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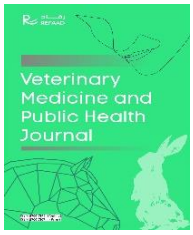

Evaluation Study of *Petroselinum Crispum* Extract Toward Pathogenic Isolates of *candida*

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Abstract:

Objectives: The aim of this study was to isolation and identify the isolated genetically if candida species from candidiasis infection and study the activity of bioactive compounds.

Methods: Samples were obtained from 28 patients from the mouth of patients with oral candidiasis. Samples were collected from the Child Specialized Hospital, Al-Faiha General Hospital Dermatologist Dr. Laith Globe Lazim Al-Kinani (Master of Dermatology, PhD in Dermatology, Iraqi Ministry of Health, Al-Fayhaa Teaching Hospital) aided in the diagnosis of clinical cases and molecular analysis then tested the activity of the mint plant extract on it and compared it with the antifungal nystatin.

Results: Mint leaf extract showed activity against *Candida* isolates. The extract was non-toxic, and *C. albicans* was the most common *Candida*.

Conclusions: The methanolic extract of *Petroselinum crispum* in this study showed good inhibition activity against the isolated *Candida* species.

Keywords: *Candidiasis; Petroselinum crispum; GC-MS; antifungal.*

1 Introduction

Candidiasis is a problem and has increased the morbidity of immunocompromised patients. (Guevara-Lora *et.al*.,2020). The genus of *Candida* is normal flora that colonizes human skin and mucosal surfaces, but under conditions of a weak host immune system it turns into pathogens (Khan, *et.al*., 2010) candidiasis is a common opportunistic infection caused by an overgrowth of *Candida* species, the commonest being *Candida albicans*. (Mamtani, *et.al*.,2020). invasive fungal diseases associated with *Candida* and candidiasis of the internal organs, which are characterized by frequent hospital origin and high mortality rates of 30-45% of affected patients (Mesini *et. al*., 2010). There are 200 species of *Candida* but only 20 species as *C. albicans,C. glabrata,C. tropicalis*(Torres, *et.al*., 2007). candidiosis treated antifungal (Ahmad Khan, *et.al*., 2020). There are four group of antifungal drugs were developed, (Nett & Andes., 2016). but both fungal and human cells are eukaryotes, and, *Candida* strains of antifungal resistance have either been intrinsic or acquired, and this is a major problem in treating candidiasis (Wiederhold., 2017).

Despite the developments of antifungal treatments against *Candida* species, there are still increasing rates of resistance to the current antifungal therapy resulting in their frustration (Tang, *et.al*., 2018). Therefore, new alternative treatments against these fungal infections (Mati, *et.al*., 2012) has been highlighted.

Natural products are preferred as biocompatible and non-toxic drugs in medicine compared to chemical and synthetic drugs. In recent years, there has been an increasing interest in natural extracts that exhibit medicinal properties that are provided to humans as food.

2 Materials and Methods

2.1 Sampling, Isolation and DNA extraction

Samples were obtained from 28 patients from the mouth of patients with oral candidiasis. Samples were collected from the Child Specialized Hospital, Al-Faiha General Hospital Dermatologist Dr. Laith Globe Lazim Al-Kinani (Master of Dermatology, PhD in Dermatology, Iraqi Ministry of Health, Al-Fayhaa Teaching Hospital) aided in the diagnosis of clinical cases. The samples were transferred to the microorganism laboratory. The swabs were culture on SDA and PDA HiMedia Co., India and incubat at 37 C° for a period of (2-5) days. Genomic DNA of each strain was extracted using the Presto™ Mini gDNA Yeast Kit (Geneaid) (White *et.al.,* 1990). was used for DNA isolation according to the company instructions. Later, isolated DNA samples were electrophoresed in 0.5% agarose gel stained with ethidium bromide and preserved in the -80 ºC for PCR amplification.

