Treatment of Endophytic Fungal Extracts in *Candida albicans* Infected Wounds with Collagen Regeneration

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Abstract

Background: Infected wounds, particularly those caused by *Candida albicans*, are difficult to treat due to the lack of effective antifungal agents with minimal toxicity. Wound healing involves several phases, including inflammation, proliferation, and remodeling, with collagen playing a crucial role in tissue repair. The use of natural compounds, such as endophytic fungal extracts rich in secondary metabolites like terpenoids, has shown promise in promoting wound healing and inhibiting microbial growth. This study aimed to evaluate the effects of endophytic fungal extracts on *Candida albicans*-infected wounds, focusing on collagen formation and tissue regeneration. Methods: Endophytic fungal extracts were applied at different concentrations (5%, 10%, 15%) to rat skin wounds infected with *Candida albicans*. Histopathological analysis was conducted at 7 and 14 days post-treatment to assess collagen density, fungal presence, and tissue regeneration. Collagen density scores were recorded, and the structure of collagen fibers was examined using computerized microscopy. Results: The negative control group exhibited normal collagen fibers and a collagen density score of 4. Infected tissues in the positive control group, without treatment, showed severe fungal infiltration and no collagen fibers, with a score of 0. At 7 days post-treatment, 5% extract showed partial collagen regeneration with a score of 1, while the 10% and 15% concentrations exhibited minimal tissue improvement. After 14 days, the 5% extract significantly improved collagen density to a score of 3, indicating better tissue regeneration. However, yeast cells were still present in the dermis, suggesting incomplete infection resolution. Conclusion: The study demonstrated that the 5% endophytic fungal extract effectively promoted collagen formation and tissue regeneration in *Candida albicans*-infected wounds. Higher concentrations did not significantly improve outcomes and may pose risks of tissue damage.

Keywords: Endophytic fungal extract, *Candida albicans*, collagen regeneration, wound healing, fungal infection

Significance | This study determines the potential wound healing therapies of endophytic fungal extracts with enhanced collagen formation and tissue regeneration in *Candida albicans*-infected wounds

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Introduction

The global challenge of antimicrobial resistance has significantly hampered the treatment of infectious diseases, with microorganisms increasingly developing resistance to conventional antimicrobial agents. This phenomenon has prompted researchers to explore alternative solutions, including the use of bioactive compounds derived from medicinal plants. These plants and their

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derivatives offer a promising avenue for combating fungal infections due to their accessibility, affordability, safety, and minimal adverse effects (Yudiana Shinta et al., 2024). Among these, *Dahlia variabilis* tubers, known for their therapeutic potential, harbor endophytic fungi capable of producing potent antimicrobial metabolites.

Endophytic fungi reside asymptomatically within plant tissues and are recognized as prolific producers of secondary metabolites with diverse biological activities, including antibacterial, antifungal, antiviral, anticancer, and antimalarial properties (Wali et al., 2019). These fungi establish a symbiotic relationship with their host plants, contributing to the production of bioactive compounds. In particular, the endophytic fungus *Aspergillus fumigatus*, isolated from *Dahlia variabilis*, has demonstrated significant antifungal activity against *Candida albicans* (Yudiana Shinta & Riau, 2019).

The *Dahlia variabilis* tubers used in this study were collected from Padang Panjang, a small city in the West Sumatra region, where they are traditionally grown for ornamental purposes. Previous in vitro research on these tubers highlighted their active antimicrobial properties, revealing the potential for their application in fungal infection therapy. This study further investigates the antifungal efficacy of *Aspergillus fumigatus* extracts, particularly focusing on their role in promoting collagen regeneration in wounds infected with *Candida albicans*.

Candida albicans is an opportunistic fungal pathogen that commonly resides as part of the natural flora on human skin and mucous membranes. However, under certain conditions, it can become invasive, leading to infections ranging from superficial mucosal conditions to systemic candidiasis (Lestari, n.d.). Individuals with compromised immune systems are particularly vulnerable to these infections. While antifungal therapies are typically employed to manage such conditions, the overuse and misuse of these drugs have led to the emergence of resistant fungal strains, posing a significant challenge to effective treatment (Wali et al., 2019).

Microorganisms, including endophytic fungi, have been a rich source of bioactive compounds, and the search for novel antifungal agents has intensified in response to growing drug resistance. *Aspergillus fumigatus* extracted from *Dahlia variabilis* has been identified as a promising candidate, exhibiting potent activity against *Candida albicans* infections.

