Academic Science Journal

Effecting of Ribosomal-Inactivating Proteins on Gene Expression of *Cdr2* Gene in Pathogenic *Candida Albicans* Isolates

Yasser Muwafaq Mehdi[1*], Hadi Rahman Rasheed Al-Taai[2], Rabab Majead Abed[1] and Ali Mohammed Sadeq[2]

[1]Department of Biology - College of Education for Pure Sciences- University of Diyala

[2]Department of Biology - College of Sciences - University of Diyala

alqrtaniyasser@gmail.com

Received: 14 December 2022 Accepted: 9 February 2023

DOI: https://doi.org/10.24237/ASJ.02.01.725D

Abstract

Twenty isolates of *Candida albicans* were collected, during the study period from December / 2020 to February / 2021. For a group of 20 married women suffering from vaginal infections in Baqubah city. All the isolates were multidrug resistance against 5 antifungals from different groups according to the susceptibility test. The result of minimum inhibition concentration (MIC) for antifungal (fluconazole) values were between (256-64) µg/ml and MIC values for ribosomal inactivating proteins, between 325–5200 µg/ml . Conventional PCR technique was used to detect the *CDR2* gene that is a molecular marker of a functional resistance for azoles antifungals group, the results inducted the presence of *CDR2* gene 100%, it presented in the 20 isolates. By using Real-time PCR technique the *CDR2* gene gave a low expression after treatment (32)µg/ml with sub MIC concentration of fluconazole with average folding 0.1once and after treatment 325 µg/ml with sub MIC ribosomal inactivating proteins concentration again of compared with control with average folding 0.6 in different degrees in two isolates were multi-resistant to antifungals.

Key word: fluconazol, *CDR2*, gene expression, RT-qPCR

Academic Science Journal

تأثير البروتينات المعطلة للريبوسومات على التعبير الجيني لجين *CDR2* في عزلتين *Candida albicans* الممرضة

ياسر موفق مهدي[1] و هادي رحمن رشيد الطائي[2] و رباب مجيد عبد[1] و علي محمد صادق[2]

[1]قسم علوم الحياة - كلية التربية للعلوم الصرفة - جامعة ديالى

[2]قسم علوم الحياة - كلية العلوم - جامعة ديالى

الخلاصة

تم جمع عشرين عزلة من *Candida albicans* خلال فترة الدراسة التي بدأت من كانون الاول / 2020 وانتهت في شباط / 2021 ل 20 امرأة متزوجة تعاني من التهابات مهبلية. من مدينة بعقوبة.كانت جميع العزلات متعددة المقاومة ضد 5 مضادات فطريات من مجموعات مختلفة حسب اختبار الحساسية. كانت نتيجة التركيز المثبط الادنى MIC لقيم المضاد الفطري (فلوكونازول) بين (64 - 256) ميكروغرام / مل وقيم MIC لبروتينات المعطلة للرايبوسوم ، ما بين 325-5200 ميكروغرام / مل. تم استخدام تقنية تفاعل البوليميراز المتسلسل (PCR) التقليدية للكشف عن جين *CDR2* الذي يعتبر علامة جزيئية للمقاومة الوظيفية لمجموعة مضادات الفطريات الازولات ، وقد اظهرت النتائج إلى وجود الجين بنسبة 100٪ ، وتم الكشف عنه في 20 عزلة. باستخدام تقنية تفاعل البوليميراز المتسلسل في الوقت الحقيقي ، أعطى الجين *CDR2* تعبيرًا منخفضًا بعد المعالجة بتركيز (32) ميكروغرام / مل (دونMIC) لمضاد فلوكونازول بمتوسط 0.1 قابلة للطي مرة وبعد العلاج بتركيز 325 ميكروغرام / مل دون MIC للبروتينات مثبطة للريبوسوم. مرة أخرى مقارنة مع السيطرة مع متوسط الطي 0.6 درجة في عزلتين كانتا متعددة المقاومة لمضادات الفطريات.

الكلمات المفتاحية : فلوكونازول، *CDR2*، التعبير الجيني ، تفاعل البلمرة المتسلسل اللحظي .

