International Journal of Molecular and Clinical Microbiology 12(1) (2022) 1643-1649

International Journal of
Molecular and Clinical Microbiology

Expression of the *HWP1* Gene in *Candida albicans* Induced by the Ethanolic Extract of Propolis

Riazalhosseini Behnaz[1] , Zahraei Salehi Taghi[2], Anvar Seyed amirali[1], Sohrabi Haghdoost Nakisa[3]*

1. Department of Food Hygiene, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2. Department of microbiology and immunology, faculty of veterinary medicine, university of Tehran, Azadi Street, Tehran, Iran.
3. Department of pathobiology, School of Veterinary, Science and Research Branch, Islamic Azad University, Tehran, Iran.

ARTICLE INFO

Article history:
Received 5 March 2022
Accepted 24 May 2022
Available online 1 June 2022

Keywords:
Hyphal Wall Protein 1,
gene expression,
propolis extract,
RT-PCR method,
hyphal form,
adherence to epithelial cells

ABSTRACT

Candida albicans is an aggressive pathogen and the main etiologic agent in the genus Candida, accounting for 42.5% of infections. The existence of hyphal form along with adherence is critical to colonize mucosal surfaces and cause infection. Adherence of *C. albicans* to host tissue is crucial to colonize cells; therefore, these microorganisms use proteins named adhesins. HWP1, a protein associated with adhesins, serves as a substrate for mammalian transglutaminases and plays an important role in adherence to epithelial cells. Due to the emergence of antifungal drug resistance, the present study aimed at determining the effect of propolis ethanolic extract (a natural product with antifungal properties) on the *HWP1* gene expression in clinically isolated *C. albicans* strains. The HWP1 gene expression was analyzed using real-time PCR, and *HWP1* expression was normalized against the *ACT1* housekeeping gene. After the treatment of clinical samples with propolis ethanolic extract, the relative level of *HWP1* gene expression was 0.88, 0.53, 0.21, and 0.66% for the standard strain, and those isolated from the oral cavity, nail, and vaginal cavity, respectively. The decrease in *HWP1* expression might affect *C. albicans* virulence, and propolis ethanolic extract might be used as an alternative remedy for antifungal drugs.

1. Introduction

Candida albicans (*C. albicans*) is a common microorganism in the normal flora of the human body, mostly living in the gastrointestinal tract, vaginal cavity, and oral cavity (Haghdoost et al., 2016; Romo & Kumamoto, 2020; Villa et al., 2020). It is also an aggressive pathogen, which often causes severe diseases in individuals with immunodeficiency disorders (Maras et al., 2021). Likewise, *C. albicans* can cause a wide range of diseases, from symptomatic candidaemia to fulminant sepsis (Pappas et al., 2018). As the etiologic agent in the genus Candida, *C. albicans* accounts for 42.5% of infections (Felipe et al., 2018) and nearly 35% of the mortality rate in vulnerable people (Khan et al., 2014). Studies demonstrated that an increase in *C. albicans* colonization is essential to transit from commensalism towards the systemic infection, which is usually associated with failure in the host immune system (d'Enfert et al., 2021; Pappas et al., 2018). Unlike other *Candida* species, *C. albicans* can shift among different forms, yeast, pseudohyphal, and hyphal (Kornitzer, 2019). Hyphae are the pathogenic

*Corresponding author: Nakisa Sohrabi Haghdoost
E-mail address: sohrabi.nakisa@gmail.com

form of *C. albicans* since they can attack epithelial cells and cause tissue damage, form biofilms, and grow in the hyphal form to escape phagocytosis and kill macrophages (Kornitzer, 2019; McKenzie et al., 2010).

