In Silico and Experimental Evaluation of Beta _glucan Effect on *SAP5* Gene Expression in *Candida albicans*

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Abstract: *Candida albicans* is a major opportunistic fungal pathogen, and its secreted *SAP* family genes contribute significantly to virulence, tissue invasion, and immune evasion. Among these, *SAP5* is rapidly induced during infection and is closely associated with pathogenicity. Resistance to traditional antifungals such as azoles and polyenes is increasing, highlighting the need for alternative therapeutic agents. β-glucan polysaccharides, present in fungal and plant cell walls, exhibit immunomodulatory and antimicrobial properties; however, their effects on *C. albicans* virulence gene expression remain insufficiently investigated. In this study, 100 breast mastitis swabs were collected from female patients, yielding 25 Candida isolates, including 13 identified as *C. albicans*. Antifungal susceptibility was tested against nystatin, fluconazole, itraconazole, and clotrimazole. β-glucan was extracted from oats, purified, and applied at serial concentrations to determine the minimum inhibitory concentration (MIC). Polymerase chain reaction (PCR) was used to confirm the presence of the *SAP5* gene, and quantitative real-time PCR (qPCR) was performed to assess *SAP5* gene expression following treatment with β-glucan. Among the 13 *C. albicans* isolates, the highest resistance rate was to nystatin (61.5%), followed by fluconazole (53.8%), itraconazole (46.1%), and the lowest to clotrimazole (38.4%). Both fully resistant and fully susceptible isolates accounted for 30.8% of the total, while the remainder exhibited intermediate resistance. Oat β-glucan demonstrated a dose-dependent inhibitory effect on *C. albicans* growth, with MIC values varying among isolates. qPCR analysis revealed a significant 2.8 ± 0.4-fold decrease in *SAP5* gene expression at 400 µg/ml β-glucan ($p < 0.001$, Student's t-test). Oat-derived β-glucan exhibits antifungal activity against *C. albicans* and suppresses *SAP5* expression, suggesting both direct inhibition and modulation of virulence. These results support its potential as a complementary antifungal agent amid increasing drug resistance.

1 INTRODUCTION

Candida albicans is a common commensal fungus in the human microbiome (oral cavity, gastrointestinal tract, and vaginal mucosa), but it can become pathogenic under conditions of immunodeficiency or microbiome disturbance [1], [2]. It can cause superficial infections (e.g., candidiasis) and invasive candidiasis, depending on the body's immunity and fungal virulence factors. A less studied clinical context is mastitis in women, where Candida species may contribute to persistent or recurrent breast infections, particularly in lactating women. The involvement of *Candida albicans* in mastitis has not been as widely documented as that of bacterial causes, but fungal etiologies deserve attention, especially when conventional antibacterial therapies fail [3].

The virulence factors of *Candida albicans* are multiple: adhesions, (dimorphism phenomenon), biofilm formation, secreted enzymes (proteases and phospholipases), and evasion of host immunity are all essential components [4]. Among the secreted enzymes, aspartic proteases (Saps) play a crucial role in tissue invasion, host cell damage, immune modulation, and nutrient acquisition [5], [6].

The *SAP* gene family in *Candida albicans* consists of at least ten homologous genes (*SAP1-SAP10*), each with distinct patterns of regulation and expression that depend on growth conditions, environmental factors, and location [7], [8]. These proteases degrade host proteins (e.g., extracellular

matrix, immunoglobulins), facilitating tissue penetration and evasion of host defenses [5].

Among these genes, *SAP5* is of particular interest. It is among the most rapidly induced *SAP* genes during infection and can strongly contribute to pathogenic phenotypes in vivo [6], [4]. In murine and in vivo studies, *SAP5* is among the most highly expressed genes during the progression of infection [4]. Furthermore, *SAP5* regulation is linked to transcription factors (such as Efg1 and Cph1) that mediate dimorphism phenomenon and virulence pathways [9]. Its early induction suggests that inhibiting *SAP5* may impair the pathogenicity of *C. albicans*. Indeed, the dynamic regulation of *SAP5* makes it a useful molecular target for monitoring virulence modulation [4].