2.2 Polymerase Chain Reaction (PCR) product

Two universal primers, ITS1(F-5-TCC GTA GGT GAA CCT GCG G-3) and ITS4(R-5-TCC TCC GCT TAT TGA TAT GC-3), were used in the PCR created by (Mirhendi *et.al*.,2006) to amplify the Internal Transcribed Spacer region (ITS 1-5.8SITS2). The PCR Master Mix (5 µl, Bioneer Co, Korea), DNA template (5 µl, Bioneer Co, Korea), and Nuclease Free Water (25 µl, Bioneer Co, Korea) were combined to create the DNA amplification solution. DNA fragments were separated in a 2% agarose gel, and the sizes of the products were compared with molecular markers (100–2000 bp, Bioneer Co., Korea). The fragments were then inspected with a UV transilluminator and compared to ladder fragments. 2.2.3. PCR Product Sequencing of ITS1- 5.8S – ITS2 rDNA region Macrogen Co, South Korea, sent the PCR products for sequencing. The NCBIs " BLAST " was used to identify the PCR sequencing products of fungi.

2.3 Study plant

The plant P. crispum were purchased from local market were collected from Basrah city.

2.4 Preparation of methanol extract of *Petroselinum crispum*

Dry P. crispum were weighed and grind to powder and a 5% w/v suspension was prepared in a flask by adding methanol cohol. The flask was then placed on a shaker (200 rpm) for 7 h and the temperature was maintained at 37°C. After shaking, the flask was brought to room temperature. The suspension was filtered through a series of Whatman filters and finally passed through 0.22 micron filter for sterilization. The filtered methanol extract was dried at room temperature and stored at −20°C until use (Nair *et.al*.,1989).

GC-MSMS analysis:

The methanol extracts for *P. crispum* were analyzed using the GC/MS Agilent/USA Compounds profile by GC-MSMS. Compounds in *P. crispum* were identified based ontheir mass spectral data by using Gas chromatography-Mass

spectroscopyat qurnah college laboratories, analysis was done in 7010B Triple Quadrupole GC-MS/MS (Agilent- US), equipped with a capillary inlet at (30m x 0.25 mmID; 0.25 in film thickness) the samples were injected in the following conditions: injector temperature 300 ℃; carriergas, helium; pressure,11.962 psi.

Cytotoxicity test:

Biocompatibility test was carried out for prepared *P. crispum* extract against human freshblood according to Nair et al 8 methods, briefly, Different concentrations of extract (10,500,1000) were prepared, then 100µl from each concentration was added to each tube of human blood solution. The tubes were incubated at room temperature and formation of turbidity of blood solution was tested at 15, 30 and 60 min. respectively (Perez, *et.al*.,1990).

Test the effectiveness of *petroselinum crispum* methanol extracts and antifungal on the isolated *candida* spp:

The well diffusion method was to study the sensitivity of antifungals and the sensitivity of isolates for the methanol extract of *P. crispum* where a number of colonies were mixed with 5 ml of physiological solution (normal saline). Sterile cotton scabs were then covered in the fungal suspension and then were plotted on the surface of the wafers and the dishes were left until the mixture was absorbed by the macaroni excavated with 6 mm diameter drills were drilled with a sterile flask and filled with 50 microliters of all concentrations (100. 500 and 1000) mg / ml. After the incubation period, the diameters of the inhibition zones are measured around the drill and the results are recorded (Samaranayake, *et.al*.,1984).

2.5 Statistical Analysis

The analysis of variance (ANOVA) L.S.D (least significant difference 0.05 was applied) was used to achieve the suitability of the analyzed parameters for botanical authentication of *P. crispum*. ANOVA was carried out using Statgraphics.

3 Result and Discussion

3.1 Morphological and Phenotypical Analysis

Microscopic direct examinations of all oral specimens showed that 42.85% (12 samples) were yeast infections by presence of true and pseudo-hyphae in wet smear with 10% KOH, while only 57.14% (16 of samples) revealed negative result of direct investigation. However, 64.28% (18 samples) of positive sample swabs were shown growth onto SDA media after incubation interval and the rest failed to grow.

