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In silico Analysis of the Synergistic Interaction Between Biosurfactants and Antifungal Agents Against *Trichophyton* spp.

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Abstract

BACKGROUND/AIMS: Dermatophytosis, caused by *Trichophyton* species such as *T. rubrum, T. mentagrophytes*, and *T. indotineae*, is a common fungal infection with rising terbinafine resistance, particularly in *T. indotineae* strains in South Asia. This study investigates the potential of biosurfactant (rhamnolipid and sophorolipid) and antifungal (terbinafine, itraconazole, fluconazole) combinations in the battle against antifungal resistance.

METHODS: Homology modeling was used to generate 3D structures of 14-alpha-demethylase and squalene epoxidase. Molecular docking and molecular mechanics/poisson-boltzmann surface area calculations were performed via GROMACS 2020.6.

RESULTS: Itraconazole/sophorolipid combination demonstrated the highest combined energy in *T. mentagrophytes* (-82.24 kJ/mol) and *T. indotineae* (-85.35 kJ/mol). The terbinafine/rhamnolipid combination exhibited strong synergistic effects in *T. mentagrophytes* (-84.23 kJ/mol), but it was found not to be an ideal combination for *T. rubrum*.

CONCLUSION: Combining biosurfactants with conventional antifungals is reported to be a promising strategy for treating resistant *Trichophyton* infections, particularly via co-administration of itraconazole/sophorolipid and terbinafine/rhamnolipid combinations.

Keywords: Dermatophytosis, *Trichophyton* species, antifungal resistance, biosurfactants, molecular docking

INTRODUCTION

Dermatophytosis is the most prevalent fungal infection worldwide, caused by various dermatophyte species. These include *Trichophyton*, *Microsporum*, and *Epidermophyton*, which infect keratinized tissues such as skin, hair, and nails. Among *Trichophyton* species, *T. rubrum*, *T. mentagrophytes*, and *T. interdigitale* are the most common in humans.[1] *T. indotineae,* a hypervirulent strain previously known as *T. mentagrophytes* genotype 8, has been responsible for persistent dermatophytosis outbreaks in South Asia.[2] *T. indotineae* manifests primarily as tinea faciei, corporis, or cruris, and is highly transmissible.[3] Increasing cases of terbinafine-resistant *T. indotineae* and other *Trichophyton* species globally have raised concerns about treatment effectiveness.[4,5]

Tinea in the genital region is rare, mostly caused by *T. rubrum*, but a new genotype of *T. mentagrophytes* (type 7) has been linked to severe infections in this part of the body.[6] These infections, reported among men who have sex with men, are suspected to be sexually transmitted, although no terbinafine resistance has been observed in this strain.[7]

Common treatments for dermatophytosis include topical and oral antifungals, with oral therapies (primarily terbinafine) reserved for

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severe cases. However, rising resistance to azoles and allylamines in *T. rubrum* and *T. indotineae* presents a significant challenge.[8] Resistance mechanisms include mutations in the *ERG11* gene (targeting azoles) and *SQLE* gene (affecting terbinafine), which alter enzyme structures, reducing drug binding.[8,9]

Biosurfactants, like rhamnolipids and sophorolipids, are regarded as promising alternatives in antifungal therapy due to their ability to disrupt biofilms and cell membranes.[10,11] Rhamnolipids reduce the minimum inhibitory concentrations of azoles and allylamines in resistant strains like *T. rubrum*, while sophorolipids enhance drug permeability, particularly in *Candida albicans* and *T. mentagrophytes*.[12] Given the rise in drug-resistant *Trichophyton* species,[13] the growing resistance underscores the urgent need for novel treatment strategies, such as combining biosurfactants with conventional antifungals to enhance therapeutic effectiveness.

This study aims to address the critical challenge of antifungal resistance by exploring the synergistic potential of combining biosurfactants, known for their biofilm-disrupting and permeability-enhancing properties, with conventional antifungal agents. Through *in silico* analysis of binding interactions with key enzymes in three *Trichophyton* species, this research seeks to identify more effective therapeutic options, providing a basis for targeted, species-specific treatment approaches for drug-resistant dermatophytosis.