In biofilm models, *Candida albicans* isolates often exhibit elevated expression of *SAP5* compared to planktonic conditions, suggesting that *SAP5* contributes to biofilm virulence [5]. Thus, assessing *SAP5* expression under antifungal treatment enables a deeper understanding not only of fungal killing but also of modulating biofilm virulence at the genetic level.

In clinical practice, the increasing resistance of *Candida albicans* to commonly used antifungals is a growing concern. Azoles (Fluconazole, Itraconazole) are widely used, but prolonged or repeated use can lead to the emergence of resistant strains via efflux pumps, target enzyme mutations, or biofilm-associated resistance. Polyene agents (e.g., Nystatin) are used topically and may also show reduced efficacy in resistant isolates [10]. Therefore, regular monitoring of antifungal susceptibility is critical for clinical and drug management. Furthermore, molecular studies of resistance (gene overexpression, target gene mutations, stress response pathways) can help uncover mechanisms of antifungal failure [11], [12].

Given the variable susceptibility of *Candida albicans* isolates, the combination of antifungal agents with adjuvant therapeutic molecules (such as immunomodulators and natural extracts) is a promising research direction [13].

Beta-glucans are polysaccharides composed of β-(1→3) and β-(1→6)-linked glucose residues and are found in the cell walls of fungi, cereals (oats and barley), and some bacteria [14], [15]. They are recognized by innate immune receptors (e.g., dectin-1), stimulating immune responses, including phagocytosis, cytokine release, and "trained immunity" [16], [17]. Their immunomodulatory properties have been explored in models of cancer, metabolic diseases, and infectious diseases [18], [19].

In addition to immune activation, some beta-glucans have shown direct or indirect antimicrobial effects. Some studies have reported that beta-glucans can enhance phagocytic function or synergize with antimicrobial agents [20], [21]. In agricultural or veterinary settings, oat beta-glucans have been used to enhance resistance to bacterial infections and protozoan parasites [22]. However, the direct antifungal effect of plant-derived beta-glucans (particularly from oats) on *Candida albicans*, and their impact on virulence gene expression, remains poorly explored.

Oat beta-glucan is a soluble fiber with satisfactory physical and chemical properties and is completely safe for humans; it has been used in human studies (2–6 g/day) without significant side effects [15]. As a food-derived molecule, its translational potential is promising.

Despite the extensive research on the immunomodulatory and pathogenic roles of beta-glucans, the following gaps remain:

1) Few studies examine plant-derived β-glucan (e.g. oat β-glucan) for direct antifungal effects against *C. albicans.*
2) Even fewer investigate how such β-glucans influence virulence gene expression (particularly *SAP5*) in *C. albicans.*
3) The interplay between antifungal resistance phenotypes and modulation of virulence genes under novel treatments is understudied in clinical isolates from human infections such as mastitis [23], [24]. This study hypothesized that oat-derived β-glucan can both inhibit the growth of *Candida albicans* and reduce its virulence by lowering *SAP5* gene expression.

Therefore, this research aims to investigate:

- The antifungal susceptibility profiles of *C. albicans* isolates (from human breast samples).
- The inhibitory effect of oat β-glucan on *C. albicans* growth.
- The presence of the *SAP5* gene in isolates, and
- The effect of oat β-glucan treatment on *SAP5* gene expression (via qPCR).

By doing so, this study seeks toexplore the therapeutic potential of oat beta-glucan as an additional antifungal agent that not only inhibits growth but may also reduce virulence.

2 MATERIALS AND METHODS

2.1 Sample Collection and Isolation

One hundred breast swab samples were collected aseptically from women clinically diagnosed with mastitis attending outpatient clinics in Al-Alam District, Salah al-Din Governorate, between September 10, 2024, and April 25, 2025. Each sample was taken with a sterile swab of inflamed breast tissue or nipple discharge, placed in sterile transport media, and transported within two hours to the microbiology laboratory under cold chain conditions. All processing was performed in a biosafety cabinet to prevent cross-contamination.