Introduction

Opportunistic pathogens are assumed to include *Candida* species. However, the human body naturally contains these microbes. *Candida albicans* and non-albicans species infections are among the most common causes of nosocomial infections, which have substantial negative effects on public health **[**1**].** Over the past ten years, these infections have rapidly grown. According to estimates, vaginal candidiasis affects 23–49% of women of reproductive age; the majority of these cases are uncomplicated (fewer than 3–4 cases in a 12-month period), but a sizeable portion of patients (6–9%) present with recurrent infections (at least 3–4 cases in a

year), which is a persistent condition with complex pathogenicity and tolerance to antifungal treatment [2 and 3]. To live and expand throughout the human body, this opportunistic fungus has evolved a complex network of adaptations. In addition to producing hydrolytic enzymes, it may form biofilms and switch between yeast and hyphal morphology [4]. However, *C. albicans* also employs several adaptive strategies, such as enhanced *CDR2* expression, to evade the effects of antifungal therapies [5]. The foregoing characteristics have all led to the pathogen's medicinal relevance. With over 700,000 invasive cases and 2,000,000 instances of oral candidiasis reported each year, *C. albicans* is one of the most prevalent causes of human fungal infections [6]. The widespread use of first-line antifungals nowadays is being connected to a variety of negative side effects, such as hepatotoxicity and nephrotoxicity, as well as a surge in strains that are resistant to this kind of treatment[7]. In attempt to uncover agents that could stop the growth of harmful bacteria, natural products have initially been extensively explored. However, more recently, emphasis has been drawn to the role of natural products as virulence inhibitors and quorum quenching agents [8]. Insecticidal, antiviral, and antifungal properties have been revealed for ribosomal inactivating proteins (RIPs). anti-cancer measures [9], as different types of RIPs can Inhibition of ribosome activity in eukaryotic and prokaryotic [10] as well as its role . As an anti-enzyme, it works to stop the protein synthesis process in the target cells and cause apoptosis of these cells occurs [11.] Known as ribosomal-inactivating proteins in short Understanding which proteins interfere with ribosome activity in target cells by focusing on the ribosome's structural makeup It is irreparable. As is widely known, damaged ribosomes As the sites of protein production in the cell, they are inhibited by any structural or functional flaws. The cell will not be able to continue its essential functions during the process of protein synthesis, leading to her death. Many plants, as well as some other species like fungi, bacteria, and some varieties of algae Stirpe and, create RIPs [12] . *Truffles* belong to hypogeous ascomycete fungi that make a mycorrhizal association with some kind of vascular plants. The desert truffles are economically important and widely distributed in arid and semi-arid regions.

Academic Science Journal

Material and methods

1. **Isolation and identification of *Candida albicans* isolates**:

Twenty specimens of high vaginal swab (H.V.S) were collected during the period from December /2020 until February /2021 to 20 ladies suffering from vaginal infections in Baquba city, their ages ranged between 20-50 years**.** The isolates were identified by their colony characteristic on the Sabouraud dextrose agar**,** germ tub formation **t**est by using a human serum, using the KOH Direct quantity method by directly examining using 10% KOH and gram stain according (Kumar, 2010) ,using (CAC) Chrom Agar Candida and incubated at 37C° for 48 hours the isolation of yeast was done according to the colony color[13].

2. **Protein extraction method(RIPs)**

Based on the method mentioned in [14], The protein extract was prepared from 180 g of the dry powder of the *Truffle* spp. after dissolving it in 1 liter of extraction solution buffer for the extraction of ribosome-inactivating proteins.

