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Original Article

Candida Berkh. (1923) Species and Their Important Secreted Aspartyl Proteinases (SAP) Genes Isolated from Diabetic Patients

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ABSTRACT

Background: *Candida* Berkh. (1923) occurs naturally in the body. But it becomes opportunistic fungi, meaning that it infects humans when there is any weakening of the immune system, such as exposure to chemotherapy, diabetes, or organ transplantation. Most species of *Candida* grow at a temperature between 20-40 °C and have a pH of 3-8. Human pathogens of *Candida* species include *C. albicans*, *C. glabrata*, *C. lusitaniae, C. parapsilosis*, *C. tropicalis* and *C. utili*. *C. albicans* has many virulence factors that facilitate injury process. Virulence factors are considered as a measure of pathogenicity, and it is in the form of fungal toxins, enzymes, or cell structures that facilitate infection, as well as pathogen resistance in different conditions. This study aimed to investigate the frequency of some secreted aspartyl proteinases (SAP) genes from some *Candida* species isolated from diabetic patients

Methods: *Candida* spp. were identified on CHROMagar medium by color of each species after incubation at 37 °C for 48 hours such as: *C. krusei*, *C. albicans*, *C. glabrata*, and *C. tropicalis*. Germ tube formation test was used to distinguish between germ tube-forming species, such as *C. albicans* and *C. dubliniensis* , from those that do not produce germ tube. A portion of the isolation was taken and placed in a test tube containing 0.5 ml of serum, and then incubated at 37 °C for 2-4 hours. A drop of serum was taken out and examined under a microscope for the presence of the germ tube. Growth at 45 °C, has the advantage of distinguishing between *C. albicans* and *C. dubliniensis* by culturing *Candida* spp. on SDA medium and, incubation for 48-72 hours at 45 °C.

Results: The results of isolation and diagnosis showed that *Candida* species grew at 37 °C for 48 hours on SDA medium, the shape of the colonies was round or oval-convex, and creamy white, shiny and smooth color. The results of the microscopic examination of the cells were spherical or oval, *C. albicans* was distinguished by its ability to grow at 45 °C and its ability to form the germ tube. The surface growth test showed the ability of *C. tropicalis* to grow surface on SDB medium. The medium of CHROMagar showed that *C. albicans* were light green in color. *C. glabrata* showed a pale pink color, while *C. tropecalis* showed a blue color and *C. dublineiensis* was dark green. DNA extracted samples, including 20 isolates, were used for four species of *Candida*, 1-9 *C. albicans*, 10-

16 *C. glabrata*, 17-18 *C. dubliniensis* and 19-20 *C. tropicalis.* Parts of the body (mouth, vagina, Urine) detect *SAP1* and *SAP7* genes by PCR, and after electrophoresis of the PCR product, the results were positive for all *Candida* species studied as all isolates that contained SAP1 (578bp) and SAP7 (466bp).

Conclusion: The SAP genes are among the main virulence factors for the occurrence of candidiasis. *SAP1* and *SAP7* genes are responsible for digesting and breaking down protein to penetrate the host's tissues.

Key words: Diabetic patients, *Candida* spp., Polymerase chain reaction, SAP1, 7 genes.

1. Introduction

Diabetes is known as an endocrine disorder that reduces or stops insulin production [1], which causes an increase in the amount of sugar in the blood for a long time and is one of the main diseases that afflict a large number of the world's population [2]. The symptoms associated with diabetes cause various body dysfunctions and organ failure, especially the heart, kidneys, blood vessels, and nerves [3]. *Candida* Berkh. (1923) occurs naturally in the body [4]. But it becomes opportunistic fungi, meaning that it infects humans when there is any weakening of the immune system, such as exposure to chemotherapy, diabetes, or organ transplantation [5]. Most species of *Candida* grow at a temperature between 20-40 °C and have a pH of 3-8 [6].