MATERIALS AND METHODS

Homology Modeling and Molecular Docking

AutoDock Vina was used to assess the binding affinities of antifungal agents (fluconazole, itraconazole, terbinafine) and biosurfactants (rhamnolipid, sophorolipid) against *Trichophyton* species. The target proteins 14-alpha-demethylase and squalene epoxidase, involved in fungal sterol biosynthesis, were modeled via homology using I-TASSER (https://zhanggroup.org/). Ligands and proteins were prepared with AutoDockTools, and docking focused on the active sites using a grid box of 28 × 28 × 28 Å. The Lamarckian genetic algorithm generated 10 docking poses per ligand, with the lowest energy pose chosen for molecular dynamics (MD) simulations.[14]

MD Simulations and MM/PBSA Calculations

MD simulations were performed using GROMACS 2020.6 to evaluate the stability of ligand-protein complexes as described in previous research.[14] Post-MD simulations, molecular mechanics/poisson-boltzmann surface area binding free energy calculations were performed using g_mmpbsa to estimate the free energy of ligand-protein complexes.[14] The stability of combination-enzyme complexes was illustrated using a root mean square deviation (RMSD) plot.

Ethical approval was not applicable for this study as no human participants or animals were involved.

RESULTS

The binding energies of antifungal agents and biosurfactants against 14-α-demethylase and squalene epoxidase in *T. mentagrophytes*, *T. indotineae*, and *T. rubrum* were calculated by AutoDock Vina. As a result of this analysis, the most effective combinations for *T. mentagrophytes* were found to be terbinafine/rhamnolipid (-84.23±1.63 kJ/mol), itraconazole/sophorolipid (-82.24±1.32 kJ/mol), and itraconazole/rhamnolipid (-73.46±1.75 kJ/mol). For *T. indotineae*, the highest interactions in terms of combined binding energies were observed in the following order: itraconazole/sophorolipid (-85.35±1.64 kJ/mol), itraconazole/rhamnolipid (-81.57±2.18 kJ/mol), and terbinafine/rhamnolipid (-72.27±2.04 kJ/mol). When compared to the other two *Trichophyton* species, the binding energies in *T. rubrum* exhibited less negative binding energies, with the highest affinity observed in the combinations of itraconazole/rhamnolipid (-74.73±1.87 kJ/mol), fluconazole/rhamnolipid (-56.23±2.05 kJ/mol), and fluconazole/sophorolipid (-48.54±1.54 kJ/mol). The binding energies of the combinations of antifungal drugs (terbinafine, itraconazole, fluconazole) with biosurfactants (rhamnolipid, sophorolipid) at the binding sites (squalene epoxidase and 14-α-demethylase) in different *Trichophyton* species are shown in Table 1.

Higher additive energy values for terbinafine/rhamnolipid and terbinafine/sophorolipid (-54.60±2.04 and -64.70±2.26 kJ/mol, respectively) were obtained in *T. rubrum* compared to the combined energy values for terbinafine/rhamnolipid and terbinafine/sophorolipid (-51.73±1.38 and -45.29±2.14 kJ/mol, respectively). This indicates that terbinafine alone is more effective than its combinations with biosurfactants, particularly for this species (Figure 1a).

RMSD analysis was performed to assess the stability of the MD simulations in the drug-biosurfactant combinations that exhibited the highest binding energy in each *Trichophyton* species. As a result, the terbinafine/rhamnolipid combination in *T. mentagrophytes* was found to be the most stable combination (Figure 1b). The itraconazole/rhamnolipid combination in *T. rubrum* was reported to be more stable than the itraconazole/sophorolipid combination in *T. indotineae*, and all MD were within the expected range (RMSD value <2).