3. **Protein Estimation**

The extracted protein was quantified according to the method described for [15] using the standard curve as shown in Figure 1

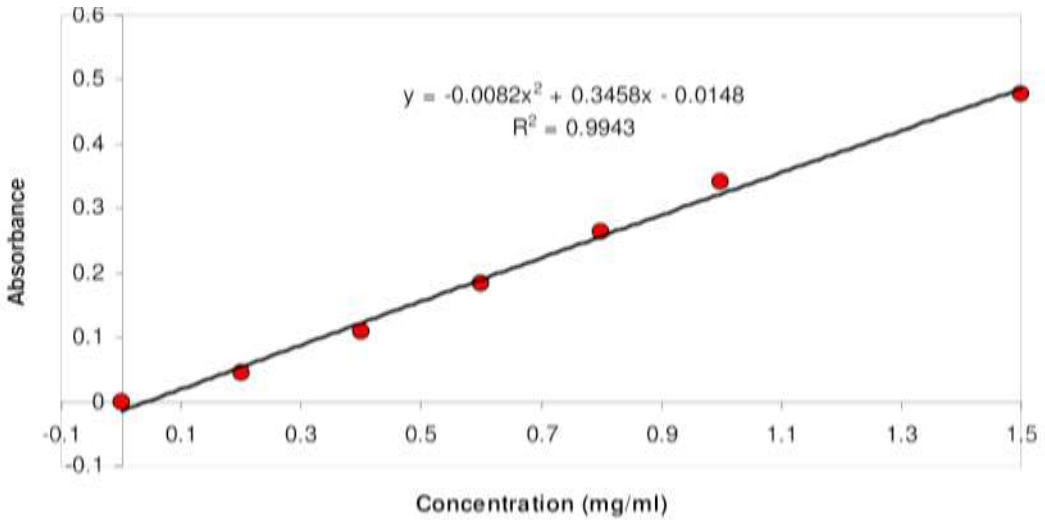

Figure 1: shown the standard curve of extracted protein

4. Antifungal susceptibility test and MIC for FLU and RIPs

To estimate the potential resistance of *C. albicans* isolates (5) antifungals from different classes were verified by Kirby-Bauer standard disk diffusion method to evaluate their resistance. , all isolates had been subjected to an antibiogram test according to CLSI 2019. [16]Antifungals were Amphotrecin B(100µg/disc), Keloconazole(30 µg/disc) , Metroconazol(100µg/disc), Fluconazole(10µg/disc) and Caspfungin (50 µg/disc) . In this investigation. The MIC for all isolates had been determined according to [17] by using the microtitre broth dilution standard method by serial dilution on Müeller Hinton Broth. All the 20 isolates were subjected to the Minimal inhibitory concentration test for Fluconazole and Ribosomal Inactivating Proteins. The reading of results had been made manually using a black card and electronically with an ELISA reader on 630 nm wavelengths.

5. DNA Extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from yeast growth according to the protocol of kite (ABIOpure) Extraction and Purification depending on the instruction of the manufacturing company of it. All 20 genomic extracts of isolates were screened by conventional polymerase chain reaction by using a specific primer to *CDR2* gene with F: **5`-**ATCTGGTGCTGGTAAGAC-3'and R: 5`-GCTGATGGTTGATGGATAG-3 and the annealing 54ºC, product length 501bp[18] . PCR reaction tubes were transferred into a thermal cycler that was programmed as follows: initial denaturation for 5 mints at 95°C (the conditions for each cycle were: 30 sec. at 940C, 30 sec. at, 55°C and 30 sec. at 72°C) and a final extension at 72°C for 5 mints. Amplified PCR products were detected by agarose gel electrophoresis.

6. RNA Extraction and quantitative RT-PCR of *CDR2* gene expression

RNA was extracted from the 2 *C. albicans* isolates from High Vaginal Swab (H.V.S) according to the protocol of TRIzol™ Reagent at several steps. The measurement of gene expression of the gene (*CDR2*) in the yeast isolates was done before the treatment with the fluconazole on 32 µg/ml concentration as sub MIC and before treatment with the Ribosomal in- activating protein on 325 µg/ml as sub MIC once , and after the treatment again , it was studied by RT-PCR. The

standard protocol was applied on extracted RNA with TRIzol™ GoTaq®(Syber green) Master Mix (1-Step RT-qPCR) System used according to manufacturer's instructions and applies to a single reaction where only template, primers, the gene expression was detection based on comparing the different values of mean CT *CDR2* gene and confirmed and normalized them by 2-mean ΔCT, where the ΔCT; is the difference in CT threshold cycle between the target and reference gene obtained from quantitative real time PCR(qRT-PCR) for *CDR2* (target gene) and 18S rRNA (reference gene) each isolate, so ΔCT= CT gene - CT reference gene, where CT; is threshold cycle value for the amplified gene**.** [19]. 18S rRNA (reference gene) F: 5′-ATA AGG GAA GTC GGC AAA ATA GAT CCG TAA-3´and R : 5′-CCT TGG CTG TGG TTT CGC TAG ATA GTA GAT-3′**.**