Human pathogens of *Candida* species consist of *C. albicans*, *C. glabrata*, *C. lusitaniae, C. parapsilosis*, *C. tropicalis* and *C. utili*. *C. albicans* has many virulence factors that facilitate injury process. Virulence factors are considered a measure of pathogenicity, and it is in the form of fungal toxins, enzymes, or cell structures that facilitate infection, as well as pathogen resistance in different conditions [7-9]. Aspartyl proteinase (SAP) has a major role in the infection through the degradation of the host surface proteins and thus the success of its operation. These enzymes are a family of ten genes (*SAP1-SAP10*), and this family has an important role in the adaptive response to *Candida* species. These enzymes work on tissue damage by decomposing their components and spreading them into the host and facilitating their adhesion and then invading [10].

Experiments that disrupted the gene encoding phospholipase demonstrated a decrease in virulence and a decrease in the ability of *C. albicans* to penetrate into host cells [11]. *C. albicans* and *C. tropicalis* have higher cell lysing capacity compared with other species such as *C. glabrata* and *C. parapsilosis*. The secreted proteins of aspartyl consist of a family with 10 species. According to their difference in pH activity, *SAP1-SAP3* has the highest activity and SAP 4-6 has a low pH activity, and this property helps *C. albicans* to exist as an opportunistic pathogen in a variety of different environments [12]. The enzymes can also counteract the host's immune response by resisting phagocytosis [13]. Therefore, this study aimed to investigate the frequency of some secreted aspartyl proteinases (*SAP*) genes from some *Candida* species isolated from diabetic patients

2. Material and Methods

2.1. Collections of samples

Twenty isolates of the *Candida* spp. were isolated from the oral, urine and vaginal mucosa. The clinical samples were collected from women and men seen at health-care units in the National

Center for Diabetes, Mustansiriyah University, and Baghdad, Iraq.

2.2. Morphological and microscopic examination

Sabourand Dextrase Agar was used to detect *Candida* spp. It was incubated at 73 °C for 48 hours, after which the isolates were examined for color and size where they appeared creamy white with round and oval shapes [14].

2.3. Diagnosis of *Candida* spp. on CHROMagar

Candida spp. were identified on CHROMagar medium by color of each species after incubation at 37 °C for 48 hours such as *C. krusei*, *C. albicans*, *C. glabrata*, and *C. tropicalis* [15].

2.4. Biochemical tests

2.4.1. Germ tube formation test

This test is used to distinguish between germ tube-forming species, such as *C. albicans* and *C. dubliniensis*, from those that do not produce germ tube. A portion of the isolation was taken and placed in a test tube containing 0.5 ml of serum, then incubated at 37 °C for 2-4 hours. A drop of serum was taken out and examined under a microscope for the presence of the germ tube [16].

2.4.2. Surface growth capability

Sterile tubes were prepared, the *Candida* spp. were inoculated onto SDB medium and mixed and then incubated for 48 hours to follow the surface growth of the *Candida* [17].

2.4.3. Growth at 45 °C

This test has the advantage of distinguishing between *C. albicans* and *C. dubliniensis* by culturing *Candida* spp. on SDA medium, then incubation for 48-72 hours at 45 °C [18] .

2.5. Molecular diagnosis of *SAP1* and *SAP7* gene by PCR technique

2.5.1. DNA methylation

The extraction process was carried out according to the directions of the manufacturer, Transgenbiotech–China.

2.5.2. Measuring the concentration and purity of the extracted DNA

A Nanodrop spectrophotometer was used to measure the concentration and purity of DNA extracted from samples, by reading the absorbance with a wavelength ranging between 260-280 nm. The extracted DNA purity was measured by the equation: DNA purity = OD260 / OD280
OD = optical density.

2.5.3. Gene selection

With regard to the PCR reaction, special primers were designed in our study to detect each gene.

2.5.4. Prepare the PCR master mix

The PCR reaction mixture was prepared using the Transgenbiotech-China kit according to the company's instructions.

The components of the reaction mixture mentioned for each sample were placed in tubes of the PCR kit, placed in a centrifuge at 12,000 rpm for 1 minute and then transferred to a PCR technology machine.

2.5.5. PCR thermocycler program

The polymerase chain reaction was carried out using a PCR technique after adjusting the thermocycler according to the program as mentioned in Table1.

Table1. Temperature program used to amplify the *SAP* genes in *Candida* species.