Table 1. Additive and combined binding energies (kJ/mol) of drug-biosurfactant combinations with squalene epoxidase and 14-α-demethylase in *Trichophyton* species

Combinations	*Trichophyton mentagrophytes*		*Trichophyton indotineae*		*Trichophyton rubrum*	
	Additive energy	Combined energy	Additive energy	Combined energy	Additive energy	Combined energy
Fluconazole + rhamnolipid	-27.00±2.04	-34.73±2.23	-28.00±2.74	-48.39±1.83	-45.00±2.94	-56.23±2.05
Fluconazole + sophorolipid	-35.00±2.26	-38.23±1.26	-25.00±2.59	-32.33±1.64	-25.00±2.46	-48.54±1.54
Itraconazole + rhamnolipid	-47.00±1.95	-73.46±1.75	-48.00±2.59	-81.57±2.18	-55.00±2.57	-74.73±1.87
Itraconazole + sophorolipid	-55.00±2.17	-82.24±1.32	-45.00±2.43	-85.35±1.64	-35.00±2.00	-41.84±1.45
Terbinafine + rhamnolipid	-70.00±1.91	-84.23±1.63	-50.00±2.33	-72.27±2.04	-54.60±2.04	-51.73±1.38
Terbinafine + sophorolipid	-55.00±2.35	-78.37±2.16	-40.00±2.07	-52.38±1.83	-64.70±2.26	-45.29±2.14

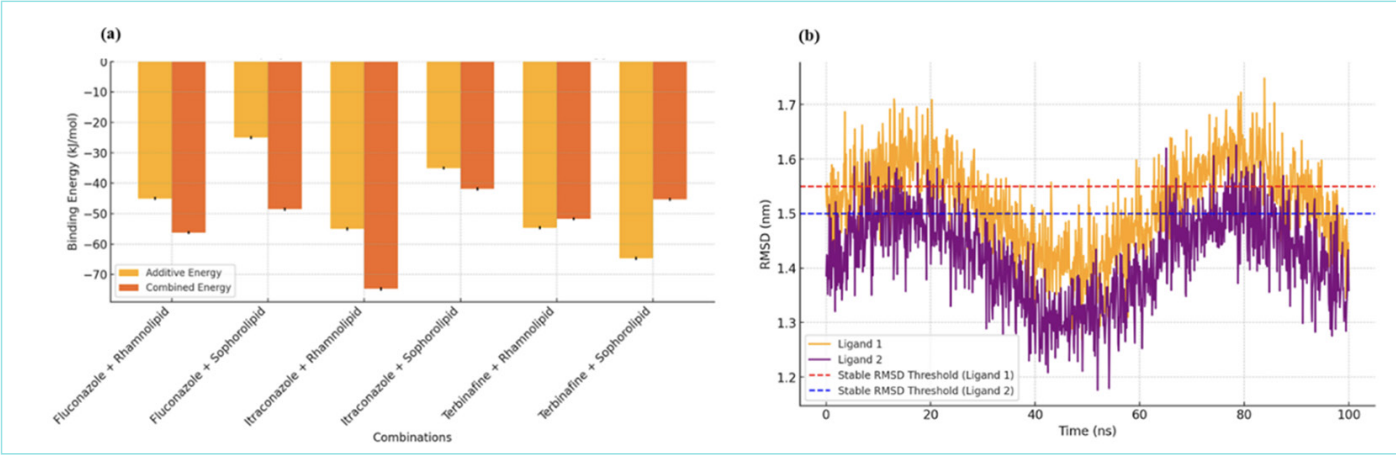

Figure 1. (a) Comparison of additive and combined binding energies for drug-biosurfactant combinations in *Trichophyton rubrum*. (b) The RMSD analysis of terbinafine (purple) and rhamnolipid (orange) in complex with *Trichophyton mentagrophytes* was conducted over a 100 ns simulation period.

RMSD: Root mean square deviation.