Results and Discussion

1. Collecting samples and recognizing *Candida albicans* from a vaginal swabs

All the twenty isolates were identified based on the morphological characteristics of yeast on Sabouraud dextrose agar ,the colonies appeared as white to cream color, round, curved, soft and smooth to wrinkled shape, characteristic yeast odor; proliferated quickly in 24 hours; and they matured in 3 days [20]. The result of germ tube production experiment was appeared as oval to spherical budding cells around epithelial cells. Numerous budding yeasts were seen on saline and KOH microscopy, however hypha components were not present .Another step in the identification process was to examine the microscopic characteristics of *Candida albicans* in gram stain. According to characteristic yeast *C. albicans* isolates were gram positive, round to oval, and had budding present as figure (2) [21]

Academic Science Journal

Figure 2: showed Gram stain of *Candida albicans* (40X)

A-germ tube formation B-Budding yeast.

Candida colonies that developed on SDA were subcultured on Hichrom agar media in order to identify *Candida albicans*. After an overnight incubation, the isolates grew well and produced colonies with different colors. According to the manufacturer's instructions, the colors of the colonies were observed and an assumptive identification was made of *C. albicans*, which was green, (Figure 3). With a sensitivity and specificity of 98%, the chromogenic medium Hichrom agar *Candida* provides a quick, easy way to identify common Candida species. [22]. The current investigation discovered that *C. albicans* could only be identified using Hichrom agar,

Figure 3: show the morphology and color of *Candida* colonies on Hichromo candida agar.

2. Result of Ribosomal inactivating proteins:

The extraction results indicated that the Ribosomal inactivating proteins extracted from the *Truffles* spp. were at a concentration of 9.800 mg/ml

3. Test for Antifungal Susceptibility and MIC of fluconazole and RIPs

Based on the inhibition zoon diameter indicated in CLSI (2019)[23]. All the 20 *Candida albicans* isolates underwent this test against five antifungal Amphotrecin B(100µg/disc), Keloconazole(30 µg/disc) , Metroconazol(100µg/disc), Fluconazole(10µg/disc) and Caspfungin (50 µg/disc**)** were Multi Drug Resistance (MDR). These findings are in agreement with Tamai [24], who found that all *Candida* isolates showed high resistance to azoles antifungal group drug and other drugs .Kaur [25],mentioned that all *Candida* isolates were (58.25%) exhibited fluconazole resistance. However, many *Candida* species, particularly *C. albicans*, now have higher rates of resistance to these antifungal medications, and the implications of this can be seen in the clinical situation. It is crucial to have antifungal medications that can treat these infections without causing greater resistance, which typically results from mutations in the pathway used for sterol biosynthesis, just like with azole treatments like fluconazole and clotrimazole. [26] . The results of minimum inhibitory concentration (MIC) of fluconazol and ribosomal inactivating proteins showed that the MIC of fluconazol was 64 µg/ml while the MIC of the ribosomal inactivating proteins was between (325-5200 µg/ml).