2.2 Isolation and Identification of *Candida albicans* Isolates

Samples were streaked onto Sabouraud dextrose agar (SDA; HiMedia, India) containing chloramphenicol (50 µg/ml) and incubated at 37°C for 48 h. Colonies exhibiting typical yeast morphology were examined via KOH direct examination, Germ tube formation in human serum at 37°C for 2–3 h was used as a presumptive identification test for *C. albicans*. Confirmation was performed using CHROMagar™ Candida (CHROMagar, France), as *C. albicans* colonies develop a characteristic green pigmentation [1].

Biochemical identification was performed with the VITEK® 2 Compact System (bioMérieux, France) using Yeast Identification Cards (YST) [2]. Of the 25 recovered Candida isolates, final identification was done using *ITS1, ITS4* genes detection via PCR, 13 were confirmed as *C. albicans* and retained for further testing.

2.3 Susceptibility to Antifungal Agents

Candida albicans isolates were tested for antifungal susceptibility using the disk diffusion method, which followed CLSI M44 recommendations. Thirteen isolates were evaluated against Fluconazole (25 µg), Itraconazole (30 µg), Nystatin (100 U), and Clotrimazole (10 µg). Mueller-Hinton agar was treated with 2% glucose and 0.5 µg/ml chloramphenicol to prevent bacterial growth and promote pure fungal culture.

Inoculum were produced from 24-48 hour-old colonies on Sabouraud Dextrose Agar, suspended in sterile saline, and adjusted to 0.5 McFarland (about 1-5 × 10^6 CFU/ml). Plates were equally inoculated, antifungal disks were applied and incubated at 35 ± 2°C for 24–48 hours. Inhibition zones were measured in millimeters and interpreted using CLSI M44-A2 criteria. Quality control was achieved using Candida parapsilosis ATCC 22019 and Candida krusei ATCC 6258 [25].

2.4 Extraction and Purification of β-Glucan from Oats

Oat β-glucan was extracted using the alkaline extraction method described by Bhatty [26], with slight modifications to optimize solubilization and minimize co-extraction of starch and proteins. Finely milled oat flour (particle size approximately 0.5 mm) was used as the raw material. The extraction solvent consisted of sodium hydroxide (NaOH) solution at a concentration between 0.1 M and 1 M, maintaining a solid-to-solvent ratio of about 1:10 to 1:20 (w/v) to ensure efficient solubilization. The suspension was stirred continuously or gently shaken for 1–2 hours at a temperature between 20°C and 40°C, while maintaining the pH within the range of 9–12 to maximize β-glucan solubility.

The alkaline treatment disrupted the oat cell wall matrix, allowing the β-glucan to dissolve while leaving behind insoluble starch, proteins, and fibrous materials. Following extraction, the suspension was centrifuged at approximately 10,000 × g for 15–30 minutes to separate the soluble and insoluble fractions, after which the supernatant containing the soluble β-glucan was filtered to remove particulate residues. The filtrate was neutralized to pH 7 using dilute hydrochloric acid or acetic acid, and β-glucan was precipitated by adding two to three volumes of ethanol or isopropanol. The mixture was then stored at 4°C for several hours or overnight to ensure complete precipitation.

The resulting precipitate was collected by centrifugation or filtration, washed with 70% ethanol to remove residual salts, and subsequently dried either by air drying or lyophilization. For samples with high fat content, an optional defatting step using ethanol or hexane was performed to enhance the purity of the extracted β-glucan. Finally, the molecular weight distribution of the purified β-glucan was determined by gel permeation chromatography (GPC).

2.5 Beta Glucan Effectivity Test

The antifungal activity of beta-glucan was evaluated using the broth microdilution method in sterile 96-well plates according to CLSI M27-A3

standard [27]. Serial two-fold dilutions of beta-glucan were prepared in Sabouraud dextrose broth to achieve final concentrations of 25–800 µg/ml. *Candida albicans* inoculums (1–5 × 10^5 CFU/ml) were added and incubated at 35°C for 48 h.

The minimum inhibitory concentration (MIC) was recorded as the lowest concentration showing no significant growth. Experiments were performed in triplicate using growth and sterility controls.