Adherence of *C. albicans* to host surfaces is a crucial event to dispersion and settle down of fungul growth which eventually result in infection in host. The existence of hyphal form along with adherence causes damage to mucosal surfaces; as a result of the combined action of secreted phospholipases and aspartyl proteases, facilitating the attack of the *C. albicans* into the epithelium (Maras et al., 2021). Earlier investigations on *C. albicans* behavior revealed that it uses a group of proteins named adhesins to escalate adherence among microorganisms, between the microorganism and host cells, or dead surfaces. Adhesins include the HWP family, which contains glycosylphosphatidylinositol-anchored proteins located on the cell wall (de Groot et al., 2013; Hoyer, 2001). The HWP1 (hyphal wall protein 1) acts as a substrate for mammalian transglutaminases and plays a pivotal role in adherence to epithelial cells (Sundstrom et al., 2002). Moreover, an investigation indicated that deletion of *HWP1* from *C. albicans* decreased its virulence using a mouse model (Nas et al., 2008). Several studies suggested that *HWP1* expression is required for germ tube and hyphae formation (Fan et al., 2013; Nas et al., 2008; Nobile et al., 2006).

Conventional antifungal drugs exert their effects by killing the fungal cells or inhibiting growth to treat various types of candidiasis; however, a high rate of mortality and morbidity associated with candidiasis revealed the emergence of antifungal drug resistance species (Chandra et al., 2001; Vila et al., 2017). Thus, there is a need to design alternative agents for antifungal drugs to target virulence factors specific to *C. albicans.* In this case, natural products recently raised more attention due to their high structural diversity and biological properties that show therapeutic activities either in natural form or their derivatives (Aldholmi et al., 2019) (Shojaee et al.). Propolis so called bee glue is a natural material composed by honey bees from various plants such as palm, apple, orange and pine secretions and leaf buds. It has been approved that the material is collected and used for crevices occurring and waterproofing cracks in honey bee hives. Basically, its antiseptic properties protects bee-hive from microbial infections as well as decomposition of intruders (Firdaus et al., 2016; Veiga et al., 2018). Moreover, bee glue has been used in traditional medicine for eras. The biologic properties of propolis depend upon its chemical structure, plant resources and seasons. In many studies, over three hundered of phenolic, aromatic acids, essential oils, waxes and amino acids compounds have been identified in this material (Ożarowski et al., 2022). Propolis, a viscous adhesive is well-known for a variety of biological activities, such as antibacterial, antiviral, and antifungal effects (Haghdoost et al., 2016). The ethanolic extract is the main fraction of propolis, and some *in vitro* studies confirmed its antifungal effects on different human pathogenic fungi, such as *C. albicans* (Firdaus et al., 2016; Veiga et al., 2018). In addition, a recent study reported the growth inhibitory effect of propolis ethanolic extract on *C. albicans* germination (Haghdoost et al., 2016; Shojaee et al.). It has been shown that extract of propolis targets the cell membrane of fungi to inhibit the activity of extracellular phospholipase to faciliate the fungi adhesion to epithelial cells and induction of cell death (Ożarowski et al., 2022).

Therefore, the present study aimed at investigating a possible association between the virulence gene *HWP1* expression in *C. albicans* strains isolated from different clinical samples (e g, vaginal, oral, nail) and the antifungal activity of propolis ethanolic extract.

2. Materials and Methods

2.1 Fungal strains

A total of 38 clinically isolated *C. albicans* strains were obtained from the Fungal Collection of Mycology Research Center, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. The strains were isolated from the vagina (n=15), oral cavity (n=15), and nail (n=8), and the Mycology Laboratory results confirmed their resistance to fluconazole. In addition, *C. albicans* ATCC 90028 strain was applied as the standard control.

