Activity of Extract Seed *Moringa Oleifera* on Gene Expression of (EFB1) Gene in *Candida dubliniensis* Isolated from oral Candidiasis to Cancer Patients

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Abstract

Gene expression of EFB1 gene in yeast *Candida dubliniensis* isolated from oral candidiasis in cancer patients was detected using by R-time PCR for some isolates before and during the yeast infection of the epithelial cells. The results showed a high rate of gene expression of T2 (42.104 ± 6.533) and a significant difference at the probability level P>0.01 compared with the treatment was c (1.034 ± 0.126) and the use of the Moringa oleifera extract was 25% to evaluate its effect on the gene expression (ALS1) before and during yeast infection of epithelial cells. The results showed that the gene expression of T1 was decreased by (0.129 ± 0.021) compared to (C) value (1.034 ± 0.126) .

As for the T2 treatment was gene expression at a rate (42.104 ± 6.533) high Compared with T3 treatment in which gene expression dropped at a rate (22.143 ± 4.176) and with a significant difference at the level of the probability of P ≤ 0.01.

***Keywords*:** *Moringa oleifera, (EFB1) Gene elongation factor 1-beta Candida dubliniensis , Candidiasis.*

Introduction

The immune system of the cancer patient is in a state of weakness and deterioration due to the disease and treatments used, especially chemotherapy and radiotherapy, which aims to eliminate cancer cells and reduce the spread within the body but It has side effects. it is transmitted through the blood circulation to all members and tissues of the body until his arrival the target, more vulnerable tissues are fast-growing tissues and permanent replacement cells such as bone marrow cells and gut tissue [1] The mouth contains the appropriate environmental conditions for the growth of colonies of yeast, which increase the proportion of acidic function of saliva, and the yeast of *Candida dubliniensis* opportunistic fungi that invade the body in the case of weakened immunity and This is aided by many of the virulence factors that are produced with the help of the (EFB1) gene[2-4] . elongation factor 1-beta (EFB1) Translation elongation factor 1 beta; stimulates nucleotide exchange to regenerate EF-1 alpha-GTP for the next elongation cycle which facilitates binding of aminoacyl-tRNA to the ribosomal A site TO Producing protein [2].

This study were conducted in order to achieve our aims, like the isolate and diagnose yeast *Candida dubliniensis* infections from oral cancer patients, the use of aqueous Extract of the seeds of *Moringa Oleifera* to test its effect on the growth of the yeast Candida dubliniensis and use seeds Extract *Moringa Oleifera* to assess its effect on gene expression of (EFB1) gene before and during a yeast infection of epithelial cells.

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Materials and Methods

Samples Collection

(100) samples (oral Scrape) was collected from patients that infected with cancer, which treated with chemotherapy and who appear to have symptoms of oral candidiasis disease and reviewers of the tumors Division in Teaching Hospital of A-Diwanyiah city for the period from the first of March 2018 to the end of August 2018.

Yeast Isolation

Candida dubliniensis was isolated from From Oral Candidiasis To Cancer Patients by culture in the media of Sabourauds dextrose agar (SDA) and incubated in degree (37)C for (7) days, and examined later the growth colonies scrupulous in terms of color, shape, textures and examined the optical microscope after staining dye gram to note the shape and size of the yeast and a growth test at a temperature of (45)C AND growth sample on chromagar candida [3] A hundred samples were collected (oral Scrape) from healthy individuals as a control to compare

Prepare an aqueous Extract of the seeds *Moringa oleifera*

It was Prepare depending on the method Price [4], and then were prepared differently than concentrations, namely, (25)% sterilized Concentrations filtration using filter paper (0.45) microns.

Detection of active compounds in the Extract:

Followed the method contained in the Fahmy [5] for the detection of alkaloids while following the method contained in the Shihata [6]for the detection of tannins and saponins, flavonoids have either followed the method contained in the AL-Khazragi [7] for disclosing them

Experience Design:

1. Prepare of Yeast stuck depending on the method of Clayton [8].

2. Preparation of epithelial cells: Preparation was done depending on the method Chritchaly and Douglas [9].

- **The first treatment (T1):** mixing (0.5 ml) of the yeast is stuck with (0.1) ml *Moringa oleifera* seed Extract concentration of (25)% in the Eppendorf tube.
- **The second treatment (T2):** mixing (0.5)ml of the yeast is stuck with (0.5)ml of the epithelial cells of the mouth is stack in the Eppendorf tube.
- **The Third treatment (T3):** mixing (0.5)ml of the yeast is stuck with (0.5)ml of the epithelial cells of the mouth is stuck in the Eppendorf tube.
- **Control (C):** put (0.5)ml of yeast cells is stuck in the Eppendorf Tube.

All transactions were incubated at a temperature $(37)^0$C four (48) hours and repeated transactions five times, then completed the steps of qRT- PCR.