2.6 Molecular Methods

2.6.1 DNA Extraction from Candida Albicans

Genomic DNA was extracted from 24-h cultures using the GeneAid Genomic DNA Mini Kit (Taiwan), following the yeast protocol. Cells (1 ml) were pelletized (10,000 rpm, 5 min) and resuspended in 200 µl lysis buffer with glass beads. After vigorous mixing and incubation at 60°C for 10 min, proteinase K and RNase A were added sequentially, followed by ethanol precipitation. DNA was extracted in 50 µl lysis buffer and quantified using a NanoDrop 2000 at 260/280 nm [28].

2.6.2 Polymerase Chain Reaction (PCR) Techniques

Molecular confirmation of *Candida albicans* isolates was performed using universal fungal primers targeting the internal transcribed spacer (*ITS*) region of ribosomal DNA, as described by White *et al*. [29]. The primers used are:

- *ITS1*: 5′-TCC GTA GGT GAA CCT GCG G-3′
- *ITS4*: 5′-TCC TCC GCT TAT TGA TAT GC-3′

The *SAP5* gene was amplified using primers described by Naglik et al. [6]:

- Forward 5′-AGA ATT TCC CGT CGA TGA GAC TGGT-3′
- Reverse 5′-CAA ATT TTG GGA AGT GCG GGA AGA-3′

Polymerase chain reaction (PCR) was performed in a 25 µL reaction containing 12.5 µL of Promega GutAc® Green Master Mix, 1 µL of each primer (10 pmol), 5 µL of DNA template, and 5.5 µL of nuclease-free water. Cycle conditions: initial denaturation at 95°C for 5 min; 35 cycles of 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s; final extension at 72°C for 5 min. Amplicons were resolved on a 2% agarose gel as described below.

2.6.3 Gene Expression Analysis (qPCR)

Quantitative real-time polymerase chain reaction (qPCR) was performed on a micPCR system version 2.10.0 (Bio Molecular Systems, Australia) to assess SAP5 expression in β-glucan-treated isolates. Total RNA was isolated using the GeneAid Yeast RNA Mini Kit, and cDNA was synthesized using Promega reverse transcriptase reagents.

qPCR reactions used SYBR® Green Master Mix (Promega) with 18S rRNA as the primer gene [30]. Reaction volumes were 20 µL; cycles: 95°C for 2 min → 40 cycles (95°C for 15 s, 60°C for 60 s); melting curve 72–95°C (0.3°C/s). The $2^{-}\Delta\Delta Cq$ method [31] was used to calculate fold change differences.

2.6.4 Preparation of 2 % Agarose Gel

Two grams of agarose (Promega) was dissolved in 100 ml of 1× TAE solution, heated until clear, cooled to approximately 60°C, and stained with ethidium bromide (0.5 µg/ml). The gel was poured into a 12-well comb-type casting tray and allowed to solidify at room temperature [28].

2.6.5 Loading of Samples and Visualization of PCR Products

Five microliters of PCR product was mixed with loading dye and electrophoresed alongside a 100-base-pair DNA ladder at 100 V for 45 min. DNA bands were imaged using a Bio-Rad gel documentation system under ultraviolet illumination. The presence of a band approximately 277 base pairs in length indicated positive amplification of the *SAP5* gene [6].

2.7 Ethical Approval and Patient Confidentiality

This study was approved by [the education hospital in Salah al-Din] under approval number [2952]. All participants provided written informed consent prior to participation.

3 RESULTS

3.1 Isolation and Identification of Candida albicans

Of 100 breast swab samples collected from women clinically diagnosed with mastitis, 25 (25%) showed

yeast growth. Initial diagnosis was confirmed by colony morphology, Gram staining, and germ tube formation as Candida species. On CHROMagar™ Candida medium, *Candida albicans* colonies displayed a characteristic green color, while non-albicans appeared pink to purple.

Further biochemical diagnosis using the VITEK® 2 Compact System (Bio-Mérieux, France) confirmed the presence of Candida albicans in 13 samples (52%). These samples were used for antifungal susceptibility testing and molecular analysis following β-glucan treatment.