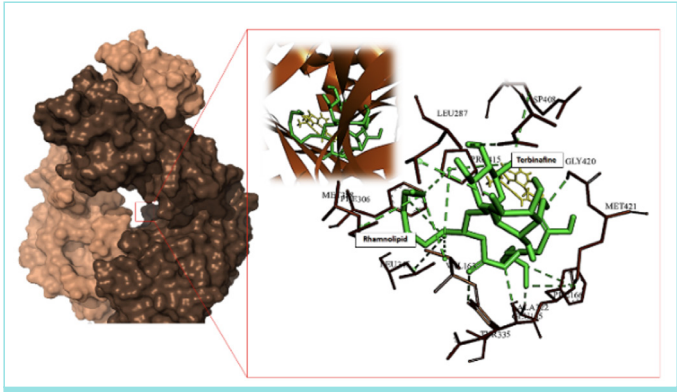

Figure 2. The interaction of terbinafine (yellow) and rhamnolipid (green) at the squalene epoxidase binding site of *Trichophyton mentagrophytes*.

DISCUSSION

In this study, we aimed to investigate the combined use of biosurfactants with the efficacy of conventionally used antifungal agents over three different *Trichophyton* species in combating antifungal resistance. For this purpose, both the additive and combined energies of each combination were calculated for specific *Trichophyton* species included in the study.

Additive energy represents the sum of the binding energies of each agent individually, whereas combined energy refers to the binding energy when both agents interact simultaneously. Data revealed that, relatively higher combined binding energies were obtained in all combinations except for the combinations of *T. rubrum* with terbinafine, meaning that the combinations with biosurfactants were shown to be more effective than conventional agents used alone. The ability of rhamnolipid to disrupt fungal biofilm and the capacity of sophorolipids to alter membrane permeability may contribute to the enhanced drug interactions, as previously reported in studies exploring biosurfactants as drug-delivery enhancers.[15-17] This could be attributed to the possibility of having higher combined binding energies in combinations over antifungals alone.

When examining the combinations that exhibited the highest combined binding energy for all three *Trichophyton* species, it is observed that the top three combinations with the highest affinity vary for each species. While the azole group combination, itraconazole/sophorolipid, and the allylamine group combination terbinafine/rhamnolipid are common options for *T. mentagrophytes* and *T. indotineae*, the itraconazole/rhamnolipid combination is shared between *T. indotineae* and *T. rubrum*. However, there is no common factor combination that shows the highest interaction for both *T. mentagrophytes* and *T. rubrum*. Interestingly, fluconazole, which is less potent than itraconazole, demonstrated better binding when combined with biosurfactants, particularly for *T. rubrum* compared to the other two species. This discrepancy might be attributed to species-specific differences in the structure of the targeted enzymes, influencing how biosurfactants interact with antifungals. These findings are consistent with previous research showing that genetic variability among *Trichophyton* species impacts drug efficacy, which might explain the varying effectiveness of certain drug combinations across different *Trichophyton* species.[18,19]

The findings of the present study indicated that the combination application of biosurfactants and antifungals demonstrated enhanced antifungal activity particularly in *T. mentagrophytes* and *T. indotineae* as they possess higher combined energy than additive energies. The combination of terbinafine and rhamnolipid demonstrated the highest synergism at the squalene epoxidase binding site, especially on *T. mentagrophytes* (Figure 2).

CONCLUSION

In conclusion, this study emphasizes the necessity of personalized antifungal treatment strategies due to species-specific responses and genetic variability among *Trichophyton* species, which influence drug efficacy. It emphasizes accurate species identification to optimize treatment and reduce antifungal resistance. This paves the way for future research on developing more precise diagnostic tools and targeted therapies to enhance efficacy.

MAIN POINTS

- Biosurfactants combined with antifungals significantly improved efficacy against *Trichophyton* species.
- Sophorolipid/itraconazole and rhamnolipid/terbinafine combinations showed strong antifungal effects.
- The combinations effectively targeted drug-resistant strains, offering a promising approach for overcoming antifungal resistance.

ETHICS

Ethics Committee Approval: Ethical approval was not applicable for this study as no human participants or animals were involved.

Informed Consent: Not available.

Footnotes

Authorship Contributions

Concept: C.S.Ö., Design: C.S.Ö., Data Collection and/or Processing: E.E., Analysis and/or Interpretation: C.B., E.E., Literature Search: C.B., Writing: C.B.

DISCLOSURES

Conflict of Interest: No conflict of interest was declared by the authors.

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