Collagen plays a crucial role in wound healing, serving as a structural framework that supports tissue repair and regeneration. It facilitates hemostasis, promotes cellular adhesion, modulates exudation, and enhances fibroblast activity, all of which contribute to the restoration of skin integrity. Additionally, collagen supports epidermal proliferation, making it a vital component in the healing process (Betriksia et al., 2018).

This study aims to evaluate the antifungal activity of *Aspergillus fumigatus* extract in *Candida albicans*-infected wounds by analyzing its impact on collagen density and wound healing in a *Rattus norvegicus* model. Increased collagen density in the dermal layers serves as an indicator of the tissue's ability to resist infection and mitigate pathological conditions associated with fungal proliferation.

The primary objective of this study is to elucidate the potential of *Aspergillus fumigatus* extract from *Dahlia variabilis* as a treatment for fungal infections, particularly those caused by *Candida albicans*. By focusing on the regeneration of collagen in infected tissues, the research seeks to demonstrate the extract's therapeutic efficacy in promoting dermal healing and reducing fungal burden. Furthermore, the study addresses the gap in in vivo research, as most prior studies have concentrated on in vitro analyses of antifungal compounds.

This research contributes to the understanding of the physiological and pathological implications of *Aspergillus fumigatus* extracts in antifungal therapy. The findings are expected to pave the way for the development of antifungal formulations, such as topical emulsions, that leverage the bioactive properties of endophytic fungi to combat resistant fungal infections effectively.

Our study highlights the potential of endophytic fungi, specifically *Aspergillus fumigatus*, in advancing antifungal therapies. By investigating its role in collagen-mediated wound healing and infection control, this research aims to establish a scientific foundation for its clinical application in managing *Candida albicans*-associated infections.

2. Materials and Methods

2.1 Experimental animals

We have integrated both in vitro and in vivo assessments to evaluate the antifungal efficacy of endophytic fungal extract (Aspergillus fumigatus). The in vitro analysis focused on quantifying the antifungal properties of the extract, while the in vivo experiments involved creating skin excisions in an animal model, applying the fungal extract, and isolating dermal tissue from various treatment groups for further analysis. This study was approved by the Universitas Perintis Indonesia Ethical Committee in Health Research, ensuring compliance with ethical standards for the use of animals in research (Approval No. 587.1/KEPK.F1/ETIK/2024).

A total of 25 male white rats (*Rattus norvegicus*), aged 2–3 months and weighing between 100–200 grams, were used as experimental subjects. Prior to the study, the rats underwent a seven-day acclimatization period to adapt to their new environment. During this time, they were provided with a standardized diet and daily water replenishment.The rats were randomly assigned to five experimental groups, each representing a distinct treatment protocol. Group 1 (negative control) comprised four rats that

received dermal incisions without *Candida albicans* infection. Group 2 (positive control) consisted of four rats with dermal incisions infected with *Candida albicans*, but without any application of the endophytic extract. Group 3 included six rats with *Candida albicans*-infected wounds treated with a 5% concentration of the fungal extract. Similarly, Group 4 consisted of six rats treated with a 10% extract concentration, and Group 5 included six rats treated with a 15% extract concentration.

This design allowed for a systematic evaluation of the antifungal extract's effectiveness at varying concentrations, providing insights into its potential for combating *Candida albicans*-induced infections and promoting wound healing.

2.2 Preparation of Fungal Suspension

The fungal suspension was prepared by adding three loops of *Candida albicans* pure culture into a test tube containing 0.9% NaCl solution. The contents were homogenized using a vortex mixer. The fungal suspension was applied to create consistent infections across the experimental groups.

Endophytic fungal extracts at 5%, 10%, and 15% concentrations were prepared using the dilution formula:

$N1.V1 = N2.V2$

where N1N_1N1 and V1V_1V1 are the initial concentration and volume of the extract, and N2N_2N2 and V2V_2V2 are the desired concentration and volume.

2.3 Identification of Secondary Metabolites

The chemical composition of the fungal extract was analyzed using a Shimadzu GC-MS Ultra QP-2010 system. A 1 µL sample was injected, with helium gas as the mobile phase and an Rtx-5MS column as the stationary phase. Endophytic fungi were cultured in potato dextrose broth (PDB) supplemented with 100 mg/L amoxicillin. After three days of incubation at room temperature, the fungi were transferred to 250 mL PDB for 14 days under agitation at 150 rpm (Abed et al., 2023; Hasiani et al., 2015).

