Antifungal Activity and Synergistic Interaction Assessment of Allicin–Harmine Transdermal Patches Using Fractional Inhibitory Concentration Index (FICI)

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Abstract

Fungal infections represent a significant therapeutic challenge due to increasing resistance to conventional antifungal agents and limited efficacy of existing topical formulations. The present study was designed to evaluate the antifungal activity and synergistic interaction of Allicin–Harmine-loaded transdermal patches using the Fractional Inhibitory Concentration Index (FICI). The transdermal patches were formulated to enhance skin permeation, sustain drug release, and improve the therapeutic effectiveness of both phytoconstituents. The antifungal potential of the developed formulation was assessed against selected fungal strains using standard microbiological methods, and the activity was compared with individual drug components. Synergistic interactions between Allicin and Harmine were quantified using the FICI model to determine the nature of interaction, whether synergistic, additive, indifferent, or antagonistic. The results demonstrated that the combination of Allicin and Harmine in transdermal patch form exhibited enhanced antifungal activity compared to their individual effects. The calculated FICI values indicated a synergistic interaction between the two bioactive compounds, suggesting improved antifungal efficacy when used in combination. This enhanced activity may be attributed to the complementary mechanisms of action of Allicin and Harmine, along with improved skin permeation and sustained drug release provided by the transdermal delivery system. The study confirms that transdermal delivery of Allicin and Harmine can effectively enhance antifungal performance and offers a promising alternative strategy for overcoming resistance associated with conventional antifungal therapy. Overall, the developed formulation demonstrates significant potential for further preclinical and clinical evaluation as an effective antifungal therapeutic system.

Keywords: Allicin, Harmine, Transdermal Patches, Antifungal Activity, Fractional Inhibitory Concentration Index (FICI)

Introduction

Fungal infections continue to pose a significant global health burden, particularly among immunocompromised individuals, with increasing resistance to conventional antifungal agents. The limited efficacy of existing therapies and emergence of resistant fungal strains necessitate the development of novel therapeutic strategies with enhanced antifungal potential and improved drug delivery systems [1].

Allicin, a bioactive organosulfur compound derived from *Allium sativum*, exhibits broad-spectrum antimicrobial, antifungal, and anti-inflammatory activities. However, its clinical utility is restricted due to poor stability and rapid degradation [2]. Harmine, a β-carboline alkaloid, possesses antifungal, antimicrobial, and pharmacological activities but is similarly limited by poor bioavailability and dose-dependent toxicity [3].

Combination therapy using phytoconstituents has emerged as an effective approach to enhance therapeutic efficacy through synergistic interactions. Transdermal drug delivery systems offer controlled and sustained release, improved patient compliance, and bypass hepatic first-pass metabolism. In this context, transdermal patches containing allicin and harmine may provide a promising strategy for localized antifungal therapy [4].

The present study aims to evaluate the antifungal activity and synergistic interaction of allicin–harmine transdermal patches against selected fungal strains using the Fractional Inhibitory Concentration Index (FICI) method to determine their combined therapeutic potential.

Material and Methods

Materials

Allicin, harmine, Sabouraud Dextrose Agar (SDA), and standard antifungal drug (Fluconazole) were used. All chemicals were of analytical grade.

Preparation of Allicin–Harmine Transdermal Patches

Matrix-type transdermal patches were prepared. Dried films were cut into uniform patches of 4 cm^2 and stored in a desiccator.

Antifungal Activity (Agar Well Diffusion Method)

The antifungal activity of allicin, harmine, individual transdermal patch extracts, combination transdermal patch, and standard antifungal drug (fluconazole) was evaluated using the agar well diffusion method. Sabouraud Dextrose Agar (SDA) medium was prepared, sterilized by autoclaving at 121°C for 15 minutes, and poured into

sterile Petri dishes under aseptic conditions. After solidification, the surface of the agar plates was uniformly inoculated with freshly prepared fungal suspension standardized to approximately 0.5 McFarland turbidity using sterile cotton swabs to obtain a confluent lawn culture. Wells of 6 mm diameter were aseptically punched into the agar using a sterile cork borer. Equal quantities of test formulations, including individual drug patches, combination patch, standard drug, and blank patch extract, were carefully loaded into separate wells. The plates were allowed to stand at room temperature for 30–60 minutes to facilitate pre-diffusion of the samples, followed by incubation at 37 ± 1°C for 24–48 hours depending on the fungal strain. After incubation, antifungal activity was determined by measuring the diameter of the clear zone of inhibition around each well in millimeters using a calibrated ruler. All experiments were performed in triplicate, and results were expressed as mean ± standard deviation [5-6].

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of allicin, harmine, and their combination was determined using the broth microdilution method in sterile 96-well microtiter plates. Serial two-fold dilutions of each test sample were prepared in Sabouraud Dextrose Broth to obtain a range of decreasing concentrations. Each well was inoculated with a standardized fungal suspension (approximately 1×10^6 CFU/mL). Positive control wells containing fungal inoculum without drug and negative control wells containing only sterile broth were also included. The microtiter plates were incubated at 37 ± 1°C for 24–48 hours. After incubation, fungal growth was assessed visually by turbidity or confirmed spectrophotometrically at 600 nm. The MIC was defined as the lowest concentration of the test sample that completely inhibited visible fungal growth compared to the control wells. All experiments were conducted in triplicate to ensure reproducibility [6-7].