4. Detection of *CDR2* gene that responsible for fluconazole resistance:

The results showed that all the 20 *C. albicans* isolates were positive to *CDR2* gene figure (4). This result is agree with Hassanpour[27] In that study five *Candida* isolates resistant to fluconazole and three sensitive to fluconazole were isolated from 60 patients who suffering from candidiasis . and that Concerning of *CDR2* gene presence, this rate varied of its appearance to 91%, 78%, and 100% in FLU resistant, respectively, and in another study the CDR2 overexpression in *C. albicans* [28] .The main reason for the emergence of isolates of Candida resistant to azoles is due to the presence of *CDR1*,*CDR2* and *MDR1* gene, according to a study

[29] Maras says that molecular mechanism responsible for resistance to the two major classes of antifungal drugs, azole and echinocandins that have been investigated. As far as azole resistance is concerned, it correlates mainly with an increased expression of drug efflux membrane transporters due to upregulation of multidrug transporters belonging to ABC (ATP-binding cassette) transporters, *CDR1* and *CDR2* or to the major facilitators as *MDR1*. Overexpression of *CDR1, CDR2,* and *MDR1* is commonly observed in azole-resistant oral, systemic, and vaginal *C. albicans* clinical isolates.

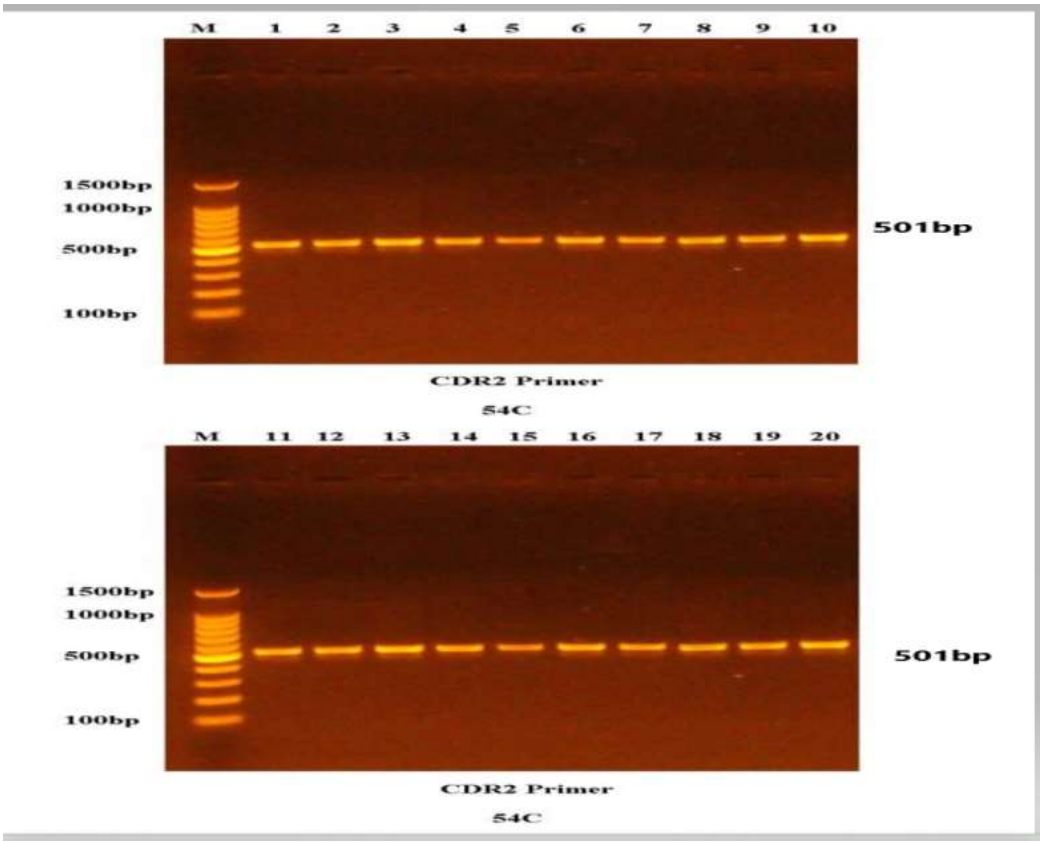

Figure 4: Gel electrophoresis for PCR product for *CDR2* gene of *Candida albicans* on 1.5% agarose gel at 7volt /cm for 1 hour with 100bp DNA ladder**).**

5. Real-time PCR Quantification of *CDR2* Gene Expression

The experiment of the quantitative RT-PCR reaction was completed by selected (2) out of the (20) of *C. albicans* isolates. The source of these isolates was (high vaginal swab). The *CDR2*

gene gave a high expression of different degrees in these 2 isolates. This variation refers to the source of isolates. The cycle of threshold (Ct) value of *CDR2* gene in the present study is shown in Tables (1a and b).