2.4 Induction of Wounds and Treatment

The rats were anesthetized with 10% ether. The lower back was shaved using a commercial depilatory cream (Veet) and sterilized with 70% alcohol. A standardized incision measuring 2 cm in length and 2 mm in depth was made on the back. A suspension of *Candida albicans* was applied to the wound to induce infection. Inflammation was observed within two days, at which point the fungal extract was applied to the wound in two drops twice daily for 14 days.

2.5 Isolation of Dermal Tissue

Tissue samples were collected at two intervals: 7 and 14 days post-treatment. Rats were anesthetized with 10% ether, and skin samples were excised using sterile scissors and tweezers.

The isolated tissues were fixed in 10% Neutral Buffered Formalin (NBF) and prepared for histopathological analysis.

2.6 Histopathological analysis

Histopathological preparations were carried out using a LEICA TP1020 automatic tissue processor, which facilitated the automated handling of tissue samples through various processing stages. The process began with the fixation phase, where samples were immersed in a 10% Neutral Buffer Formalin (NBF) solution for two hours to preserve tissue morphology. Following fixation, dehydration was performed sequentially with graded alcohol concentrations: 70% alcohol for 1.5 hours, 80% alcohol for 1.5 hours, 96% alcohol for 1.5 hours, absolute alcohol 1 for 1 hour, absolute alcohol 2 for 1.5 hours, and absolute alcohol 3 for 2 hours. The dehydrated tissues then underwent a clearing stage, where xylol solutions were applied to remove residual alcohol. This involved immersion in xylol 1 for 1 hour, xylol 2 for 1.5 hours, and xylol 3 for another 1.5 hours. After clearing, the tissues were soaked in liquid paraffin at 60°C, with two consecutive immersions lasting 1–1.5 hours and 2 hours, respectively. The paraffin was poured over the tissues in cassettes to embed them, and the blocks were left to solidify on a cold surface for approximately one hour.

During the staining phase, tissue sections were prepared and stained using hematoxylin and eosin to visualize histological structures. The sections were processed as follows: initial deparaffinization in xylol I, II, and III for 5 minutes each, followed by rehydration in 96% alcohol for 3 minutes, 80% alcohol for 3 minutes, and 70% alcohol for 3 minutes. The sections were rinsed in running tap water for 3 minutes before staining with Meyer hematoxylin for 5–7 minutes. After staining, the slides were rinsed again in tap water for 3 minutes, counterstained with eosin for 2–3 dips, and sequentially dehydrated using 70% ethanol, 80% ethanol, 96% ethanol, and absolute alcohol, each for 2–3 dips. The sections were cleared in xylol I and II for 2 minutes each, dried, and mounted.

The stained tissue slides were examined under a LEICA DM2000 microscope to analyze collagen density and assess histological images of collagen tissue. Additionally, yeast cell accumulation in the skin barrier of the experimental rats was evaluated and categorized using a semi-quantitative scoring system to measure collagen density.

2.7 Collagen Density Scoring

Collagen density was assessed semi-quantitatively using histological slides. Scores were assigned based on the relative density of collagen fibers observed microscopically, as shown in Table 1.

3. Results

The results demonstrate the differential effects of various concentrations of endophytic fungal extract on dermal collagen regeneration and fungal infection control. While higher concentrations showed some improvement in tissue regeneration, yeast cells remained evident in all treated groups, indicating that the

treatment concentration and duration require optimization for enhanced efficacy.

3.1 Secondary Metabolite Chemicals

The analysis of secondary metabolites, as depicted in Figure 1, revealed that the terpenoid group comprised the highest number of chemical compositions, while flavonoids, alkaloids, and saponins were present in lower concentrations. These metabolites, primarily utilized for defense mechanisms, are synthesized by both plants and bacteria. Secondary metabolites are not produced during the logarithmic phase of microbial growth but rather during the stationary phase, when cell population equilibrium is reached, and microbial cells exhibit increased resistance to environmental stresses, including radiation, extreme temperatures, chemicals, and self-produced metabolites (Abed et al., 2023).