Cycle	Time	Temperature	Step	Stage number
1	3 m	94ºC	Denaturation	Stage 1
	30 S	94ºC	Denaturation	Stage 2
٣٠	30 S	59ºC	annealing	
	1 min	72ºC	Extension	
١	5 min	72ºC	Extension	Stage 3

2.5.6. Gel electrophoresis

The gel migration method was adopted to ensure the quality of the extracted DNA and to detect the polymerase chain reaction products by comparison with standard DNA volumes.

2.5.7. Amplification of *SAP1* and *SAP7*genes

A set of primer (Table 2) was used for amplification of *SAP1* and *SAP*7genes by using a single reaction mixture with final volume of (25 µL); the resulting PCR products were electrophoresis in 2% agarose gel to yield amplicon of 578bp for *SAP1* gene and 644bp for *SAP*7gene.

Table2. Primer sets used for amplification of *SAP1* and *SAP7* genes in *Candida* spp.

Genes	Primer	Sequence (5'→3' direction)	Product bp.
SAP1	Forward	AGATCCCCAGGTTTTGTCACT	578
	Reverse	GCAGCATTGGGAGAGTTGAG	
SAP7	Forward	TCGTGATGCTGTCCAAGGTT	644
	Reverse	CGATAGGAACAACGGCATGG	

3. Results

3.1. The Morphology and Diagnosis Characteristics of the isolated *Candida* spp.

The results of Table3 indicate that the species of *Candida* grew at 37 °C for 48 hours on SDA medium. The shape of the colonies was round or oval-convex, with a creamy white, shiny and smooth color. The results of the microscopic examination after staining with lactophenol showed that the cells were spherical or oval with greenish-blue borders, as a result of accumulation of the dye on the gram-positive wall. The growth test at 45 °C was used to distinguish between *C. albicans* and *C. dubliniensis* in particular for the great similarity in color and other characteristics. The test was also used to differentiate with other species in general. It was observed that only *C. albicans* among all species had the ability to grow at 45 °C. Formation gram tube distinguished *C. albicans* from other species, *C. albicans* form the germ tube when grown in serum for 2-3 hours. The result was positive when examined under a microscope. The surface growth test was used to distinguish the *C. tropicalis* from the other species due to its ability to grow surface on an SDB medium. The growth of *Candida* spp. on CHROMagar medium showed that *C. albicans* had a light green color, and *C. glabrata* was a pale pink color, while *C. tropicalis* showed a blue color and *C. dubliniensis* was dark green.

Table 3. Morphology and diagnosis characters of the isolated *Candida* spp.

***Candida* spp.**	**Colored on SDA**	**Growth at 45 °C**	**Formation gram tube**	**Surface growth formation**	**Colony color on CHROMagar**
C. albicans	Creamy Whit	Positive	Positive	Negative	Light Green
C. glabrata	Creamy White	Negative	Negative	Negative	Light Pink
C. tropicalis	Creamy White	Negative	Negative	Positive	Metallic Blue
C. dubliniensis	Creamy White	Negative	Positive	Negative	Dark Green

3.2. Molecular diagnostics

3.2.1. DNA extraction

The DNA was extracted by freezing and heating method. Figure 1 shows the electrophoresis process after DNA extraction using 1% agarose gel for 10 minutes at a voltage of 100 volts, then reduced to 70 volts for 60 minutes. The result of the DNA concentration, after measuring it in the Specteophotometer Nanodrop at the wavelengths 260 and 280 nanometers, was in ideal concentration to complete the PCR process (Table 4).

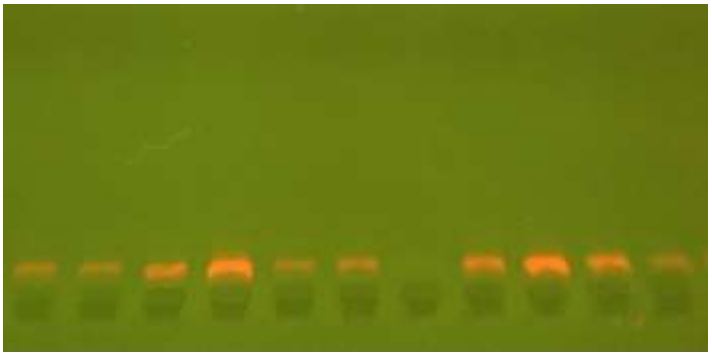

Figure 1. Extraction by freezing and heating from *Candida* spp.