Table (1a): Ct values and fold of gene expression of *CDR2* gene of *C. albicans* that was treated with antifungal (fluconazole)

SOURS	SAMPLES	CT H.K 18S RRNA	CT *CDR2*	ΔCT	ΔΔCT	FOLDING= $2^{-\Delta\Delta CT}$	AVERAGE OF FOLDING
HVS	Ca18	34.18	29.52	-4.65	0.00	1.00	1
	Ca20	35.6	29.92	-5.14	0.00	1.00	
After treated with antifungal							
HVS	Ca18	33.36	31.14	-2.21	2.44	0.18	0.1
	Ca20	33.70	33.94	0.23	5.37	0.02	

Table (1b): Ct values and fold of gene expression of *CDR2* gene of *C. albicans* that was treated with ribosomal inactivating protein

SOURS	SAMPLES	CT H.K 18S RRNA	CT CDR2	ΔCT	ΔΔCT	FOLDING= 2-ΔΔCT	AVERAGE OF FOLDING
HVS	Ca18	34.18	29.52	-4.65	0.00	1.00	1
	Ca20	35.6	29.92	-5.14	0.00	1.00	
After treated with ribosomal-inactivating proteins							
HVS	Ca18	35.60	32.28	-3.33	1.33	0.40	0.6
	Ca20	36.68	32.00	-4.69	0.45	0.73	

According to the qRT-PCR results of gene*CDR2* it is noticed there was a change in the Ct of the gene after the treated and this leads to decrease the expression in *CDR2* as formula ($^{\Delta\Delta CT}$), so that it showed lower level of folding with the average of 0.1 after treated with fluconazole at the concentration of32µg/ml . Also it gave lower level of folding with an average 0.6 after treated with ribosomal-inactivating proteins at the concentration of325µg/ml . This result disagree with [30] Hajar that showed the expression of *CDR2* increased by 1.42 - fold after the treatment with fluconazole . According to this study, the gene expression of the *CDR2*gene was supposed to increase *CDR2* gene but the results showed a decrease after the *C. albicans* isolate was treated with the antifungal (Fluconazol) and this indicates that the resistance of the isolate

to the antifungal is phenotypic, not genetic. On the other hand, the conditions of the experiment may have affected the isolate, where the *C. albicans* isolate was stored for three months after The experience of susceptibility test to antifungal in the refrigerator, which may weaken its ability to resist even after the isolation is activated the inhibition of the gene expression of the *CDR2* gene was a result of treatment with RIPs that can be attributed This leads to an obstruction or stopping of the gene production process in the fungus cells, due to the activited enzyme for RIPs, which is of several types, including the type N-glycosidases, which target bonds C-N glycosidic nucleotide that binds the nitrogenous bases (adenine) at position 4324 of the large unit in rRNA, This activity is called glycosylase-N rRNA, and it leads to a stop in the interaction of factors Elongation of EF1 and EF2 dependent on GTPases with the ricin/sarcin-α loop and thus obstruction occurs in the functioning of the ribosome and its participation in protein synthesis makes the ribosome unable to bind EF-2 and stops protein synthesis in the transport step [31] and [32]. To our knowledge, this is the first study to report detection of gene expression for *CDR2* gene in Iraq after treated with ribosomal inactivating proteins, so there are no available results for comparison. On the other hand, the results of the current study partially agreed with[33].

Conclusions

The most studied antifungals that were resistant to *Candida albicans* were azoles such as fluconazole and ketoconazole. The gene under study (*CDR2*) was detected with a percentage 100% in isolates of *Candida albicans* isolated from clinical samples (HVS).

Recommendations

Performing additional molecular research to identify changes in the local *Candida albicans'* patterns using cutting-edge molecular techniques, and MLST analysis to confirm the pathogenicity and diversity of other *Candida albicans*. Moreover,developing a technique for extracting ribosome- inactivating proteins from multiple genera of fungi such as mushrooms and mixing these proteins with antifungals in calculated proportions to reduce the possibility of *Candida* resistance to these antibiotics and testing them *in vivo*.