In the in vitro assessment, two active compounds were identified within the extract. Terpenoids, representing the highest number of detected metabolites, were associated with four main chemical constituents: Octadecanoic acid, n-Hexadecanoid acid, Digitoxin, and Gibberellic acid. These results are summarized in Table 2.

3.2 Collagen Tissue Density Scores

The experimental groups displayed varying collagen density scores, as indicated in Table 2. The negative control group and the group treated with a 5% concentration of endophytic extract following *Candida albicans* infection showed similar collagen density scores. Conversely, the positive control group and other intervention groups recorded scores approximately one point lower, highlighting the impact of fungal infection and treatment concentration on collagen density.

3.3 Histopathological Features of Dermal Collagen Tissue

Histopathological analysis conducted at seven and 14 days post-treatment revealed notable differences in collagen structure and tissue conditions among the experimental groups (Figure 2). In the negative control group (A), normal collagen fibers with thick density were observed, resulting in a collagen density score of 4. Conversely, the positive control group (B), which consisted of infected skin without treatment, exhibited fungal infiltration penetrating the epidermis layer (indicated by the red arrow) and an accumulation of inflammatory lymphocytes (R) in the dermal layer. Collagen fibers were entirely absent in this group, leading to a collagen density score of 0.

In the group treated with a 5% concentration of endophytic fungal extract, different results were observed at varying time points. After seven days (C), treated skin showed epidermal and dermal tissue damage caused by fungal infiltration (red arrow), corresponding to a collagen density score of 1. By 14 days (E), some tissue regeneration was noted, and collagen density improved to a score of 3, although yeast fungal cells (blue arrow) were still present.

The group treated with a 10% concentration of fungal extract displayed partial improvement after seven days (D), with slight thickening of collagen tissue and a reduction in inflammation, although degenerative changes persisted, maintaining a collagen density score of 1. After 14 days (F), further improvements were observed in the epidermis and sub-dermis layers, yet yeast cells (blue arrow) were still present in the endodermis layer, and the collagen density score remained at 1.

For the group treated with a 15% concentration of fungal extract, histopathological images after seven days (G) revealed the presence of yeast cells (blue arrow) and fungal infiltration (red arrow) surrounding the collagen tissue and within the dermal layer, causing delayed collagen regeneration and a collagen density score of 1. By 14 days (H), some positive tissue regeneration was evident, as collagen density (CI) increased; however, yeast cells (blue arrow) persisted in the endodermis area, and the collagen density score remained unchanged at 1. These findings highlight the varying impacts of different concentrations of endophytic fungal extract on collagen tissue regeneration and fungal infection persistence.

4. Discussion

Infected wounds result from harmful microbes or pathogens penetrating the body and its tissues. These wounds can arise from various microorganisms, including bacteria, fungi, viruses, and parasites, and may develop in both open and closed wounds, with open wounds being more susceptible to infection. Pathogens infiltrate the dermal and cutaneous layers, leading to rapid colonization, particularly by bacteria or fungi (Patria et al., n.d.). The healing process of infected wounds typically follows three phases: inflammation, proliferation, and remodeling. The proliferation phase plays a central role in tissue repair, involving re-epithelialization, cellular migration, and fibroblast activity. Fibroblasts migrate to the wound site, proliferating and releasing essential substances such as collagen, which are critical for wound repair (Betriksia et al., 2018).

4.1 Role of Terpenes and Collagen in Wound Healing

Terpenes, particularly those with anti-inflammatory properties, have shown promise in medical research. According to Kim et al. (2020), terpenes influence a wide range of targets, including transcription factors and inflammatory mediators, making them desirable for developing anti-inflammatory medications. While the precise mechanisms of many terpenes remain unclear, they are effective when combined with existing treatments to combat inflammatory conditions.

Collagen is pivotal at every stage of wound healing. It facilitates hemostasis, platelet aggregation, fluid modulation, cellular interactions, growth factor induction, and fibroplasia. Collagen regeneration accelerates the formation of new tissue while removing damaged or necrotic tissue, supporting rapid tissue recovery. Furthermore, collagen maintains tissue integrity and structure, allowing consistent regeneration, even in adverse

Table 1. Collagen tissue density score.

Density score	Descriptions
0	No collagen fibers were discovered
1	Low collagen fiber density
2	Medium collagen fiber density
3	The density of collagen fibers was tight
4	The density of collagen fibers was very tight

Data are expressed in numeric scores to identify the histology of collagen tissue that appeared in the histopathological preparation from the rat's dermal tissue experimented.