3.2 Molecular idefinition for isolates

The amplified and sequenced the ITS region of the fungal isolates in particular. Between the rRNA genes in the fungal genome is the conserved ITS region, which is frequently used to identify fungi(Alrubayae and Kadhim ., 2020) All positive isolates in vitro culture (18 isolates) were subjected for molecular identification. The process was completed by amplifying and sequencing the ITS region for sequencing as (Figure 1). The results of the ITS region sequences showed that 50% of the isolates were *C. albicans*, *C. tropicalis* 27.77%, 22.22% *C. Glabrata* and 5.55% *C. dubliniensis* as figure (2). In this study, molecular methods were applied to identify yeast isolates. The morphological methods are time consuming and imprecise compared to the accurate and reliable molecular approach (Kurtzman *at.al*., 2011). The plainness and reliability of molecular identification due to the availability of ITS region sequences in the general database is optimal, so the interpretation was based on the molecular method (Alrubayae *et.al*., 2020). The molecular results showed the prevalence of *C. albicans* as in the study (Al-Dabbagh *et.al*., 2019) This is not compatible with (Alrubayae and Kadhim., 2020). Our results may be inconsistent with other studies due to the small sample. In general, the development of suitable diagnostic and treatment approaches can benefit from understanding the prevalence and distribution of fungus through molecular identification(Millon *et.al.,* 2019). Based on the susceptibility patterns of the detected species, it enables medical professionals to precisely identify the fungi that are the cause of fungal illnesses and select the most effective antifungal treatments (Reinel, 2021).

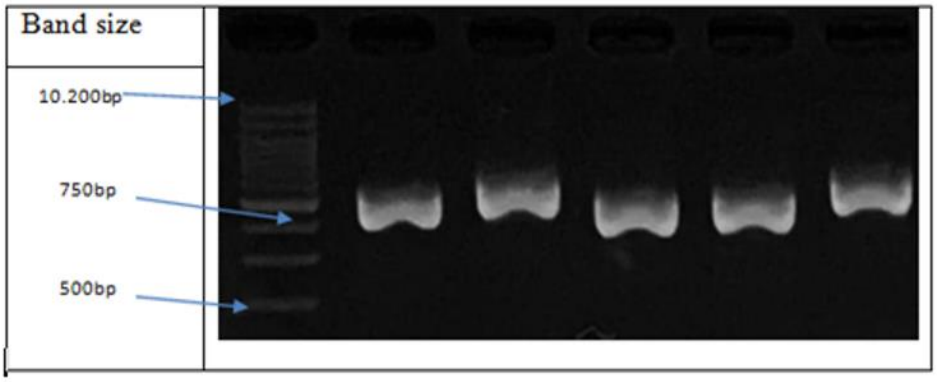

Figure 1: Results of PCR technology for some isolated yeasts

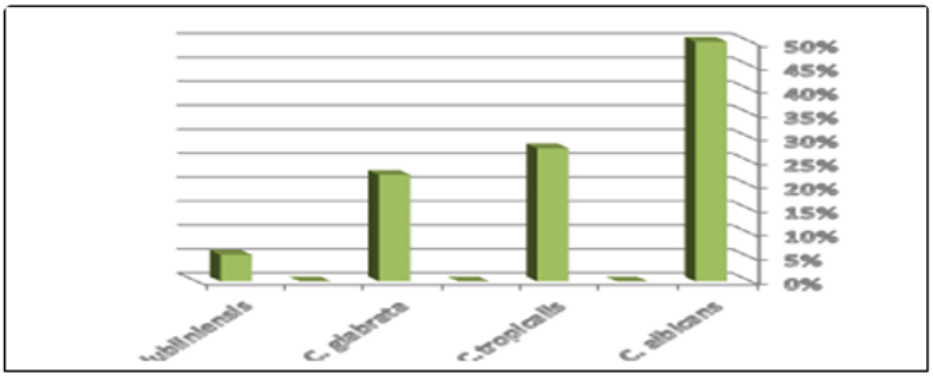

Figure 2: Candida proportions in molecular analysis

3.3 GC-MSMS Analysis

A large number of compounds were detected in the GC/MS analysis of the *P. crispum* plant. The major components and percentage are summarized in (Table 1) P. crispum contain at least 9 anti-microbial agent. Various pharmacological activities for plants extracts this is consisent with (Denning., 2003) that *P. crispum* contain activity compounds, which are due to It has biological activity against pathogens, including *Candida* spp Figure 3 explainthe rotation timeand area peckof all compounds in *P. crispum*.

