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Department of Biology



# **Effect of Ribosomal Inactivated Protein Extracted from *Terfezia* spp. on Some Virulence Gene Expression in *Candida albicans* Isolated from Different Clinical Sources**

A Thesis

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By

**Yasser Mowafaq Mehdi Alqrtani**

B.Sc. Microbiology /College of Science / Mustansiria University, 2002  
M.Sc. Potony-Mycology / College of Education for Pure Science / Diyala  
University, 2013

Supervised by

Asst. Professor.

Professor.

**Rabab Majeed Abed (Ph.D)**

**Hadi Rahman Rasheed Al-Taai (Ph.D)**

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## 1.1-Introduction

*Candida* species are considered opportunistic pathogens. However, these microorganisms are normally found in the human body. Infection resulting from *Candida albicans* and non-*albicans* species have increase significantly in the last decade and are among the leading causes of nosocomial infections, which result in serious public health problems (Gajdacs *et. al*, 2019).

The finding of *Candida* species in urine samples (candiduria) represents a diagnostic and therapeutic challenge for physicians working in both primary care or infectious diseases, as well as intensive medicine and surgery (Dias,2020). In healthy individuals, candidiasis occurs as a result of dysfunction in epithelial barrier of normal flora. The clinical manifestations can be acute, sub-acute, chronic to episodic.

The location of infection is usually localized to the mouth, throat, skin, scalp, vagina, finger, nails, bronchi, lungs, or the gastrointestinal tract, or becomes more complicated in systemic septicemia, endocarditis and meningitis (Zozo *et al.*, 2021). although Vaginal candidiasis is one of the commonest medical conditions affecting otherwise healthy women of reproductive age.

It was estimated that 23–49% of women of reproductive age suffer from vaginal candidiasis, with the majority having uncomplicated infections (less than 3–4 episodes within a 12-month period) and a considerable number (6–9% of the patients) presenting with recurrent infections (at least 3–4 episodes in 1 year); the latter is a stubborn condition characterized by complex pathogenicity and tolerance to antifungal treatment (Sobel, 2007; Blostein *et al.*, 2017). This opportunistic fungus has developed a wide network of adaptations in order to survive and spread throughout human organism. It can

group into biofilms, transit between yeast and hyphal morphology, and produce hydrolytic enzymes (Mba and Nweze, 2020).

On the other hand, *C. albicans* also uses certain adaptation traits, including increased expression of *CDR2* to avoid elimination by antifungal therapeutics (Fan *et al.*, 2022). All of the abovementioned traits have contributed to the medical significance of this pathogen. *C. albicans* is worldwide listed as one of the most common causes of human fungal infections with around 700,000 cases of invasive and 2,000,000 cases of oral candidiasis recorded annually (Bongomin *et al.*, 2017). The extensive usage of current first line antifungals is being linked to different side effects, including hepatotoxicity and nephrotoxicity, along with increased frequency of strains resistant to this type of treatment (Ivanov *et al.*, 2020), urging the development of novel therapies. Natural products have been extensively studied—at first in order to find agents that could inhibit growth of pathogenic microorganisms, but recently attention is focused on the role of natural products as inhibitors of virulence and quorum quenching agents (Wright *et al.*, 2022).

Ribosomal Inactivated Proteins (RIPs) have shown antiviral, antifungal, and insecticidal activities. Anticancer activities (Wani *et al.*, 2020), as different types of RIPs can inhibit ribosome activity in eukaryotic and prokaryotic (Luo *et al.*, 2021) 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 (Weise *et al.*, 2020).

Abbreviated as RIPs, know that they are those proteins that inhibit the function of ribosomes in target cells through targeting the structural structure of the ribosome. It can't be repaired. As is well known, ribosomes remnants of

damage They are places of protein synthesis in the cell, so any structural or functional defect in them leads to inhibition

In the process of protein synthesis in the cell, the cell will not be able to maintain its vital functions Hence her death. RIPs are produced by many plants, as well as some other organisms such as fungi bacteria, and some types of algae. ( Stripe and Oriol-Gilabert *et al*, 2017).