Table 2. Secondary metabolite chemicals from the experimental fungus.

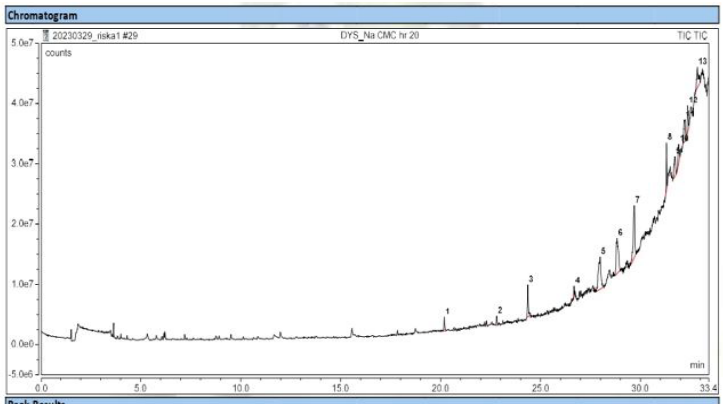
Chemical's composition	Chemicals group
Retinal	1 (Quercetin)
Octadecanoic acid	1 (Terpenoid)
n-Hexadecanoid acid	1 (Terpenoid)
Ricinoleic acid	0
9-Octadecenoic acid (Z)-, methyl ester	0
9-Hexadecen-1-Oi, (Z)-	0
Isopropyl palmitate	0
Hexanedioic acid, dioctyl ester	0
Digitoxin	5 (Terpenoid)
Beclomethasone	0
Gibberellic acid	6 (Terpenoid)
Hydrocortisone acetate	0

Data are expressed the main composition of the secondary metabolite extract contained. The chemical groups indicate the active ingredients analyzed from the extract.

Table 3. Collagen density scores of experimental animal dermal tissue

Assessment groups of animal model	Extract concentrations for interventions	Intervention days	Collagen density scores
Negative control	-	-	4
Positive control with *Candida albicans*-infected wounds	-	-	0
Candida albicans-infected wounds	5%	Seven days	1
	5%	Fourteen days	3
	10%	Seven days	1
	10%	Fourteen days	1
	15%	Seven days	1
	15%	Fourteen days	1

Data are expressed from the 5 groups of research interventions or assessments entangle 25 rats with different length of intervention days (7 to 14 days limitation).



Peak Results

No.	Peak Name	Retention Time min	Area counts*min	Height counts	MS Quant m/z	Rel.Area %	Rel.Height %
1	Retinal	20.192	98077.922	2283401.101	137.066	2.22	4.05
2	Gibberellic Acid	22.808	56787.048	1531325.019	109.022	1.29	2.72
3	Octadecanoic acid	24.366	234946.360	5321498.186	129.053	5.32	9.44
4	Ricinoleic acid	26.682	65970.420	1849487.169	55.060	1.49	3.28
5	Hydrocortisone Acetate	27.981	816367.789	5354848.114	81.045	18.50	9.50
6	Hydrocortisone Acetate	28.831	737103.140	5864787.213	124.951	16.70	10.40
7	Cholesteryl benzoate	29.688	798669.593	8637171.025	368.240	18.10	15.32
8	Beclomethasone	31.304	234244.644	7706329.841	148.955	5.31	13.67
9	Beclomethasone	31.715	268552.181	3309653.802	206.958	6.08	5.87
10	Beclomethasone	31.913	236399.334	3136267.490	206.967	5.36	5.56
11	Beclomethasone	32.229	293117.230	3549891.764	206.941	6.64	6.30
12	Gamabufotalin	32.368	235360.589	4427409.013	206.965	5.33	7.85
13	Beclomethasone	32.848	337919.356	3416908.851	206.920	7.66	6.06

Figure 1. The graph indicates the GC-MS performed the extract highest compound group. The endophytic fungus' metabolite, 1 loop, was cultivated with 5 mL of PDB and 100 mg/L of amoxicillin. The pure fungus was cultured for three days at room temperature.