Table 1: The chemical compounds of the methanol extract for flowering of the *P. crispum*

NO.	Chemical compounds	Chemical formula	Percentage %	Retention time
1	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	$C_6H_8O_3$	6.123	15.539
2	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	5.123	16.049
3	Thiocyanic acid, 2-propenyl ester	C_4H_5NS	5.83	17.724
4	1-(1'-pyrrolidinyl)-2-propanone	$C_{11}H_{21}N$	8.232	18.505
5	4H_Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-mEthyl	C18H32O16	5.83	19.411
6	2H-Pyran-3-ol, tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S[3.alpha.,6.alpha	C15H26O2	38.47	20.815
7	Benzene, 1,3-dimethyl-	C12H25N3	1.3045	21.042
8	Cyclopentanone, 2-(1-methylheptyl)	C13H24O	1.1965	22.829
9	Phenol, 3-methyl-6-propyl-	C10H14O	22.159	23.010

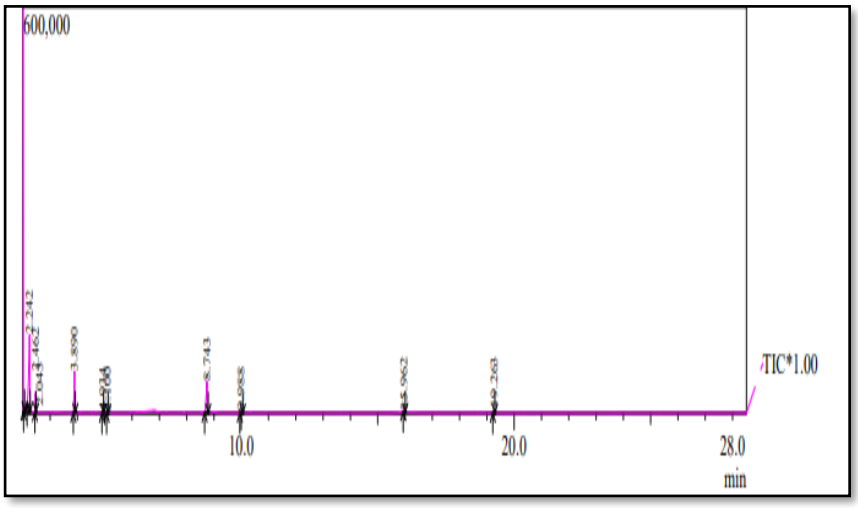

Figure 3: GC-MSMS P. *crispum*

3.4 Cytotoxicity

The cytotoxic activity of methanol extract was assessed by using different concentrations of human blood solution , the result showed no turbidity formation after 15, 30 and 60 min from adding 100µl of *P. crispum* extract to all concentrations of human blood solution, indicated no cytotoxic effect of fungal extract so the extract used in the study are safe and can be described as a therapeutic alternative to antifungals with adverse side effects on the host and this is in agreement with (Seyedjavadi, *et.al*., 2019).

3.5 Antifungal activity

The acquired results show that, within the permitted dose range, all tested fungal isolates responded differently to various doses of the flavonoid extract taken from *P. crispum*. (Hajifattahi, *et.al*., 2019) findings are consistent with the ME ability to effectively control the fungus. Depending on the extract content and the source from which the sample was taken, the isolates had varying degrees of fungal growth inhibition. This implies that various fungi may be more or less susceptible to the extract, and that the origin of the sample may also affect how they react. Antibiotics should never be used without contacting a doctor, as doing so can result in fungal colonization of the injured area.

This may account for changes in the fungi's sensitivity and resistance patterns. Antibiotics can be poisonous to fungi, but they can also create resistance in them to lessen their effectst(Denning., 2003) Based on the results obtained, different concentrations of the extract extracted from *P. crispum* affected the growth of all *Candida* isolates oral according to the approved dose. The methanol extract showed good efficacy in controlling *Candida*, and this is in agreement with (Hajifattahi, *et.al*., 2017). who concluded that *P. crispum* were effective against *Candida* and indicated that *P. crispum* could be a promising candidate as an anti-fungal agent and for controlling pathogens responsible for invasive fungal infections. phytochemical have inhibitory activity for all isolated *Candida* species.