3.2 Antifungal Susceptibility Profiles

Antifungal susceptibility testing of 13 *Candida albicans* samples against four agents (nystatin, fluconazole, itraconazole, and clotrimazole) showed varying levels of resistance as shown in Table 1.

Table 1: Resistance pattern of *Candida albicans* isolates (n = 13) to antifungal agents.

Antifungal agent	Resistant (%)	Intermediate (%)	Sensitive (%)
Nystatin	61.5	7.7	30.8
Fluconazole	53.8	15.4	30.8
Itraconazole	46.1	23.1	30.8
Clotrimazole	38.4	30.8	30.8

Resistance was most pronounced to nystatin (61.5%), followed by fluconazole (53.8%) and itraconazole (46.1%), while clotrimazole (38.4%) showed the least resistance. Approximately 30.8% of isolates showed resistance to all four antifungals, while another 30.8% maintained full sensitivity. This pattern reflects an increasing trend towards azole and polyene resistance among clinical *C. albicans* isolates [10].

3.3 Effect of β-Glucan on Candida albicans Growth

Oat beta-glucan demonstrated a clear concentration-dependent inhibitory effect against *Candida albicans* isolates in a broth microdilution assay. Visual inspection of the wells showed a progressive decrease in turbidity with increasing beta-glucan concentrations, reflecting inhibition of fungal growth. Complete inhibition was observed at 400 µg/ml, which represents the minimum inhibitory concentration (MIC) for most isolates. Partial inhibition occurred at 200 µg/ml, while concentrations of 100 µg/ml or lower had no significant effect on growth compared to the control group.

These results confirm that oat beta-glucan exerts dose-dependent antifungal activity, consistent with its proposed cell wall modification and anti-virulence mechanisms.

3.4 Molecular Identification of Candida Albicans (ITS Region)

Polymerase chain reaction (PCR) amplification using primers *ITS1* and *ITS4* successfully amplified the internal transcribed spacer (*ITS*) region in all isolates. The isolates that didn't show the specific band where ruled out and the remaining were 13 *Candida albicans* isolates. Each produced a single, distinct band at approximately 500 base pairs, confirming their molecular identity as *Candida albicans* isolates as shown in Figure 1.

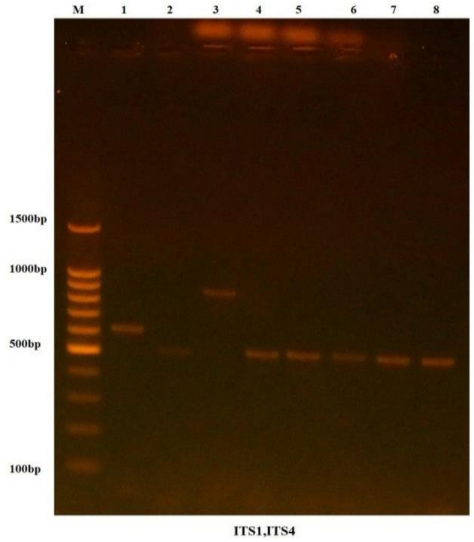

Figure 1: Agarose gel electrophoresis showing *ITS* amplicons (approximately 500 base pairs) in *C. albicans* isolates. M = 100 base pair DNA ladder; lanes 1,3 = negative for *candida albicans*; lanes 2,4,5,6,7,8 = *C. albicans* isolates.

3.5 Detection of the SAP5 Gene by Conventional PCR

All thirteen *Candida albicans* isolates treated with beta-glucan were screened for the *SAP5* virulence gene using specific primers. A single 277-base-pair band was detected in only four isolates (30.7%), while nine isolates showed no amplification signal.

The PCR products were analyzed on 2% ethidium bromide-stained agarose gel. The gel electrophoresis

image as shown in Figure 2 shows the presence of *SAP5*-positive bands among the tested isolates.