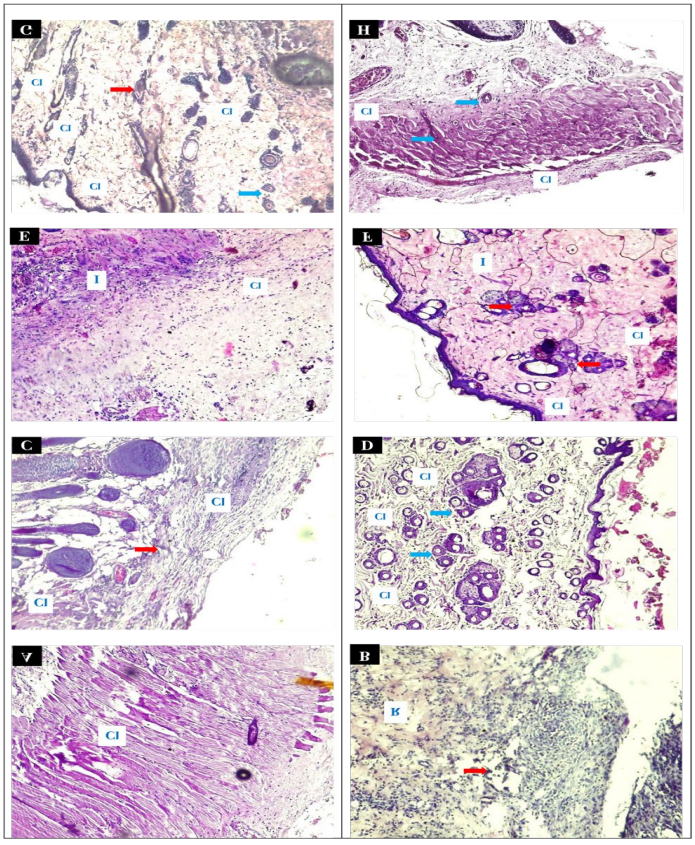


Figure 2. The feature of rat's dermal and cutaneous histopathological tissue of negative control group (A), positive control group (B), 5% extract with seven days intervention group (C), 5% extract with 14 days intervention group (D), 10% extract with seven days intervention group (E), 10% extract with 14 days intervention group (F), 15% extract with seven days intervention group (G), 15% extract with 14 days intervention group (H); H&E staining with 40x magnification.

Data are expressed from the LEICA DM2000 computerized microscope with 40x magnification for each histopathology preparates. Cl indicates the collagen layer histology, while I and R indicate the inflammation area and the inflammatory cells (leucocyte) manifestation respectively.

conditions such as chemical exposure, radiation, or trauma (Prastika et al., 2020).

4.2 Collagen Density and Fungal Extract Intervention

The study's findings underscore the critical role of endophytic fungal extracts in collagen density restoration. In the negative control group, where no infection or treatment was applied, collagen fibers appeared dense and uniformly distributed, resulting in the highest density score of 4. In contrast, the positive control group, infected with *Candida albicans* without treatment, exhibited severe tissue damage. Fungal infiltration into the epidermal and dermal layers, combined with inflammatory cell accumulation, eliminated collagen fibers entirely, yielding a score of 0.

Treatment with a 5% endophytic fungal extract showed promising results. By day 7, the extract mitigated some tissue damage, yielding a collagen density score of 1. By day 14, notable regeneration was evident, and collagen density improved to a score of 3, although residual yeast cells persisted (Table 3). The 10% extract concentration resulted in marginal improvements, with a consistent score of 1 on both days 7 and 14. Similarly, the 15% extract concentration showed limited efficacy, with minimal collagen regeneration and persistent fungal infiltration.

4.3 Supporting Literature on Antifungal and Anti-Inflammatory Effects

The findings align with earlier research on the therapeutic potential of plant and fungal extracts. Kang et al. (2023) highlighted the anti-inflammatory and antimicrobial properties of *Rhododendron brachycarpum* leaf extract, attributing its efficacy to flavonoids. These bioactive compounds enhance tissue recovery after infection and mitigate inflammation, suggesting an interrelation with the efficacy of fungal extracts.

Bhat et al. (2020) demonstrated the antifungal properties of ketoconazole and fluconazole when combined with tissue conditioners, significantly inhibiting *Candida albicans*. However, the observed effect depended on the interaction between the antifungal agents and tissue conditioners, emphasizing the need for optimized formulations. These insights align with the current study, where endophytic fungal extracts initially inhibited fungal growth and supported tissue regeneration. However, the residual presence of yeast cells by day 14 suggests that extended treatment durations or enhanced formulations may be necessary for complete recovery.