The largest diameter of the inhibition zone is shown by *C.glabrata*. As for the antifungal agents used in the current study nystatin to compare it with the methanol extract, the effect was variable according to type This is similar to (Abdullah, *et.al*., 2022), and 5 of the isolates belonging to *C. albicanis* were resistant to nystatin and this is in agreement with (Kamath & Nayak., 2013). The other isolates differed in the diameter of inhibition according to the concentration used. Due to the indiscriminate use of antibiotics without consulting a physician, which encourages *Candida* colonization, changes in sensitivity and resistance can also be explained by the yeast's ability to withstand the toxic effects of the antibiotic and its ability to resist the development of resistance mechanisms (Hajifattahi, *et.al*., 2017).

Data in (Table 2) and (Figure 4) indicated that, all studied control treatmens superior on inhibition zone parameter of *Candida* isolates compared with the pathogens alone, the highest value was in 1000 ml concentrate of *P. crispum* plant and the antifungal properties in all of the *Candida* isolates.

Table 2: Effectiveness of the methanol extract for flowering of the *p. crispum* plant and the antifungal nystatin properties of the isolated *candida spp.*

isolated	Inhibition zone (mm)						
	***P. crispum* extract (µl/l)**				**Nystatin (µl/l)**		
	control	**100**	**500**	**1000**	**100**	**500**	**1000**
C. albicans	0.00	13.333±0.47	15.667±0.47	23.000 ±0.81	0.00	0.00	0.000
C. albicans	0.00	18.333 ±0.47	20.333±0.47	29.000±0.81	16.66±0.61	16.16±1.3	26.00±0.57
C. glabrata	0.00	15.667±0.47	23.333±1.2	29.667 ±0.77	16.66±0.81	20.66±0.33	26.66±0.43
C.tropicalis	0.00	11.333±10.94	12.667±0.41	15.667 ±0.99	15.30±0.81	18.13±0.67	20.16±0.47
C.dubliniensis	0.00	14.667±0.81	19.333±0.87	21.000±0.55	12.20±0.81	17.10±0.78	24.26±0.67

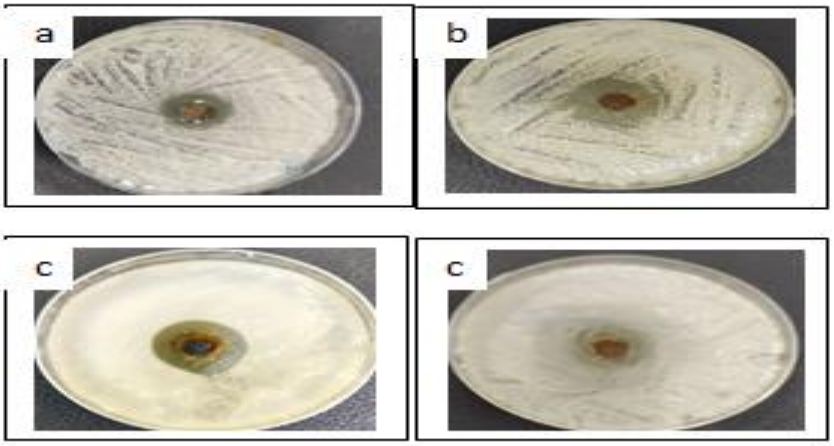

Figure 4: Antifungal activity of *p. crispum* against pathogenic isolates a(*C. albican*), b(*C.tropicalis*), c(c. glabrat), d(c.dubliniensis) for concitraction 100

4 Conclusions

The methanolic extract of *Petroselinum crispum* in this study showed good inhibition activity against the isolated *Candida* species.

Recommendations:

Further research is required to explore the potential applications of plant extracts in fungal infection treatment.