Table 4. Concentration and purity of DNA of *Candida* spp. using the Nanodrop specteophotometer system.

No.	***Candida* spp.**	**The degree of purity**	**Concentrate DNA after freezing nanogram**	**The degree of purity**	**Samples sources**
1	*C. albicans*	1.62	12.09	1.68	Vagina
2	*C. albicans*	1.70	14.10	1.67	Mouth
3	*C. albicans*	1.70	20.60	1.86	Vagina
4	*C. albicans*	1.68	12.24	1.58	urine
5	*C. albicans*	1.63	16.41	1.63	Mouth
6	*C. albicans*	1.69	33.79	1.62	urine
7	*C. albicans*	1.75	32.52	1.72	urine
8	*C. albicans*	1.73	13.10	1.66	Mouth
9	*C. albicans*	1.72	13.20	1.79	Vagina
10	*C. glabrata*	1.72	21.48	1.68	Mouth
11	*C. glabrata*	1.53	22.90	1.69	Mouth

12	*C. glabrata*	1.84	23.85	1.80	urine
13	*C. glabrata*	1.69	250.79	1.71	Mouth
14	*C. glabrata*	1.74	82.08	1.84	Mouth
15	*C. glabrata*	1.58	5.77	1.70	Mouth
16	*C. glabrata*	1.56	2.49	1.79	Mouth
17	*C. dubliniensis*	1.42	17.33	1.82	Mouth
18	*C. dubliniensis*	1.62	18.13	1.77	Mouth
19	*C. tropicalis*	1.62	6.45	1.78	Vagina
20	*C. tropicalis*	1.66	6.45	1.70	Urine

3.3. Polymerase chain reaction (PCR)

The extracted DNA samples used included 20 isolates for four species of *Candida*, 1-9 *C. albicans*, 10-16 *C. galabrata*, 17-18 *C. dubliniensis* and 19-20 *C. tropicals*, which were isolated from three different parts of the body (Mouth, vagina, urine) within PCR to detect *SAP1* and *SAP7* genes, and after electrophoresis of the PCR product, the results were positive as all isolates contained *SAP1* (578bp) and *SAP7* (466bp) (Figures 2, 3, 4, 5, 6, 7)

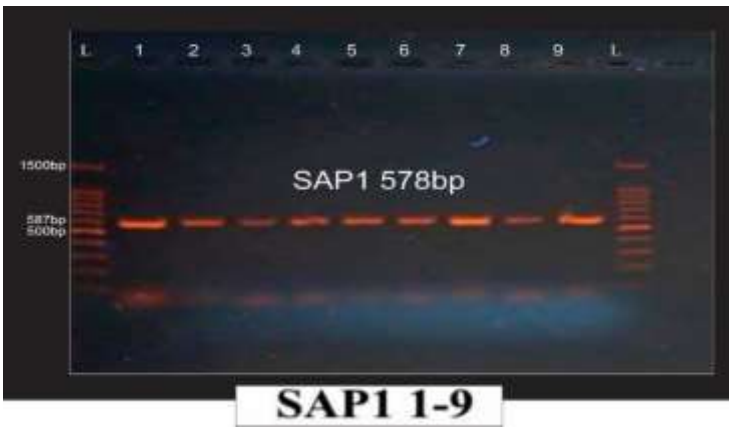

Figure 2. Process of electrophoresis of the PCR product of the SAP1 gene of *C. albicans* isolates aquarose gel at a concentration of 2% for 45 minutes under 100 volts at a temperature of 57 °C and photographed under ultraviolet irradiation after staining it with the standard DNA dye ethidium bromide (100-1500bp).