References

1. Gajdács, M., Dóczi, I., Ábrók, M., Lázár, A., & Burián, K. (2019). Epidemiology of candiduria and Candida urinary tract infections in inpatients and outpatients: results from a 10-year retrospective survey. *Central European journal of urology*, *72*(2), 209.
2. Sobel, J. D. (2007). "Vulvovaginal candidosis." Lancet 369(9577): 1961-1971.
3. Blostein, F., Levin-Sparenberg, E., Wagner, J., & Foxman, B. (2017). Recurrent vulvovaginal candidiasis. *Annals of Epidemiology*, *27*(9), 575-582.
4. Baldewijns**,** S. (2021). Investigation and *in vivo* validation of the anti-Candida activity of probiotic metabolites in the vaginal niche (Doctoral dissertation, KU Leuven).
5. Ford**,** C. B., Funt, J. M., Abbey, D., Issi, L., Guiducci, C., Martinez, D. A., ... & Regev, A. (2015). The evolution of drug resistance in clinical isolates of *Candida albicans*. *elife*, *4*, e00662.
6. Bongomin**,** F., Gago, S., Oladele, R. O., & Denning, D. W. (2017). Global and multi-national prevalence of fungal diseases—estimate precision. *Journal of fungi*, *3*(4), 57.
7. Kably, B., Launay, M., Derobertmasure, A., Lefeuvre, S., Dannaoui, E., & Billaud, E. M. (2022). Antifungal drugs TDM: trends and update. *Therapeutic Drug Monitoring*, *44*(1), 166-197.
8. Rello, J., Parisella, F. R., & Perez, A. (2019). Alternatives to antibiotics in an era of difficult-to-treat resistance: new insights. *Expert Review of Clinical Pharmacology*, *12*(7), 635-642.
9. Wani, S. S.; Dar, P. A.; Zargar, S. M. and Dar, T. A. (2020). Therapeutic Potential of Medicinal Plant Proteins: Present Status and Future Perspectives.Current Protein and Pept. Sci., : 21(5): 443-487.
10. Luo, S., Coutinho, B. G., Dadhwal, P., Oda, Y., Ren, J., Schaefer, A. L., ... & Tong, L. (2021). Structural basis for a bacterial Pip system plant effector recognition protein. *Proceedings of the National Academy of Sciences*, *118*(10), e2019462118.

11. Weise, C.; Schrot, A.; Wuerger, L. T .; Adolf, J.; Gilabert-Oriol, R.; Sama, S.; Melzig, M. F. and Weng, A. (2020). An unusual type I ribosome-inactivating protein from Agrostemma githago L. Scientific Reports ,10:15377

12. Pizzo, E., Pane, K., Bosso, A., Landi, N., Ragucci, S., Russo, R., ... & Di Maro, A. (2018). Novel bioactive peptides from PD-L1/2, a type 1 ribosome inactivating protein from Phytolacca dioica L. Evaluation of their antimicrobial properties and anti-biofilm activities. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, *1860*(7), 1425-1435.

13. Marinho, S. A., Teixeira, A. B., Santos, O. S., Cazanova, R. F., Ferreira, C. A. S., Cherubini, K., & Oliveira, S. D. D. (2010). Identification of Candida spp. by phenotypic tests and PCR. *Brazilian Journal of Microbiology*, *41*, 286-294.

14. Park, S.W.; Prithiviraj, B.; Vepachedu, R. and Vivanco, J. M. (2006). Isolation and Purification of Ribosome-Inactivating Proteins. Methods Mol. Biol., 318:335-47

15. Bradford**,** j .A. and Heath, E. C. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein -dye binding. Analytical. Bioch., 72:248 -254.

16. Weinstein, M.P. and Lewis, J.S. (2019). Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing.* 30th ed. CLSI supplement M100 (ISBN 978-1-68440-066-9 [Print]; ISBN 978-1-68440-067-6 [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA:47..

