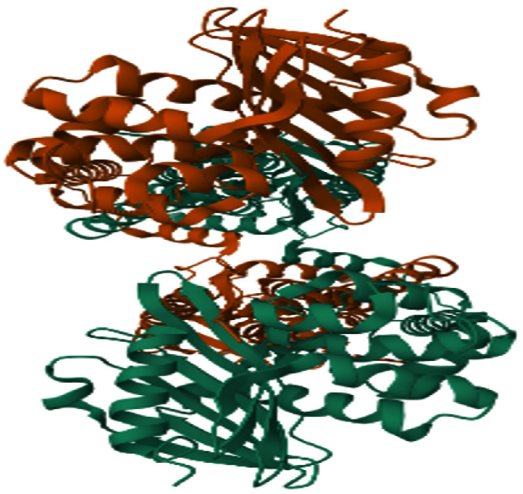

Figure 2. Protein structure of BlaR1 obtained from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB)[22]

RESULT

Unexplored environments have been suggested as a strategy in bioprospecting for unique chemical diversity. *Aureobasidium* sp. TD-062 was isolated from Thar Desert's red rocky soil, (GenBank accession number JAKSGJ000000000). The aqueous and EA extracts demonstrated antimicrobial activity. As shown in Table 1, the aqueous extract exhibited activity against clinical pathogens - *P. aeruginosa* - 1728, and *E. coli* - 1894, 2647 whereas the EA extract showed activity against *P. aeruginosa* - 1728, and *E. coli* - 1894, 1610, 2646, 2647, but no activity against *Proteus*. Zones of inhibition were comparable to or more than that of positive control tetracycline.

In our previous work, GC-MS analysis of compounds from second purification cycle of ethyl acetate extract showed the presence of three bioactive compounds categorized as tocopherols and triterpenes.[10]

Toxicity prediction of bioactive compounds

The Protox-II toxicity prediction analysis

Table 3. Prediction of active site for targeted blar1 protein with CASTp server

Protein	Volume (SA)	Area (SA)	Aa residues at predicted active site	Total aa residue in chain a
Blar1	14251.82	7625.88	22	226

Table 4. BlaR1 protein interactions with selected compounds obtained from *Aureobasidum* sp. TD-062

Protein and ligand	Molecular docking Binding affinity (kcal/mol)
Blar1 with squalene	-7.2
Blar1with stigmasterol	-8.9
Blar1 with delta-tocopherol	-7.9
Blar1 with clavulanic acid	-6.7

was used to evaluate the safety profiles of compounds obtained from the second purification cycle of the ethyl acetate extract. As shown in Table 2, the bioactive compounds analyzed were squalene, stigmasterol, and delta-tocopherol. Squalene (molecular weight 410) demonstrated a favorable safety profile, with predicted inactivity against hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, mutagenicity, cytotoxicity, and clinical toxicity. However, it exhibited activity against CYP2C9, indicating a potential for drug interactions. Stigmasterol and delta-tocopherol exhibited promising safety profiles, with minimal risks for neurotoxicity, nephrotoxicity, cardiotoxicity, and carcinogenicity. However, both stigmasterol and delta-tocopherol were found to interact with CYP2C9, a liver enzyme essential for the metabolism of various drugs. This interaction indicates a potential for drug-drug interactions, as CYP2C9 modulation. Both compounds showed inactivity against other key cytochrome P450 enzymes, reducing the likelihood of widespread metabolic disruptions.

Clavulanic acid exhibits low risks for hepatotoxicity, neurotoxicity, cardiotoxicity, and respiratory toxicity but shows activity for nephrotoxicity and clinical toxicity. Inactive for most cytochrome P450 enzymes and unable to cross the blood-brain barrier, it presents a favorable profile for combating antimicrobial resistance with minimal metabolic interactions.

Active site prediction for the targeted blar1 proteins was carried out using CASTp 3.0,[21] which identified 22 amino acid residues at the predicted site, with this, the grid box identified active site for subsequent docking studies, as illustrated in Table 3.

Docking studies on BlaR1 protein

Molecular docking was performed using autodock 1.5.6 to assess the binding affinities of the bioactive compounds from *Aureobasidium* sp. TD-062 with the BlaR1 protein, a potential target for antimicrobial resistance. Listed in Table 4. stigmasterol exhibited the strongest binding affinity (-8.9 kcal/mol), suggesting a stable and effective interaction within the blar1 binding pocket. delta-tocopherol showed a moderate binding affinity (-7.9 kcal/mol), making it a promising candidate for optimization. Squalene demonstrated the weakest binding affinity (-7.2 kcal/mol) among the compounds, suggesting it may require further optimization for efficacy.