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References:

Abdullah, A. T., Jafar, F. N., & Saleh, A. A. (2022, December). Evaluation study of Matricaria chamomilla extract toward pathogenic isolates of Candida. *In AIP Conference Proceedings*, 254 (1). AIP Publishing. https://doi.org/10.1063/5.0121876

Ahmad Khan, M. S., Alshehrei, F., Al-Ghamdi, S. B., Bamaga, M. A., Al-Thubiani, A. S., & Alam, M. Z. (2020). Virulence and biofilms as promising targets in developing antipathogenic drugs against candidiasis. *Future science OA*, 6(2), FSO440. https://doi.org/10.2144/fsoa-2019-0027

Al-Dabbagh, B., Elhaty, I. A., Elhaw, M., Murali, C., Al Mansoori, A., Awad, B., et al. (2019). Antioxidant and anticancer activities of chamomile (Matricaria recutita L.). *BMC Research Notes,* 12 (1), 3. https://doi.org/10.1186/s13104-018-3960-y

AL-Maliki, Rehab. S. & AL-Ani, Zouhair. I. (2017). Antifungal resistance of Candida species isolated from Iraqi women infected with vulvovaginal Candidiasis. *Qmj,* 7 (11), 117-127. https://doi.org/10.28922/qmj.2011.7.11.117-127

Alrubayae, I. M., & Kadhim, K. F. (2020, November). Determination of oleaginous from non-oleaginous fungi using enzymatic and microscopic techniques. *In IOP Conference Series: Materials Science and Engineering*, 928 (6), 062011. https://doi.org/10.1088/1757-899X/928/6/062011

Alrubayae, I. M., Al-laaeiby, A., Minati, M. H., & ALibraheem, S. A. (2020). Determination of genetic relationships and pathogenicity of oral candidiasis etiological agents in pediatric malignant patients in Basrah Province, Iraq. *Systematic Reviews in Pharmacy*, 11(12), 180-188.

Arismunandar, R. N., Nosartika, I., Purnomo, B. N. R., & Antari, A. L. (2021). The Effectivity of Parsley (Petroselinum crispum) Extract on The Growth Inhibition of Candida Albicans. *Journal of Biomedicine and Translational Research*, *7*(3), 123-128. https://doi.org/10.14710/jbtr.v7i3.12555

Denning, D. W. (2003). Echinocandin antifungal drugs. *The Lancet*, 362 (9390), 1142-1151. https://doi.org/10.1016/s0140-6736(03)14472-8

Geneaid. PrestoTM Mini gDNA Yeast Kit [Internet]. p.1–7. Available from: http://www.geneaid.com/downloads/genomicdna/prestotm-mini-gdna-yeast-kit

Guevara-Lora, I., Bras, G., Karkowska-Kuleta, J., González-González, M., Ceballos, K., Sidlo, W., Rapala-Kozik, M. (2020). Plant-derived substances in the fight against infections caused by Candida species. *International journal of molecular sciences*, 21 (17), 6131. https://doi.org/10.3390/ijms21176131

Hajifattahi, F., Vand Yousefi, J., & Lesan, S. I. M. I. N. (2017). Comparison between the effect of *Petroselinum Crispum* with Nystatin on growth of Candida albicans (in vitro). *Res Dent Sci*, *14*(3), 157-161.

Kamath, P.; Pais, M., & Nayak, M. (2013). Risk of vaginal candidiasis among pregnant women. *Int. J. Curr. Microbiol. App. Sci*, 2, 141-146

Khan, M. S. A., Ahmad, I., Aqil, F., Owais, M., Shahid, M., & Musarrat, J. (2010). *Virulence and pathogenicity of fungal pathogens with special reference to Candida albicans*. In Combating fungal infections (pp. 21-45). Springer, Berlin, Heidelberg.

Kurtzman C.P., Fell J.W., Boekhout T., Robert V. & Robert, V. (2011). Methods for isolation, phenotypic characterization andvmaintenance of yeasts. *The Yeasts*. 87–110. http://dx.doi.org/10.1016/B978-0-444-52149-1.00007-0

Mamtani, S., Aljanabi, N.M., Rauniyar, R.P.G, Acharya, A. & Malik, B.H. (2020). Candida endocarditis: a review of the pathogenesis, morphology, risk factors, and management of an emerging and serious condition. *Cureus*, 12 (1). https://doi.org/10.7759/cureus.6695