17. HANCOCK, Robert EW; CHAPPLE, Daniel S. (1999) Peptide antibiotics. *Antimicrobial agents and chemotherapy*, 43.6: 1317-1323.

18. Zhang, M., Yan, H., Lu, M., Wang, D., & Sun, S. (2020). Antifungal activity of ribavirin used alone or in combination with fluconazole against *Candida albicans* is mediated by reduced virulence. *International journal of antimicrobial agents*, *55*(1), 105804.

19. Schmittgen T and Livak K (2008) Analyzing real-time PCR data by the comparative (CT) method. Nat. Protoc. 3, 1101–1108.

20. Bhavan**,** P. S., Rajkumar, R., Radhakrishnan, S., Seenivasan, C. and Kannan, S. (2010). Culture and identification of *Candida albicans* from vaginal ulcer and separation of enolase on SDS-PAGE. Inter. J. Bio. 2 (1): 84-93.

21. Alshaikh**,** N. A., & Perveen, K. (2021). Susceptibility of Fluconazole-Resistant *Candida albicans* to Thyme Essential Oil. *Microorganisms*, *9*(12), 2454.

22. Agarwal, A., Xie, B., Vovsha, I., Rambow, O., & Passonneau, R. J. (2011,). Sentiment analysis of twitter data. In *Proceedings of the workshop on language in social media (LSM 2011)* (pp. 30-38).

23. Wayne, R. O. (2019). *Light and video microscopy*. Academic Press.

24. Tamai, I. A., Pakbin, B., & Fasaei, B. N. (2021). Genetic diversity and antifungal susceptibility of Candida albicans isolates from Iranian HIV-infected patients with oral candidiasis. *BMC research notes*, *14*(1), 1-7.

25. Kaur, R., Goyal, R.; Dhakad, M. S., Bhalla, P. and Kumar, R. (2014). Epidemiology and Virulence Determinants including Biofilm Profile of Candida Infections in an ICU in a Tertiary Hospital in India,‖ Journal of Mycology, vol. 2014, Article ID 303491, 8 pages, 2014.

26. Rigo, G. V., & Tasca, T. (2020). Vaginitis: Review on drug resistance. *Current Drug Targets*, *21*(16), 1672-1686.

27. Hassanpour, P., Hamishehkar, H., Bahari Baroughi, B., Baradaran, B., Sandoghchian Shotorbani, S., Mohammadi, M., ... & Nami, S. (2021). Antifungal Effects of Voriconazole-Loaded Nano-Liposome on Fluconazole-Resistant Clinical Isolates of *Candida albicans*, Biological Activity and *ERG11, CDR1, and CDR2* Gene Expression. *ASSAY and Drug Development Technologies*, *19*(7), 453-462.

28. Espinel-Ingroff, A., Turnidge, J., Alastruey-Izquierdo, A., Botterel, F., Canton, E., Castro, C.Tejada, M. (2019). Method-dependent epidemiological cutoff values for detection of triazole resistance in Candida and Aspergillus species for the Sensititre YeastOne colorimetric broth and Etest agar diffusion methods. *Antimicrobial agents and chemotherapy*, *63*(1), e01651-18..

29. 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.

30. Hajar, A. A., Dakhili, M., Saghazadeh, M., Aghaei, S., & Nazari, R. (2022). Changes of Gene Expression in Candida albicans Isolates from Vaginal Infections by Effects of Zinc Oxide Nanoparticles and Fluconazole. *Jundishapur Journal of Microbiology*, *15*(6).

31. Fan**,** X.a. ;Wang, Y.; Guo, F.; Zhang, Y. and Jin, T. 2020. Atomicresolution structures of type I ribosome inactivating protein alphamomorcharin with different substrate analogs. International J. of Biolo., Macromolecules, 164: 265–276..

32. Lu, J.; Zhu, Z.; Zheng, Y. and Shaw, P. (2020). Engineering of Ribosomeinactivating Proteins for Improving Pharmacological Properties, Rev. Toxins, 12:167.

33. Kumar, R. (2009). Generation of monoclonal antibodies against covalently linked cell wall proteins of *Candida albicans* (Doctoral dissertation).