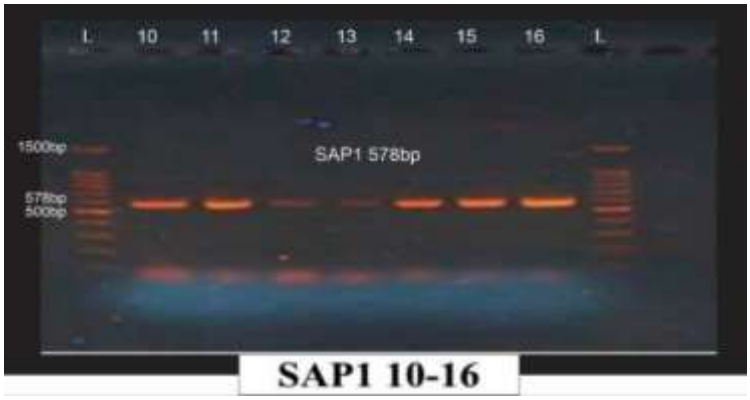

Figure 3. Process of electrophoresis of the PCR product of the *SAP1* gene of *C. glabrata* isolates aquarose gel at a concentration of 2% for 45 minutes under 100 volts at a temperature of 57 °C and photographed under ultraviolet irradiation after staining it with the standard DNA dye ethidium bromide (100-1500bp).

Figure 4. Process of electrophoresis of the PCR product of the *SAP1* gene of *C. dubliniensis* and *C. tropicalis* isolates aquarose gel at a concentration of 2% for 45 minutes under 100 volts at a temperature of 57 °C and photographed under ultraviolet irradiation after staining it with the standard DNA dye ethidium bromide (100-1500bp).

Figure 5. Process of electrophoresis of the PCR product of the SAP7 gene of *C. albicans* isolates aquarose gel at a concentration of 2% for 45 minutes under 100 volts at a temperature of 57 °C and photographed under ultraviolet irradiation after staining it with the standard DNA dye ethidium bromide (100-1500bp).

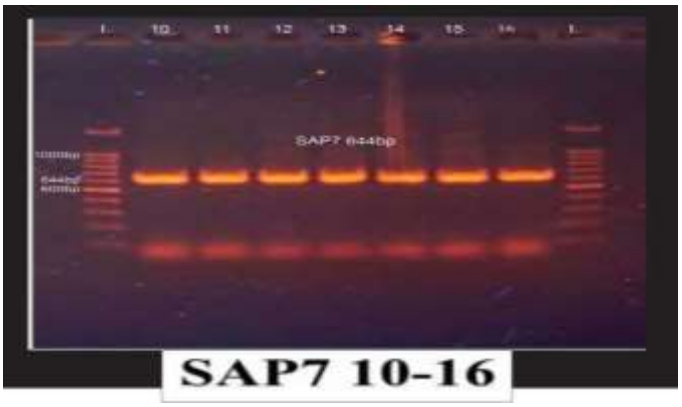

Figure 6. Process of electrophoresis of the PCR product of the SAP7gene of *C. glabrata* isolates aquarose gel at a concentration of 2% for 45 minutes under 100 volts at a temperature of 57 °C and photographed under ultraviolet irradiation after staining it with the standard DNA dye ethidium bromide (100-1500bp).

Figure 7. Process of electrophoresis of the PCR product of the *SAP7* gene of *C. dubliniensis* and *C. tropicalis* isolates aquarose gel at a concentration of 2% for 45 minutes under 100 volts at a temperature of 57 °C and photographed under ultraviolet irradiation after staining it with the standard DNA dye ethidium bromide (100-1500bp).

4. Discussion

Growth results on SDA are in agreement with [19], who confirmed that *Candida* spp. Colonies are white with a creamy, shiny and smooth color. As for the results of microscopy, they are in agreement with [20], indicating that the same characteristics were observed under a microscope. Growth test results at 45 °C agree with [21] noting that *C. albicans* is the only species that can grow at 45 °C. Our results on germ tube formation in *C. albicans* are also in agreement with [22, 23], observing that *C. albicans* is the only one capable of forming germ tube that is activated by the presence of a serum that has a role in causing pathogenicity. The surface growth test agreed with [24] who observed that *Candida tropicals* has the ability to grow surface differently from the other species. Diagnostic results on CHROMagar medium agree with [25, 26], showing that *Candida* spp. differ in their colors when grown on the medium that is considered a differential medium, and the mechanism of action depends on the chromogenic material that contains special enzymes that interact with different species of *Candida* and show different colors.