Matić, I.Z., Juranić, Z., Savikin, K., Zdunić, G., Nađvinski, N. & Gođevac, D. (2012). Chamomile and marigold tea: chemical characterization and evaluation of anticancer activity; *Phytotherapy Research*. https://doi.org/10.1002/ptr.4807

Mesini, A., Mikulska, M., Giacobbe, D.R., Del Puente, F., Gandolfo, N., Codda G, Orsi, A., Tassinari, F., Beltramini, S. & Marchese, A. (2020). Changing epidemiology of candidaemia: Increase in fluconazole-resistant Candida parapsilosis. *Mycoses*, 63 (4), 361-368. https://doi.org/10.1111/myc.13050

Millon, L., Scherer, E., Rocchi, S., & Bellanger, A. P. (2019). Molecular strategies to diagnose mucormycosis. *Journal of Fungi*, 5(1), 24. https://doi.org/10.3390/jof501002

Mirhendi, H., Makimura, K., Khoramizadeh, M., & Yamaguchi, H. (2006). A one-enzyme PCR-RFLP assay for identification of six medically important Candida species. *Nippon Ishinkin Gakkai Zasshi*, 47(3), 225-229. https://doi.org/10.3314/jjmm.47.225

Nett, J. E., & Andes, D. R. (2016). Antifungal agents: spectrum of activity, pharmacology, and clinical indications. *Infectious Disease Clinics*, 30(1), 51-83.

Osman, M. Y., Taie, H. A., Helmy, W. A., & Amer, H. (2016). Screening for antioxidant, antifungal, and antitumor activities of aqueous extracts of chamomile (Matricaria chamomilla). *Egyptian Pharmaceutical Journal*, 15(2), 55. https://doi.org/10.4103/1687-4315.190402

Perez, C., Pauli, M., & Bazerque, P. (1990). An antibiotic assay by the well agar method. Acta Biol Med Exp, 15, 113-115 Perez, C., Pauli, M., & Bazerque, P. (1990). An antibiotic assay by the well agar method. *Acta Biol Med Exp*, 15, 113-115

Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., & Rai, M. (2013). Antifungal metabolites from plants. *Berlin, Heidelberg: Springer* (pp. 27–57). https://doi.org/10.1007/978-3-642-38076-1

Reinel, D. (2021). Non-dermatophyte fungi in onychomycosis—Epidemiology and consequences for clinical practice. *Mycoses*, 64(7), 694-700. https://doi.org/10.1111/myc.13251

Samaranayake, L.P., Raeside, J.M., Macfarlane, T.W., (1984). Factors affecting the phospholipase activity of Candida species in vitro. *J Med Vet Mycol.*, 22 201–7. https://doi.org/10.1080/00362178485380331

Seyedjavadi, S. S., Khani, S., Zare Zardini, H., Halabian, R., Goudarzi, M., Khatami, S., & Razzaghi-Abyaneh, M. (2019). Isolation, functional characterization, and biological properties of MCh-AMP1, a novel antifungal peptide from Matricaria chamomilla L. *Chemical biology & drug design*, 93(5), 949-959. https://doi.org/10.1111/cbdd.13500

Srivastava, J. K., & Gupta, S. (2009). Extraction, characterization, stability and biological activity of flavonoids isolated from chamomile flowers. *Molecular and cellular pharmacology*, 1(3), 138. https://doi.org/10.4255/mcpharmacol.09.18

Tang, S. S., Prodhan, Z. H., Biswas, S. K., Le, C. F., & Sekaran, S.D. (2018). Antimicrobial peptides from different plant sources:Isolation, characterisation, and purification. Phytochemistry, 154, 94–105. https://doi.org/10.1016/j.phytochem.2018.07.002

Torres, SR., Peixoto, CB., Caldas, DM., Akiti, T., Barreiros, MGC., Uzeda, Md. & Nucci, M. (2007). prospective randomized trial to reduce oral Candida spp. colonization in patients with hyposalivation. *Brazilian Oral Research*, 21 (2), 182-187. https://doi.org/10.1590/s1806-83242007000200015

White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols*, 18(1), 315-322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1

Wiederhold, N. P. (2017). Antifungal resistance: Current trends and future strategies to combat. *Infection and Drug Resistance*, 10, 249. https://doi.org/10.2147/IDR