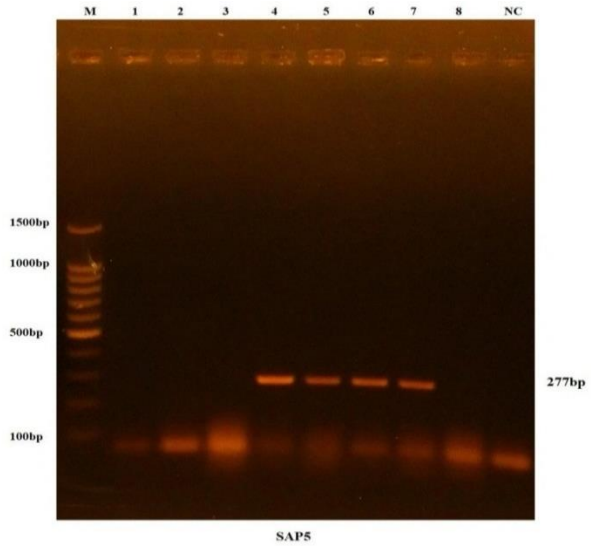

Figure 2: Agarose gel electrophoresis showing *SAP5* amplicons (277 base pairs) in β-glucan-treated *Candida albicans* isolates. M = 100 base pairs of DNA; lanes 4,5,6,7 = positive isolates; lanes 1,2,3,8 = negative isolates; NC = no-template control.

3.6 SAP5 Gene Expression Analysis (qPCR)

Quantitative real-time polymerase chain reaction (qPCR) was performed to assess *SAP5* gene expression in β-glucan-treated isolates using 18S rRNA as a reference gene. Melting curve analysis showed a single sharp peak at ≈80.9 °C, confirming specific amplification, and a slight non-specific signal (~78.2 °C) in the negative controls. The expression of *SAP5* decreased noticeably as the concentration of β-glucan increased as shown in Table 2.

Table 2: Mean fold change ($2^{-}\Delta\Delta Cq \pm SD$) of *SAP5* expression in response to increasing concentrations of oat β-glucan.

Treatment	Mean Fold Change ($2^{-}\Delta\Delta Cq$) ± SD	Significance (p)
Control	1.00 ± 0.00	—
β-glucan 100 µg/ml	1.6 ± 0.3	< 0.05
β-glucan 200 µg/ml	2.1 ± 0.4	< 0.05
β-glucan 400 µg/ml	2.8 ± 0.4	< 0.01

Amplification plots showed consistent exponential curves without primer dimer formation. *SAP5* Cq values ranged from 31.2 to 34.7 in all replicates as shown in Figure 3. Relative quantification using the $2^{-}\Delta\Delta Cq$ method indicated an average decrease in *SAP5* gene expression of ≈2.8-fold compared to untreated baseline assays.

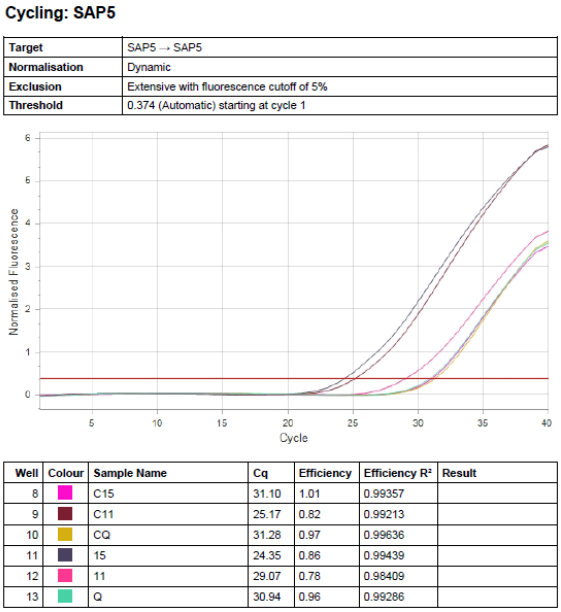

Figure 3: Relative expression of the *SAP5* gene in β-glucan-treated *Candida albicans* isolates standardized for 18S rRNA using the $2^{-}\Delta\Delta Cq$ method.