DISCUSSION

Antimicrobial activity

The extracts from *Aureobasidium* sp. TD-062 exhibited significant antimicrobial activity against clinical pathogens. The results suggest that the extracts contain bioactive compounds capable of inhibiting microbial growth, with activity levels comparable or more than that of standard antibiotic tetracycline, considering that the extracts used were not completely purified, unlike tetracycline. Authors suggest that this may be due to the synergistic activities of the compounds in the extracts.

Toxicity prediction and safety profiles

The toxicity prediction analysis revealed that the compounds squalene, stigmasterol, and delta-tocopherol from *Aureobasidium* sp. TD-062 has promising safety profiles. Squalene, a component of shark liver oil, serves as an intermediate metabolite in the synthesis of cholesterol.[26] It is recognized as a skin protectant, is resistant to lipid peroxidation and possesses antimicrobial activity on *E. coli* B-8208.[27] Squalene is predicted to cross the blood-brain barrier

(BBB) without neurotoxic effects, highlighting its potential CNS compatibility.[28] However, squalene shows activity against CYP2C9, which is a liver enzyme critical for metabolizing drugs, such as warfarin, and plays a key role in drug detoxification.[29] Its variations or inhibition can affect drug efficacy, safety, and interactions, indicating the possibility of drug-drug interactions while being inactive for other cytochrome P450 enzymes that are crucial for metabolizing drugs, hormones, and xenobiotics.[30] They catalyze oxidation reactions, aid detoxification, drug clearance, and biosynthesis. Variations in CYPs can affect drug metabolism, efficacy, and safety.[31] These findings suggest minimal risk across multiple endpoints.

Stigmasterol, also referred to as stigmasterin, is a kind of steroid that belongs to the tetracyclic triterpene class.[32] Stigmasterol has the potential to possess antiparasitic qualities against specific parasitic strains, including Leishmania and Trypanosoma congolense.[33] Vitamin E, also known as tocopherol, is a collective term for a set of fat-soluble substances comprising four tocopherols and four tocotrienols.[34] Delta-tocopherol has demonstrated antimicrobial activity on Pseudomonas aeruginosa and methicillin-resistant *Staphylococcus aureus* (MRSA).[35]

Stigmasterol and delta-tocopherol exhibited encouraging safety profiles, with minimal predicted risks for neurotoxicity, nephrotoxicity, cardiotoxicity, and carcinogenicity, supporting their potential for therapeutic applications. These findings are significant as they suggest that both compounds may have a wide safety margin when used in clinical or pharmaceutical formulations.

The interaction of both compounds with CYP2C9, a crucial liver enzyme involved in drug metabolism, is another key finding. CYP2C9 plays a vital role in the metabolism of various drugs, including anticoagulants such as warfarin and nonsteroidal anti-inflammatory drugs (NSAIDs).[36] The modulation or inhibition of CYP2C9 by stigmasterol and delta-tocopherol could lead to altered drug metabolism, resulting in either enhanced toxicity or reduced efficacy of co-administered medications. This indicates a potential for drug-drug interactions that could have clinical implications.

Clavulanic acid, a β-lactam compound, inhibits β-lactamase enzymes, enhancing the efficacy of antibiotics like penicillin and cephalosporins against resistant pathogens such as *Staphylococcus aureus* and *Escherichia coli*.[37] Toxicity predictions indicate a favorable safety profile with low risks for hepatotoxicity, neurotoxicity, cardiotoxicity, and respiratory toxicity, though nephrotoxicity and clinical toxicity remain concerns, particularly in patients with pre-existing kidney conditions. Its inactivity against most cytochrome P450 enzymes reduces the likelihood of drug-drug interactions, and its inability to cross the blood-brain barrier minimizes central nervous system-related risks.

Binding affinity with BlaR1 protein

The results from the molecular docking studies indicated that stigmasterol exhibited the strongest binding affinity to BlaR1, suggesting its potential as a highly effective inhibitor of this protein, which plays a central role in β-lactam antibiotic resistance.[38] This strong interaction supports the idea that stigmasterol could be a promising candidate for further development as a therapeutic agent targeting BlaR1. In contrast, delta-tocopherol displayed a moderate binding affinity, indicating its potential as an inhibitor as well, but suggesting that structural optimization may be necessary to enhance its binding strength and efficacy.[39] On the other hand, squalene showed the weakest binding affinity among the compounds tested. Despite this, it still holds promise as a candidate for further studies, as modifications or optimizations may improve its binding affinity and inhibitory activity against BlaR1. These findings underscore the need for these compounds and explore their potential as novel therapeutic agents in combating antibiotic resistance.

CONCLUSION

Antimicrobial compounds from *Aureobasidium* TD-062 have exhibited activity against clinical pathogens, with good safety profiles. Amongst them, stigmasterol has demonstrated potential in inhibiting BlaR1, with high binding affinity of -8.9 kcal/mol, thereby

indicating its effectiveness in control of both Gram-positive and Gram-negative pathogens via inhibition of beta-lactamase activity. Hence, we conclude that natural compounds continue to offer promise in targeting antimicrobial resistance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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