2.2 Propolis origin and preparation of its ethanolic extract

The propolis samples were purchased from hives of *Apis mellifera* L. (honey bees) located in Kurdistan Province, Western Iran. Samples were transferred to the laboratory while keeping in a clean-packed plastic bag and stored at -20°C until use. To extract, 10 g of grounded and mashed crude propolis were put in an Erlenmeyer™ flask (250 mL), and 70% ethanol was added until reaching a final volume of 100 mL. Then, propolis was mixed entirely with ethanol on a shaker until obtaining a solution and kept under standard conditions (in a dark place with 30°C temperature for two weeks). Then, to eliminate waxes and some insoluble ingredients, the suspension was kept at -20°C for 24 hours. Then, it was passed in triplicates through a filter paper, and the last filtered solution was considered the ethanolic extract of propolis. Afterward, the solution was heated until almost dry (under compact pressure, using a rotary evaporator at 40°C) and then freeze-dried. To prepare a 10% w/v stock solution of propolis ethanolic extract, the produced powder was dissolved in ethanol/water with an 80:20 ratio (Pratami et al., 2018) (Sayyadi et al., 2020).

2.3 Molecular experiments

The following steps were performed to evaluate the effect of the propolis ethanolic extract on the *HWP1* gene expression: RNA extraction: clinical samples and the Total RNA Purification Kit (Qiagen) were used according to the manufacturer's instructions. The purity of extracted RNAs was examined using a Nano-drop Spectrophotometer, adjusted to the measure absorbance ratios of A260/A280 (value range 1.6 – 1.9) and A260/A230 (value range 0.53 – 1.34). The cDNA synthesis: approximately 5 µL of total RNA was used as a template to synthesize the cDNA, applying the RevertAid First Strand cDNA Synthesis Kit (Fermentas), according to the manufacturer's instructions. Prior to RT- PCR, a pilot PCR was performed to examine the accuracy of primers and their optimum concentration and determine annealing temperature. PCR products were electrophoresed on a 2.5% agarose gel, and the bands were UV-visualized using a transilluminator. The RT-PCR was performed according to the manufacturer's protocol, using qPCR GreenMaster with the LowRox Kit (Bioneer).

PCR amplification was performed as follows: initial denaturation at 95°C for two minutes in one cycle, denaturation (95°C for 15 seconds), annealing (at 65°C for one minute) in 45 cycles, and extension (at 65°C for one minute), and final extension at 72°C for three minutes. The β-actin1 gene was used as the internal control, and expression of the *HWP1* gene was normalized against the *ACT1* housekeeping gene. The *ACT1* gene was applied as the positive control and confirmation of the PCR procedure. The sequences of primers used in RT-PCR, including the reference actin gene *ACT1,* are presented in Table 1.

To prevent contamination, all experiments, including RNA extraction, cDNA synthesis, and RT-PCR, were performed under sterile conditions. Microcentrifuge tubes were autoclaved for sterilization and also were UV-irradiated together with the nuclease-free tubes. The nuclease-free water was utilized for all experiments. Filter pipette tips were used for PCR.

Table 1. The HWP1 and ACT1 specific primers for RT-PCR

Gene	Primer	primer length	Sequence	PCR product length (bp)
HWP1	Forward	20	5'- GCCCAGAAAGTTCTGTTCCA-3'	121
	Reverse	24	5'- TTTGGTTTCAGTAGTAGTGGTTGG-3'	
ACT1	Forward	22	5'-ACTACCATGTTCCCAGGTATTG-3`	100
	Reverse	22	5'-TCAAGTTACCAGCCAAATC-3'	

HWP1: Hyphal wall protein 1 gene, ACT: Actin (housekeeping gene).

2.4 Statistical analysis

All experiments were performed in triplicate, and data were expressed as mean ± SEM. A comparative threshold cycle (CT) method was applied to analyze RT-PCR results. Statistical analyses were performed using one-way analysis of variance (ANOVA) and SPSS version 18, and P-value <0.05 was considered the level of significance.

3. Results

3.1 Evaluation of extracted RNA

The concentration of extracted RNAs was quantified 630-980 µg/mL. To obtain the same RNA concentrations, sufficient DEPC water was added to each sample, and then RNAs were used for R-PCR.