4 DISCUSSION

This study evaluated the antifungal activity of oat beta-glucan against *Candida albicans* isolates recovered from mastitis cases in women and analyzed its effect on the expression of the virulence-associated gene *SAP5*. By combining apparent resistance testing with molecular confirmation and gene expression analysis, while measuring fungal growth or viability inhibition indicates the lethal or suppressive effect of an agent, measuring changes in virulence gene expression via quantitative PCR provides a deeper understanding of the mechanism. If an agent reduces the expression of the *SAP5* gene, this indicates not only a fungicidal or inhibitory effect, but also a modification of the pathogen's ability to cause damage or evade immunity. In other words, a dual benefit can be demonstrated: killing the fungus while weakening it. Therefore, in this study, after confirming antifungal susceptibility and growth inhibition by oat beta-glucan, the current study

measured the expression levels of the *SAP5* gene in treated versus untreated Candida. spp [32], [33].

Candida albicans remains an opportunistic yeast capable of colonizing mucosal and epithelial surfaces and causing superficial or invasive infections. In lactational mastitis, its role is often overlooked because its symptoms often mimic bacterial infection, and fungal influence may persist despite antibiotic treatment [34], [35]. Studies have documented *Candida albicans* as a causative agent of chronic and recurrent mastitis, particularly among lactating women receiving broad-spectrum antibiotics [34], [35]. The 25% isolation rate obtained in this study is consistent with previous observations in similar patient cohorts, where breast swabs- cultures have yielded *C. albicans* in roughly a comparable proportion [3].

The current isolates showed high resistance to nystatin and fluconazole. This finding is consistent with reports that the extensive empirical use of azoles and polyenes contributes to the selection of resistant strains of *Candida albicans* [2], [10]. Fluconazole resistance is often attributed to overexpression of efflux pumps (CDR1 and MDR1), mutations in the ERG11 gene, and increased biofilm formation [36]. Similar resistance trends have been described in Iraqi clinical isolates and throughout the Middle East, where antifungal susceptibility testing is rarely performed before treatment [37]. The relatively low resistance to itraconazole and clotrimazole suggests that these agents retain some efficacy but emphasizes the need for continued monitoring.

Beta-glucans are polysaccharides with immunomodulatory and antimicrobial properties [21]. The present results demonstrate that oat-derived beta-glucan inhibits the growth of *Candida albicans* in a concentration-dependent manner, with maximum inhibition at 400 µg/ml. similar activity of cereal- and yeast-derived beta-glucans has been reported against *Candida tropicalis* and *Aspergillus niger*, likely through disruption of cell wall integrity or interference with ergosterol synthesis [38], [39].

In addition, beta-glucans enhance host immunity by activating macrophages via dectin-1 and Toll-like receptor signaling [16], [40]. Thus, beta-glucans may provide a dual mechanism: direct fungal inhibition and stimulation of host immunity, supporting their potential use as an antifungal adjuvant.

All isolates were molecularly confirmed as white fungi by PCR amplification of the internal transcribed spacer (*ITS*) region using primers *ITS1*/*ITS4*, producing a single 500-base-pair fragment. The *ITS* region is the most reliable genetic marker for fungal identification due to its interspecies variability and robust
amplification [30]. This confirmation validated the phenotypic identification and ensured that subsequent *SAP5* analysis targeted the correct species.

Only four out of thirteen isolates (30.7%) carried the *SAP5* gene, indicating a variable distribution of this virulence factor among clinical strains. The *SAP* family consists of at least ten isoenzymes (SAP1-SAP10) whose expression is tightly regulated by environmental and morphological cues [7]. *SAP5* is particularly associated with fungal hyphae formation and tissue invasion [5]. The limited detection of *SAP5* in this study may reflect a strain-specific adaptation to the breast environment or an inhibition of *SAP5* transcription under beta-glucan exposure. Similar variability was reported by Naglic et al., who observed a difference in *SAP5* expression between oral and vaginal candidiasis isolates [6].

Real-time quantitative PCR showed an average 2.8-fold decrease in *SAP5* gene expression after treatment with β-glucan. This decrease suggests that β-glucan not only inhibits fungal proliferation but also mitigates its virulence by modulating gene expression.