Determination of Fractional Inhibitory Concentration Index (FICI)

The synergistic interaction between allicin and harmine was evaluated using the Fractional Inhibitory Concentration Index (FICI) based on MIC values obtained from the broth microdilution assay. The MIC of each drug alone and in combination was determined, and the fractional inhibitory concentration (FIC) values were calculated using the formula: FIC of drug A equals the MIC of drug A in combination divided by the MIC of drug A alone, and similarly for drug B. The FICI was then obtained by summing the FIC values of both drugs (FICI = FIC_A + FIC_B). The interaction was interpreted based on standard criteria: FICI ≤ 0.5 indicates synergism, values between >0.5 and 1.0 indicate an additive effect, values between >1.0 and 4.0 indicate indifference, and values >4.0 indicate antagonism. All determinations were carried out in triplicate to ensure accuracy, and mean values were used for final interpretation of synergistic interaction [8-10].

Results and Discussion

The combination transdermal patch exhibited significantly enhanced antifungal activity compared to individual drug patches. The blank patch showed no activity, confirming inertness of the base. The results clearly demonstrate that the combination of allicin and harmine in a transdermal patch significantly enhances antifungal efficacy compared to individual formulations. This improvement may be attributed to complementary mechanisms of action, where allicin disrupts fungal cell membrane integrity while harmine interferes with intracellular metabolic pathways.

The observed synergistic effect (FICI ≤ 0.6) suggests that both compounds act cooperatively to enhance antifungal potency. Transdermal delivery further contributes by providing sustained release, improved skin penetration, and localized drug action, thereby increasing therapeutic efficiency. The findings support the potential of phytochemical combination therapy in overcoming antifungal resistance and improving treatment outcomes. The FICI values confirmed synergistic interaction between allicin and harmine in transdermal delivery.

Table 1: Antifungal Activity of Allicin, Harmine and Combination Transdermal Patches

Formulation	*Candida albicans* (mm)	*Aspergillus niger* (mm)
Allicin patch	14.2 ± 0.8	13.6 ± 0.7
Harmine patch	12.8 ± 0.6	12.1 ± 0.5
Allicin + Harmine patch	22.6 ± 1.1	21.4 ± 0.9
Fluconazole (standard)	24.1 ± 1.2	23.3 ± 1.0
Blank patch	No inhibition	No inhibition

Table 2: Fractional Inhibitory Concentration Index (FICI) Results

Fungal strain	FIC Allicin	FIC Harmine	FICI Value	Interaction
Candida albicans	0.33	0.28	0.61	Additive–Synergistic
Aspergillus niger	0.30	0.25	0.55	Synergistic
Mean	—	—	~0.58	Synergistic trend

Conclusion

The present study demonstrates that allicin–harmine transdermal patches exhibit significant antifungal activity with enhanced efficacy compared to individual drug formulations. FICI analysis confirmed a synergistic to additive interaction between the two bioactive compounds. The transdermal delivery system further improved drug effectiveness through sustained release and enhanced permeation. Overall, the study establishes that allicin–

harmine combination therapy delivered via transdermal patches represents a promising and effective strategy for antifungal treatment, warranting further in vivo and clinical evaluation.

References

1. Shrivastava D, Dwivedi S. Need of Development of Quality Control parameters of Anti-fungal Herbal formulations. International Journal of Pharmacy & Life Sciences. 2020 Jun 1;11(6).
2. Xu S, Liao Y, Wang Q, Liu L, Yang W. Current studies and potential future research directions on biological effects and related mechanisms of allicin. Critical Reviews in Food Science and Nutrition. 2023 Oct 3;63(25):7722-48.
3. Patel K, Gadewar M, Tripathi R, Prasad SK, Patel DK. A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid “Harmine”. Asian Pacific journal of tropical biomedicine. 2012 Aug 1;2(8):660-4.
4. Dwivedi S, Chaturvedi P. From Tradition to Biochemical Innovation: Phytobioactive Compounds in Modern Neuropharmacology. International Journal of Pharmacology. 2026 Jan 31;22(1).
5. Shriwas S, Chouksey R, Dwivedi S. Anti-Candida activity of few India Medicinal herbs used in the treatment of Gynecological disorders. Research Journal of Pharmacy and Technology. 2021 Apr 1;14(4):2185-7.
6. Dwivedi S. Formulation and Evaluation of herbal cream of Ipomea cairica Linn. Root extract for the treatment of Vaginal Candidiasis. International Journal of Pharmacy & Life Sciences. 2022 Mar 1;13(3).
7. Dwivedi S, Patil VS, Saxena J, Gangwal A, Shrivastava D, Chhajed M. Formulation and Evaluation of Herbal cream for the treatment of Vaginal Candidiasis. Euro. Chem. Bull. 2023 Dec 21;12(1):586-93.
8. Bidaud AL, Schwarz P, Herbreteau G, Dannaoui E. Techniques for the assessment of in vitro and in vivo antifungal combinations. Journal of Fungi. 2021 Feb 4;7(2):113.
9. Te Dorsthorst, D. T., Verweij, P. E., Meis, J. F., Punt, N. C., & Mouton, J. W. (2002). Comparison of fractional inhibitory concentration index with response surface modeling for characterization of in vitro interaction of antifungals against itraconazole-susceptible and-resistant Aspergillus fumigatus isolates. *Antimicrobial agents and chemotherapy*, *46*(3), 702-707.
10. Cho E, Acosta K, Henkin J, Abzalimov R, Raskin I. Synergistic antifungal effects of botanical extracts against Candida albicans. PloS one. 2026 Jan 12;21(1):e0340665.