The SAP genes are one of the main virulence factors for the occurrence of candidiasis, and the results of our study are in agreement with [27], who mentioned that *Candida* spp. isolated from the mouth has several genes, the most important of which are *SAP1* and *SAP7*, which produce secreted aspartyl proteinases. They are responsible for digesting and breaking down protein to penetrate the host's tissues. Also they have shown [28] that the presence of these genes in epithelial cells is a stimulus to produce the enzymes necessary for *Candida* pathology. After the adhesion process the activity of the *Candida* spp. increases and it needs to secrete enzymes to penetrate and break down the host's tissues, including SAP enzymes encoded by the SAP family genes that help penetrate and invade different parts of the body[29].

Our study also agreed with [30, 31] who confirmed that *SAP7* is found in mucosal surfaces and it can also be found in other places, but its genetic expression is clear in oral and vaginal candidiasis. This was confirmed by our study, that the presence of the *SAP7* gene in *Candida* isolated from mouth, vagina, and urine and expression of *candida albicans* aspartyl proteinase genes SAP7, SAP8, SAP9, and SAP10 in human serum. Our

study also agrees with [32] who mentioned that the *SAP* genes are produced during infection, and this is what was observed from the results of the study, where it was found that only pathogenic isolates produce SAP genes through the experiment of forming a germ tube that is associated with the presence of these genes.

5. Conclusions

The *SAP* genes are one of the main virulence factors for the occurrence of candidiasis. Isolated from the mouth, it has several genes, the most important of which are *SAP1* and *SAP7*, to produce secreted aspartyl proteinases, which are responsible for digesting and breaking down protein to penetrate the host's tissues.

Authors' contributions

Thamer A.A. Muhsen, Mohsen Hashim Risan, Nawras N. Alqaysi designed this study, obtained and analyzed the data. Nawras N. Alqaysi supervised the collection of samples. Thamer A.A. Muhsen, Mohsen Hashim Risan proceeded to the data quality control and the manuscript drafting. Thamer A.A. Muhsen revised the final version.

Consent for publications

All authors agree to have read the manuscript and authorize the publication of the final version of the manuscript.

Conflict declaration

The authors declare that there is no conflict.

Conflict of interest

None of the authors have any conflict of interest to declare.

Availability of data and material

Data are available on request from the authors.

Funding/Support

The experiment was done based on the equipment of National Center for Diabetes Treatment and Research in Baghdad.

Ethics approval and consent to participate

Were obtained from oral, urine and vaginal diabetic patients (humans) were obtained from the National Center for Diabetes Treatment and Research in Baghdad, Iraq.

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References

1. Organization W H. (2016). World health statistics 2016: monitoring health for the SDGs sustainable development goals: *World Health Organization*. 161 pages,
2. Okur M E, Karantas I D, Siafaka P I. (2017). Diabetes Mellitus: a review on pathophysiology, current status of oral pathophysiology, current status of oral medications and future perspectives. *ACTA Pharmaceutica Sciencia*, 55(1): 61-82. https://doi.org/10.23893/1307-2080.APS.0555
3. khajeh h, Bahari A, Rashki A. (2021). TCF7L2 Polymorphisms in Type 2 Diabetes, Insight from HRM and ARMS Techniques. *Int. J. Adv. Biol. Biomed. Res.*, 9(3): 204-214. https://doi.org/10.22034/ijabbr.2021.525681.1351
4. Akortha E, Nwaugo V, Chikwe N. (2009). Antifungal resistance among *Candida* species from patients with