### **1.2- The aims of the current study :**

1- phenotypic detection of some virulence factors of *C. albicans* (Biofilm formation , Protase activity ,Phospholipase activity, Heamolytic activity) and detection of Multi Drug Resistance strains of *C. albicans* from different clinical specimens which were under study.

2- detection of some genes those related with virulence factors in presents in *C. albicans* which were : *ALS1, ALS3, SAP5, PLB1, CDR2, ERG11*.

3-study the effecting of RIPs on gene expression by using RT-PCR technique of two genes among in genes which are found in second aim before and after treated two *C. albicans* isolates with Fluconazol and Ribosomal Inactivated Proteins which it was extracted from *Terfezia* spp.

4- Molecular profiling for *C. albicans* .

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### الخلاصة :

تم جمع (238) عينة سريرية من المرضى (المراجعين والراقدين) ، تضمنت عينات من (حروق البراز - البول) من الذكور والإناث ومسحات مهبلية من نساء متزوجات ، بفئات عمرية مختلفة ، خلال فترة الدراسة التي بدأت من ايلول 2020 / وانتهت في شباط / 2021. في المستشفيات الحكومية في بعقوبة / ديالى من اصل 238 عينة ، أعطت (18.9% ) 45( عزلة نتيجة إيجابية لعزلات *Candida albicans* وزعت في(16، 11، 13، 5) عزلة من مسحات المهبل والبراز والبول والحرق على التوالي .

. تم التحقق من مقاومة وحساسية عزلات *C. albicans* لـ (5) مضادات فطريات من مجاميع مختلفة بوساطة طريقة Kirby-Bauer القياسية للانتشار القرصي. اوضحت النتائج بان نسبة المقاومة من قبل خميرة *C. albicans* لمضادات الفطريات المختلفة. كانت (91%) إلى (87%) ، Metroconazol (MZ) ، إلى (87%) ، Fluconazol (FLU) ، (69% ) إلى Ketoconazol (KCT) ، بينما اشارة النتائج الى انخفاض كبير في مقاومتها للأمفوتريسين B (27%) و (44.4%) إلى Caspofungin (CAS) ، كما بينت النتائج من *C. albicans* أن العزلات كانت 25 (55.6%) حساسة للأدوية المتعددة (multi drug sensitive) و20 (44.4%) (multi drug resistance) متعددة المقاومة للمضادات.

تم التحري عن عوامل الضراوة مظهرها من خلال تجارب تكوين الأغشية الحيوية Biofilm formation ، والنشاط الانحلالي ، و انتاج انزيمي Protase و Phospholipase ، ففي تجربة تكوين الغشاء الحيوي أظهرت النتائج ان *C. albicans* منتجة للغشاء الحيوي ، كانت 32/15 (46.9%) ضعيفة الانتاج للغشاء الحيوي و32/15 (46.9%) متوسطة و32/2 (6.3%) عالية الانتاج للغشاء الحيوي. كذلك بينت النتائج ان العزلات المهبلية هي الأعلى من بين العينات الأخرى في انتاجها للغشاء الحيوي ، وأظهرت نتيجة تجربة التحلل الدموي أن جميع العزلات أعطت تحلل دموي نوع ألفا. بينما اشارت نتائج تجربة انتاج انزيم Phospholipase أن جميع العزلات ظهرت منطقة مترسبة حول المستعمرة *Candida albicans* ، وبنسبة (84.4%) من اصل 32 عزلة *C. albicans* أظهرت قدرة انتاج عالية لانزيم Phospholipase بينما 5 وبنسبة (15.6%) كانت ضعيفة. في تجربة انتاج انزيم Protase أظهرت النتائج قدرة خمير *C.*