3.2 Evaluation of primers utilized in the study by PCR

The optimum concentration of primers was 10 pmol, and the annealing temperature for *HWP1* and *Act1* was 50°C.

3.3 Real-time PCR results

The RT-PCR data were acquired by the EvaGreen Kit, and the results were obtained using the Livak method. The RT-PCR results are shown in Table 2. After the treatment of isolates with the propolis ethanolic extract, a decrease was observed in the *HWP1* gene expression. The expression of the *HWP1* gene was 0.88, 0.53, 0.21, and 0.66 for the standard strain, and those isolated from the oral cavity, nail, and vaginal cavity samples, respectively. In other words, a decreasing trend was observed in the expression of the *HWP1* gene for the standard strains and those isolated from the oral cavity, nail, and vaginal cavity by 12.3%, 47.4%, 78.6%, and 33.9% respectively. According to Table 2, propolis ethanolic extract had a descending effect on the expression of the *HWP1* gene in the studied clinical strains.

Table 1. RT-PCR Results for HWP1 and ACT1 genes in C. albicans, as well as clinical samples (oral, nail and vaginal) and effect of ethanolic extract of propolis (concentration= ½ MIC) on gene expression

C. albicans	Average CT	HWP1[#]	ACT[!]	ΔCT*	ΔΔCT	RFC[$]
ATCC 90028	control	16.87	17.59	0.71	0	1
	sample	20.58	21.48	0.9	0.19	0.88
oral isolates	control	16.99	18.22	-1.23	0	1
	sample	22.65	23.58	-0.92	0.92	0.53
nail isolates	control	23.46	18.39	5.07	0	1
	sample	29.1	21.81	7.29	2.22	0.21
vaginal isolates	control	16.96	16.42	0.53	0	1
	sample	21.35	20.21	1.13	0.59	0.66

ΔCT*: Threshold cycle, HWP1[#]: Hyphal wall protein 1 gene, ACT[!]: Actin (housekeeping gene), RFC[$]: Relative fold change

4. Discussion

Infections caused by *C. albicans* represent a public health challenge worldwide, especially in individuals who are on treatment with extended-spectrum antibiotics or immunocompromised patients. Resistance of *C. albicans* strains to antifungal drugs has become an increasing problem during the past years. Natural products with various chemical diversities and biological activities raised attention due to their therapeutic potentials. Among natural products with antifungal properties, propolis was examined in some investigations (Dota et al., 2011; Veiga et al., 2018). For instance, the antifungal properties of stingless bee propolis against *C. albicans* and

C. neoformans were confirmed in a study by Shehu *et al* (Shehu et al., 2016). The findings of a study demonstrated the growth inhibitory effect of both ethanolic extract and microparticles of propolis on *C. albicans* isolated from patients with vulvovaginal candidiasis, although the propolis microparticles were more efficient than the ethanolic extract in terms of fungicidal activities (Dota et al., 2011). In the present study, the effect of propolis ethanolic extract was examined on the *HWP1* gene expression in *C. albicans* strains isolated from clinical samples. The finding revealed a decrease in the expression of the *HWP1* gene by 12.3%, 47.4%, 78.6%, and 33.9% for the standard strain, as well as oral cavity, nail, and vagina cavity, respectively. The result of the present study was in agreement with those of research by Prawiro *et al.*, revealing the decreasing effect of propolis ethanolic extract on biofilm formation as well as down-expression of the *EFG1* gene which regulates the expression of some genes, including HWP1 in *C. albicans* (Prawiro et al., 2021).