genitourinary tract infection isolated in Benin City, Edo state, Nigeria. *African Journal of Microbiology Research*, 3(11): 694-699.
5. Al-Oebady M A H. (2015). Isolation and identification of Candida species from vaginal, urine and oral swabs by chromagar Candida. *International Journal of Advanced Research*, 3(1): 948-956.
6. Muhsen T A, Hawar S N, Mahdi T S, Khaleel R. (2020). Effect of eucalyptus and myrtus extracts identification by gas chromatography-mass spectrometry on some species of candida as a model of medical plants. *Ann. Trop. Med. and Public Health*, 23(S10): 1-11. http://doi.org/10.36295/ASRO.2020.231032
7. Silva S, Negri M, Henriques M, Oliveira R, Williams D W, Azeredo J. (2011). Adherence and biofilm formation of non-Candida albicans Candida species. *Trends in microbiology*, 19(5): 241-247. https://doi.org/10.1016/j.tim.2011.02.003
8. Fazeli-Nasab B, Rahmani A F, Valizadeh M, Khajeh H, Beigomi M. (2021). Evaluation of Antimicrobial Activity of Some Medicinal Plants on Human Standard Bacteria and *Candida albicans*. [Research Article]. *Gene Cell Tissue*, 8(3): e113092. https://doi.org/10.5812/gct.113092
9. Fazeli-Nasab B, Sayyed R Z, Sobhanizadeh A. (2021). In Silico Molecular Docking Analysis of α-Pinene: An Antioxidant and Anticancer Drug Obtained from *Myrtus communis*. *Int. J. Cancer Manag.*, 14(2): e89116. https://doi.org/10.5812/ijcm.89116
10. Sariguzel F M, Berk E, Koc A N, Sav H, Demir G. (2015). Investigation of the relationship between virulence factors and genotype of Candida spp. isolated from blood cultures. *The Journal of Infection in Developing Countries*, 9(08): 857-864. https://doi.org/10.3855/jidc.5359
11. Pandey N, Gupta M K, Tilak R. (2018). Extracellular hydrolytic enzyme activities of the different Candida spp. isolated from the blood of the Intensive Care Unit-admitted patients. *Journal of laboratory physicians*, 10(04): 392-396. https://doi.org/10.4103/JLP.JLP_81_18
12. Naglik J R, Rodgers C A, Shirlaw P J, Dobbie J L, Fernandes-Naglik L L, Greenspan D, Agabian N, Challacombe S J. (2003). Differential expression of *Candida albicans* secreted aspartyl proteinase and phospholipase B genes in humans correlates with active oral and vaginal infections. *The Journal of infectious diseases*, 188(3): 469-479. https://doi.org/10.1086/376536
13. Macdonald F, Odds F. (1983). Virulence for mice of a proteinase-secreting strain of *Candida albicans* and a proteinase-deficient mutant. *Microbiology*, 129(2): 431-438. https://doi.org/10.1099/00221287-129-2-431
14. Deorukhkar S C, Saini S. (2014). Laboratory approach for diagnosis of candidiasis through ages. *International Journal of Current Microbiology and Applied Sciences*, 3(1): 206-218.
15. Hospenthal D R, Beckius M L, Floyd K L, Horvath L L, Murray C K. (2006). Presumptive identification of Candida species other than *C. albicans*, *C. krusei*, and *C. tropicalis* with the chromogenic medium CHROMagar Candida. *Annals of clinical microbiology and antimicrobials*, 5(1): 1-5. https://doi.org/10.1186/1476-0711-5-1
16. PM T. (2017). Bailey & Scott's Diagnostic Microbiology, St. Louis (pp. 1056 Pages): Elsevier.
17. Van Leekwijck W, Kerre E E. (1999). Defuzzification: criteria and classification. *Fuzzy sets and systems*,