4.4 Insights on Terpenes and Medicinal Applications

Terpenes and terpenoids from fungal sources have garnered attention for their potential in treating dermal ailments. According to Trepa et al. (2024), terpenes exhibit diverse therapeutic properties, including anti-inflammatory, antimicrobial, and antioxidant effects. Some isolated terpenes outperform conventional therapies in managing inflammatory and infectious conditions. Notably, terpenoid compounds also show promise in addressing pigmentation disorders and skin cancer prevention. However, potential side effects associated with terpenes, often dose-dependent, warrant cautious application (Kim et al., 2020; Trepa et al., 2024).

The current study's findings support the regenerative capabilities of terpenoid-rich extracts, which enhanced collagen density and mitigated inflammation in *Candida albicans*-infected wounds. However, the higher concentrations (10% and 15%) yielded limited benefits, potentially due to cytotoxic effects or delayed tissue repair mechanisms.

4.5 Mechanisms of Secondary Metabolites in Tissue Repair

Secondary metabolites such as alkaloids, flavonoids, saponins, and tannins, found in fungal extracts, contribute to their medicinal properties. These compounds enhance membrane permeability, causing microbial cell lysis and promoting tissue regeneration (Ahriyasna & Primal, 2023; Bhat et al., 2020). Flavonoids, in particular, have been shown to facilitate tissue repair through specific proliferation processes, improving cellular adaptation and structural integrity. In diabetic animal models, flavonoids stimulated macrophage activity, fibroblast proliferation, and collagen synthesis, further validating their therapeutic potential (Ahriyasna & Primal, 2023; Aprilliani et al., 2021).

4.6 Innovative Antifungal Approaches

Recent advancements in antifungal therapies highlight the potential of nanotechnology. Zhou et al. (2021) developed Iturin-AgNPs, a nanocomposite with potent antifungal activity against *Candida albicans*. This composite enhanced membrane permeability, disrupted cellular integrity, and accelerated wound healing in infected skin. Similar to the current study, the nanocomposite demonstrated efficacy in promoting tissue recovery without significant toxicity. These findings underscore the potential for integrating fungal extracts with nanotechnology to optimize therapeutic outcomes.

Optimized Concentrations for Therapeutic Efficacy

The results of this study suggest that a 5% concentration of endophytic fungal extract is optimal for managing *Candida albicans*-infected wounds. Higher concentrations, while containing more active ingredients, may induce adverse effects, such as cytotoxicity or delayed wound healing, thereby compromising therapeutic efficacy. The 5% extract facilitated collagen formation and tissue regeneration without evident side effects, making it suitable for clinical applications.

4.7 Future Directions

The study highlights the need for prolonged treatment durations and advanced formulations to enhance the efficacy of fungal extracts. Further research should explore the molecular mechanisms underlying the regenerative effects of secondary metabolites and investigate their potential synergistic interactions with other therapeutic agents. Additionally, studies on optimizing

extract concentrations and delivery methods, such as nanocarriers or hydrogel systems, could provide valuable insights for improving treatment outcomes.

5. Conclusion

This study demonstrates the therapeutic potential of endophytic fungal extracts, particularly at a 5% concentration, for promoting collagen regeneration and mitigating inflammation in *Candida albicans*-infected wounds. The findings align with existing literature on the medicinal properties of secondary metabolites and terpenes, emphasizing their role in wound healing. However, the persistence of yeast cells at higher extract concentrations and limited tissue recovery underscore the importance of optimized formulations and treatment durations. Future research should focus on leveraging advanced technologies and exploring synergistic therapies to maximize the therapeutic benefits of fungal extracts. By integrating insights from secondary metabolite research and novel antifungal strategies, this study contributes to the growing body of evidence supporting the use of natural compounds in wound management and tissue regeneration. These findings suggest that low-concentration endophytic fungal extracts hold potential as an adjunctive treatment for fungal infections and wound healing. Further investigation into the long-term effects and underlying mechanisms is warranted.

Author contributions

D.Y.S. conceptualized the study, performed the initial literature review, and drafted the manuscript. W.W. contributed to data collection, analysis, and interpretation. D.P. supervised the project, provided critical revisions, and approved the final manuscript. H.S.M.S. assisted in data analysis and contributed to methodological design. S.S. provided guidance on statistical approaches and contributed to manuscript editing. All authors read and approved the final manuscript.

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Competing financial interests

The authors have no conflict of interest.

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