The pathogenesis of *C. albicans* involves invasion of the host tissue surfaces by hyphal forms, which can penetrate the epithelium and grow (Staab et al., 1999). Adherence of *C. albicans* to host tissue is a vital stage in biofilm formation and consequently the start of infection (Nikoomanesh et al., 2016). The signaling pathway, involved in infection-associated virulence factors, such as adhesins, consists of several genes encoding proteins with adhesive properties**.** Some of them are expressed during both the yeast and hyphal forms of *C. albicans,* while the *HWP1* gene is mostly expressed in hyphal cells. The HWP1 protein is located on the external surface of the cell wall and forms a tight attachment to the host tissue and thus is considered a critical virulence factor (Maras et al., 2021). The overexpression of the *HWP1* gene was reported in association with *C. albicans* pathogenicity. Results of some studies revealed that this hyphal adhesion protein is highly expressed during colonization and infection in the oral epithelium and its deletion reduced virulence in a murine model of oropharyngeal candidiasis (Naglik et al., 2006; Zakikhany et al., 2007).

The *HWP1* encodes an external mannoprotein with a cell surface-exposed NH2-terminal domain and COOH-terminal features, conferring covalent integration into cell wall β-glucan. Unlike most microorganisms that form several weak attachments to host cells, *C. albicans* form strong attachments to human buccal epithelial cells through a transglutaminase-mediated mechanism, that is vital in the growth of mucosal candidiasis. (Staab et al., 1999). A probable role for *HWP1* in the formation of strong and steady attachments to buccal epithelial cells was proposed by the amino acid sequence of the NH2-terminal domain that is similar to the mammalian transglutaminase substrate (Staab et al., 1999). Furthermore, regulation of *HWP1* expression depends on the activity (suppression or activation) of two transcription factors, Nrg1p and Efg1p, located on the promoter termed HWP1 control region (Maras et al., 2021).

Conclusion

In conclusion, the study findings revealed that *HWP1,* as a reliable marker of *C. albicans* infection, can be down-expressed by propolis ethanolic extract, and consequently, the pathogenicity of *C. albicans* is reduced.

Author Contributions: Study concept and design: B.R and S.A.; analysis and

interpretation of data: T.Z.; drafting of the manuscript: N.S.H.; critical revision of the manuscript for important intellectual content: A.A, B.R, and N.S.H, statistical

analysis: T.Z

Conflicts of interest: None declared.

Acknowledge: This study was supported by Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Refereces

Aldholmi, M., Marchand, P., Ourliac-Garnier, I., Le Pape, P., & Ganesan, A. (2019). A Decade of Antifungal Leads from Natural Products: 2010-2019. Pharmaceuticals (Basel, Switzerland), 12(4), 182. https://doi.org/10.3390/ph12040182

Chandra, J., Kuhn Duncan, M., Mukherjee Pranab, K., Hoyer Lois, L., McCormick, T., & Ghannoum Mahmoud, A. (2001). Biofilm Formation by the Fungal PathogenCandida albicans:

Development, Architecture, and Drug Resistance. Journal of Bacteriology, 183(18), 5385-5394. https://doi.org/10.1128/JB.183.18.5385-5394.2001

d'Enfert, C., Kaune, A.-K., Alaban, L.-R., Chakraborty, S., Cole, N., Delavy, M., Kosmala, D., Marsaux, B., Fróis-Martins, R., Morelli, M., Rosati, D., Valentine, M., Xie, Z., Emritloll, Y., Warn, P. A., Bequet, F., Bougnoux, M.-E., Bornes, S., Gresnigt, M. S., Hube, B., Jacobsen, I. D., Legrand, M., Leibundgut-Landmann, S., Manichanh, C., Munro, C. A., Netea, M. G., Queiroz, K., Roget, K., Thomas, V., Thoral, C., Van den Abbeele, P., Walker, A. W., & Brown, A. J. P. (2021). The impact of the Fungus-Host-Microbiota interplay upon Candida albicans infections: current knowledge and new perspectives. FEMS Microbiology Reviews, 45(3). https://doi.org/10.1093/femsre/fuaa060

de Groot, P.W., Bader, O., de Boer, A. D., Weig, M., & Chauhan, N. (2013). Adhesins in human fungal pathogens: glue with plenty of stick. Eukaryotic cell. 12(4): 470-481.