108(2): 159-178. https://doi.org/10.1016/S0165-0114(97)00337-0

18. Pinjon E, Sullivan D, Salkin I, Shanley D, Coleman D. (1998). Simple, inexpensive, reliable method for differentiation of *Candida dubliniensis* from Candida albicans. *Journal of Clinical Microbiology*, 36(7): 2093-2095. https://doi.org/10.1128/JCM.36.7.2093-2095.1998
19. Abdulla H, Mustafa E A A. (2020). Rapid Detection of *Candida* species Isolated from Denture Stomatitis Patients using Phenotypic methods and Chromogenic agar media. *Al-Rafidain Dental Journal*, 20(1): 125-133. http://dx.doi.org/10.33899/rden.2020.126821.1029
20. Wibawa T, Aman A T. (2015). Virulence of *Candida albicans* isolated from HIV infected and non infected individuals. *SpringerPlus*, 4(1): 1-10. https://doi.org/10.1186/s40064-015-1215-0
21. Singh M, Chakraborty A. (2017). Antifungal Drug Resistance among *Candida albicans* and Non-albicans *Candida* Species Isolates from a Tertiary Care Centre at Allahabad. *J Antimicrob Agents*, 3(150): 2472-1212.1000150. https://doi.org/10.4172/2472-1212.1000150
22. Mehta A, Kumar M, Bhumbla U, Vyas A, Dalal A. (2018). Comparison of Different Media for Germ Tube Production by *Candida albicans*: A Retrospective Study. *Int. J. Curr. Microbiol. App. Sci*, 7(6): 819-823. https://doi.org/10.20546/ijcmas.2018.706.096
23. Salah N S, Muhsen T A, Risan M H. (2020). Antifungal Activity of Silver Nanoparticles Using Penicillium Chrysogenum Extract Against The Formation of Biofilm for *Candida Glabrata*. *Indian J. of Forensic Medicine and Toxicology*, 14(2): 306-311.
24. Hussein H, Dheeb B, Hamada T. (2019). Studying the *candida* resistance and sensitivity for some antifungals. *Journal of Biotechnology Research Center*, 13(2): 26-34.
25. Wohlmeister D, Vianna D R B, Helfer V E, Calil L N, Buffon A, Fuentefria A M, Corbellini V A, Pilger D A. (2017). Differentiation of *Candida albicans*, *Candida glabrata*, and *Candida krusei* by FT-IR and chemometrics by CHROMagar™ Candida. *Journal of Microbiological Methods*, 141: 121-125. https://doi.org/10.1016/j.mimet.2017.08.013
26. Ghasemi R, Rabiei M M, Lotfali E, Abolghasemi S, Ansari S. (2020). Candiduria: Prevalence, identification of isolated *candida* species and trends in antifungal susceptibility in hospitalized patients. *Novelty in Biomedicine*, 8(2): 71-76. https://dx.doi.org/10.22037/nbm.v1i1.26963
27. De Backer M D, Ilyina T, Ma X-J, Vandoninck S, Luyten W H, Vanden Bossche H. (2001). Genomic profiling of the response of *Candida albicans* to itraconazole treatment using a DNA microarray. *Antimicrobial agents and chemotherapy*, 45(6): 1660-1670. https://doi.org/10.1128/AAC.45.6.1660-1670.2001
28. Zhao X, Pujol C, Soll D R, Hoyer L L. (2003). Allelic variation in the contiguous loci encoding *Candida albicans* ALS5, ALS1 and ALS9. *Microbiology*, 149(10): 2947-2960. https://doi.org/10.1099/mic.0.26495-0
29. Naglik J R, Challacombe S J, Hube B. (2003). Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. *Microbiology and Molecular Biology Reviews*, 67(3): 400-428.

https://doi.org/10.1128/MMBR.67.3.400-428.2003

30. Ilkhanizadeh-Qomi M, Nejatbakhsh S, Jahanshiri Z, Razzaghi-Abyaneh M. (2020). Aspartyl Proteinase and Phospholipase Activities of *Candida albicans* Isolated From Oropharyngeal Candidiasis in Head and Neck Cancer Patients. *Jundishapur Journal of Microbiology*, 13(9). https://dx.doi.org/10.5812/jjm.105200
31. Mothibe J V, Patel M. (2017). Pathogenic characteristics of *Candida albicans* isolated from oral cavities of denture wearers and cancer patients wearing oral prostheses. *Microbial pathogenesis*, 110: 128-134. https://doi.org/10.1016/j.micpath.2017.06.036
32. Ganesan K, Banerjee A, Datta A. (1991). Molecular cloning of the secretory acid proteinase gene from *Candida albicans* and its use as a species-specific probe. *Infection and immunity*, 59(9): 2972-2977. https://doi.org/10.1128/iai.59.9.2972-2977.1991

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