Exposure to antifungal or stress-inducing agents is known to alter SAP gene transcription through cAMP-PKA and MAPK signaling cascades [41], [42]. Current data suggest that β-glucan may interfere with these pathways or cause structural stress to the fungal cell wall, leading to the inhibition of protease-encoding genes.

Previous studies [43] have indicated that some natural compounds and polysaccharides may interfere with the growth of *Candida albicans* biofilms by modulating virulence pathways, including those related to the expression of the SAP gene. The melting curve pattern at ≈ 80.9 °C in the current study confirmed the accuracy of gene expression amplification and measurements. The decrease in *SAP5* expression matched the observed reduction in virulence, consistent with *SAP5*'s known role in tissue invasion, and biofilm formation. A strong negative correlation ($r = -0.87$) was found between *SAP5* levels and the size of antifungal inhibition zones, suggesting that β-glucan's anti-virulence effect parallels its growth-inhibitory activity.

Together, these results confirm three key findings:

1) The high antifungal resistance among *Candida albicans* isolates underscores the need for local epidemiological surveillance.

2) Beta-glucan exhibited dual antifungal and anti-virulence properties, inhibiting the growth and expression of the *SAP5* gene.
3) Accurate molecular confirmation using *ITS1*/*ITS4* ensures reliable diagnosis, which is critical for targeted antifungal therapy.

Given the increasing incidence of drug-resistant Candida infections, oat-derived beta-glucan may represent a safe natural supplement with therapeutic and preventive potential. However, further studies should include untreated controls, evaluate additional SAP genes, and explore the synergistic interaction of beta-glucan with conventional antifungal drugs.

5 CONCLUSIONS

This research provides comprehensive experimental and molecular evidence supporting the antifungal and virulence-inhibiting activity of oat-derived β-glucan against *Candida albicans* isolated from cases of mastitis in women. The results confirm that *C. albicans* remains a clinically significant fungal pathogen in mastitis, with a high prevalence of multidrug-resistant strains, particularly to commonly used antifungal agents such as nystatin and fluconazole. This pattern of resistance reflects a growing clinical concern and underscores the urgent need for alternative or complementary treatment strategies.

The results of this study demonstrated that oat-derived β-glucan exhibits a clear, concentration-dependent inhibitory effect against *Candida albicans* growth, with a minimum inhibition concentration (MIC) of 400 µg/ml for most isolates. In addition to growth inhibition, molecular analyses showed that β-glucan significantly reduced the expression of the virulence gene *SAP5* by approximately 2.8-fold. Since *SAP5* is a key gene responsible for tissue invasion, hyphal development, biofilm formation, and immune evasion, this reduction in gene expression suggests that β-glucan not only inhibits fungal growth but also weakens the fungus's pathogenicity at the molecular level. This dual action (anti-growth and anti-virulence) represents a significant therapeutic advantage compared to drugs that target only fungal viability.

Furthermore, the heterogeneous distribution of the *SAP5* gene among the clinical isolates in this study highlights the high genetic and pathological variability among *C. albicans* strains. This underscores the importance of combining phenotypic susceptibility testing with molecular assays for a more accurate assessment of infection severity and treatment response. Additionally, the strong inverse correlation between *SAP5* expression levels and the diameters of inhibition zones supports the hypothesis that virulence gene inhibition directly contributes to enhanced antifungal activity.

A key aspect of this study is that the use of oat-derived β-glucan as a naturally occurring food-grade compound lends significant practical value to the research. Unlike many synthetic antifungal agents, β-glucan exhibits a high safety profile, enhanced immunomodulatory activity, and a low likelihood of developing drug resistance. These properties make it a promising candidate as an adjunctive therapy, particularly in cases of recurrent, treatment-resistant, or mucosal candidiasis where the immune system and biofilm play a crucial role.

Despite these positive results, the study has some limitations, including the relatively small number of isolates examined, the restriction of gene expression analysis to *SAP5* only, and the absence of in vivo experiments. Furthermore, the study did not address intracellular signaling pathways that β-glucan might influence in regulating gene expression. Nevertheless, these limitations open important avenues for future research.

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