Dota, K. F. D., Consolaro, M. E. L., Svidzinski, T. I. E., & Bruschi, M. L. (2011). Antifungal activity of Brazilian propolis microparticles against yeasts isolated from vulvovaginal candidiasis. Evidence-based complementary and alternative medicine, 2011.

Fan, Y., He, H., Dong, Y., & Pan, H. (2013). Hyphae-specific genes HGC1, ALS3, HWP1, and ECE1 and relevant signaling pathways in Candida albicans. Mycopathologia, 176(5-6), 329-335.

Felipe, L. d. O., Silva, W. F. d., Araújo, K. C. d., & Fabrino, D. L. (2018). Lactoferrin, chitosan and Melaleuca alternifolia-natural products that show promise in candidiasis treatment. brazilian journal of microbiology, 49, 212-219.

Firdaus, S., Ali, F., & Sultana, N. (2016). Dermatophytosis (Qooba) a misnomer infection and its management in modern and unani perspective-A comparative review. J Med Plants Stud, 4, 109-114.

Haghdoost, N., Salehi, T., Khosravi, A., & Sharifzadeh, A. (2016). Antifungal activity and influence of propolis against germ tube formation as a critical virulence attribute by clinical isolates of Candida albicans. Journal de mycologie medicale, 26(4), 298-305.

Hoyer, L. L. (2001). The ALS gene family of Candida albicans. Trends in microbiology. 9(4): 176-180.

Khan, M. S. A., Ahmad, I., Cameotra, S. S., & Botha, F. (2014). Sub-MICs of Carum copticum and Thymus vulgaris influence virulence factors and biofilm formation in Candida spp. BMC Complementary and Alternative Medicine, 14(1), 337. https://doi.org/10.1186/1472-6882-14-337.

Kornitzer, D. (2019). Regulation of Candida albicans Hyphal Morphogenesis by Endogenous Signals. Journal of fungi (Basel, Switzerland), 5(1), 21. https://doi.org/10.3390/jof5010021

Maras, B., Maggiore, A., Mignogna, G., D'Erme, M., & Angiolella, L. (2021). Hyperexpression of CDRs and HWP1 genes negatively impacts on Candida albicans virulence. PloS one, 16(6), e0252555.

McKenzie, C., Koser, U., Lewis, L., Bain, J., Mora-Montes, H., Barker, R., Gow, N., & Erwig, L. (2010). Contribution of Candida albicans cell wall components to recognition by and escape from murine macrophages. Infection and immunity, 78(4), 1650-1658.

Naglik, J.R., Fostira, F., Ruprai, J., Staab, J.F., Challacombe, S. J., & Sundstrom, P. (2006). *Candida albicans* HWP1 gene expression and host antibody responses in colonization and disease. Journal of medical microbiology, 55(Pt 10), 1323.

Nas, T., Kalkanci, A., Fidan, I., Hizel, K., Bolat, S., Yolbakan, S., Yilmaz, E., Ozkan, S., & Kustimur, S. (2008). Expression of ALS1, HWP1 and SAP4 genes in Candida albicans strains isolated from women with vaginitis. Folia Microbiol (Praha), 53(2), 179-183.

Nikoomanesh, F., Roudbarmohammadi, S., Roudbary, M., Bayat, M., & Heidari, G. (2016). Investigation Of Bcr1 Gene Expression In Candida Albicans Isolates By Rtpcr Technique And Its Impact On Biofilm Formation.

Nobile, C.J., Nett, J.E., Andes, D.R., & Mitchell, A.P. (2006). Function of Candida albicans adhesin Hwp1 in biofilm formation. Eukaryotic cell. 5(10): 1604-1610.

Ożarowski, M., Karpiński, T.M., Alam, R., & Łochyńska, M. (2022). Antifungal Properties of Chemically Defined Propolis from Various Geographical Regions. Microorganisms, 10(2), 364.

Pappas, P.G., Lionakis, M.S., Arendrup, M.C., Ostrosky-Zeichner, L., & Kullberg, B.J. (2018). Invasive candidiasis. Nature Reviews Disease Primers. 4(1): 18026. https://doi.org/10.1038/nrdp.2018.26

Pratami, D. K., Mun'im, A., Sundowo, A., & Sahlan, M. (2018). Phytochemical profile and antioxidant activity of propolis ethanolic extract from Tetragonula bee. Pharmacognosy Journal, 10(1).

Prawiro, S.R., Dewi, A. S., Diniayuningrum, A., Maghfiroh, D. O., Nurdiana, N., Endharti, A. T., & Rahardjo, B. (2021). Propolis ethanolic extract influenced biofilm formation and gene expression level of EFG1 in Candida albicans. AIP Conference Proceedings,

Romo, J.A., & Kumamoto, C. A. (2020). On Commensalism of Candida. Journal of Fungi, 6(1). https://doi.org/10.3390/jof6010016.

Sayyadi, F., Mahdavi, S., Moghadamnia, A. A., Moslemi, D., Shirzad, A., & Motallebnejad, M. (2020). The effect of aqueous and ethanolic extract of Iranian propolis on Candida Albicans isolated from the mouth of patients with colorectal malignancy undergone chemotherapy: An in-vitro study. Caspian journal of internal medicine, 11(1), 62.

Shehu, A., Ismail, S., Rohin, M.A.K., Harun, A., Aziz, A.A., & Haque, M. (2016). Antifungal properties of Malaysian Tualang honey and stingless bee propolis against Candida albicans and Cryptococcus neoformans. Journal of Applied Pharmaceutical Science, 6(2), 044-050.

Shojaee, A., Anvar, S.A., Hamedi, H., Sohrabi Haghdoost, N., & Bahmani, S. Antifungal and antioxidant activity of two essential oils and Lactococcus lactis subsp. lactis extract on selected Iranian white cheese contaminating fungi. International Journal of Dairy Technology.

Staab, J. F., Bradway, S. D., Fidel, P. L., & Sundstrom, P. (1999). Adhesive and mammalian transglutaminase substrate properties of Candida albicans Hwp1. Science. 283(5407): 1535-1538.

Sundstrom, P., Balish, E., & Allen, C. M. (2002). Essential Role of the Candida albicans Transglutaminase Substrate, Hyphal Wall Protein 1, in Lethal Oroesophageal Candidiasis in Immunodeficient Mice. The Journal of Infectious Diseases. 185(4): 521-530. https://doi.org/10.1086/338836

Veiga, F.F., Gadelha, M.C., da Silva, M.R., Costa, M.I., Kischkel, B., de Castro-Hoshino, L. V., Sato, F., Baesso, M L., Voidaleski, M.F., & Vasconcellos-Pontello, V. (2018). Propolis extract for onychomycosis topical treatment: From bench to clinic. Frontiers in microbiology. 9: 779.

Vila, T., Romo, J. A., Pierce, C. G., McHardy, S. F., Saville, S. P., & Lopez-Ribot, J. L. (2017). Targeting Candida albicans filamentation for antifungal drug development. Virulence, 8(2), 150-158.

Villa, S., Hamideh, M., Weinstock, A., Qasim, M. N., Hazbun, T. R., Sellam, A., Hernday, A. D., & Thangamani, S. (2020). Transcriptional control of hyphal morphogenesis in Candida albicans. FEMS Yeast Research, 20(1). https://doi.org/10.1093/femsyr/foaa005

Zakikhany, K., Naglik, J.R., Schmidt Westhausen, A., Holland, G., Schaller, M., & Hube, B. (2007). In vivo transcript profiling of Candida albicans identifies a gene essential for interepithelial dissemination. Cellular microbiology. 9(12): 2938-2954.