Quantitative Reverse Transcription Real- Time PCR (RT-qPCR) test:

It is an examination of a series of polymerase chain reaction in real-time quantitative (reverse reproduction) to measure the levels of quantitative DNA sender (mRNA) to denote the amount of gene expression of genes EFB1 gene expression as well as the use of gene of (Act1) structured record to calculate gene expression, has this test was conducted four transactions T1, T2, T3, C depending on the method in Tavanti [10].

Table (1) DNA sequencing primers and their Nitrogenous base**s of Bioneer Corporation - South Korea**

Primer	Sequence		Amplicon
EFB1	F	ATACGCTGCCAAGAAAGCTG -3¢ 5¢-	127bp
	R	TTCGACGGCTTTAACGTTGG -3¢ 5¢-	
Act1	F	5¢- TGTGTAAAGCCGGTTTTGCC -3¢	136bp
	R	TTGGATTGGGCTTCATCACC -3¢ 5¢-	

Genbank code EFB1: XM_002420026.1 and *Act1*: XM_717232.1.

Real-Time PCR data analysis:

The results were analyzed chain reaction in real time through using the use of quantitative way livak method, which was developed by Livak and schmittgen [11]. Statistical analysis, Results were analyzed statistically using the gene expression of the way one way ANOVA LSD at the level of probability of (P<0.01) using SPSS software.

Results

Isolation and diagnosis

Type *C dublineinsis*, (30 (30%), was diagnosed in cancer patients, while *C dublineinsis*1 (1%) was diagnosed in healthy people. The isolated species have been confirmed using growth on chromagar candida and the Candida system . The use of this type of diagnosis is preferred because it is fast and accurate, but it is very expensive and sensitive to the pollution **.**

Qualitative detection of some of the active compounds of aqueous Extracts of the seeds of *Moringa oleifera* .The table shows (2) qualitative detection results of the active ingredient in the aqueous Extract of the seeds of *Moringa oleifera*, which pointed to the presence of saponins and flavonoids and tannins

Table (2): Quality Detection for some active compound for *Moringa oleifera*

Substance	Tannins	Alkaloids	Flavonoids	Saponins
aqueous extracts of the seeds of Moringa oleifera	+	-	+	+

Gene expression of EFB1**gene using the method (2-ΔΔCT Livak method)**

The results shown in Table (3) and Figure (1,2) indicate a high rate of gene expression (42.104 ± 6.533) in T2 and a significant difference at P ≤ 0.01compared to group C (1.034 ± 0.126 (

Effect of aqueous extracts of the seeds of *Moringa oleifera* on gene expression of EFB1gene before and during yeast infection of epithelial cells The results in Table (3) and Figure (1,2) indicate that the gene expression of EFB1gene was in group C (1.034 ± 0.126) To 0.129 ± 0.021in the treatment T1 and significant difference at the probability level of P ≤ 0.01, while for the treatment T2 the value of gene expression (42.104 ± 6.533) was high compared to the treatment T3, which decreased the expression of gene and (22.143 ± 4.176) with a significant difference at P ≤ 0.01.

Table (3) :Gene Expression of EFB1**gene using the(Livak method $2^{-\Delta\Delta CT}$)**

Treatment Isolation	CT (EFB1 gene)	CT (actin)	ΔCT (Test)	ΔCT (control)	ΔΔCT	Fold change (2^-ΔΔCT)	Mean + Std.Error
T1	34.67	35.26	-0.59	-3.46	2.87	0.137	0.129 ± 0.021
T1	34.00	34.61	-0.61	-3.46	2.85	0.139	
T1	34.07	35.15	-1.08	-3.46	2.38	0.192	
T1	34.83	35.17	-0.34	-3.46	3.12	0.115	
T1	35.30	34.74	0.56	-3.46	4.02	0.062	
T2	27.65	35.28	-7.63	-3.46	-4.17	17.952	42.104 ± 6.533
T2	26.71	35.53	-8.82	-3.46	-5.36	41.016	
T2	26.31	35.29	-8.98	-3.46	-5.52	45.913	
T2	26.38	35.46	-9.08	-3.46	-5.62	49.279	
T2	26.48	35.76	-9.28	-3.46	-5.82	56.359	
T3	27.23	35.71	-8.48	-3.46	-5.02	32.465	22.143 ± 4.176
T3	28.07	35.42	-7.35	-3.46	-3.89	14.790	
T3	27.44	35.58	-8.14	-3.46	-4.68	25.649	
T3	27.45	35.70	-8.25	-3.46	-4.79	27.707	
T3	27.60	34.40	-6.80	-3.46	-3.34	10.102	
C	31.63	35.31	-3.68	-3.46	-0.22	1.166	1.034 ± 0.126
C	31.63	35.56	-3.93	-3.46	-0.47	1.387	
C	31.74	35.15	-3.41	-3.46	0.05	0.967	
C	31.74	34.51	-2.77	-3.46	0.69	0.622	
C	31.88	35.38	-3.50	-3.46	-0.04	1.028	
Mean C	31.724	35.18	-3.46				

T1 *Candida dubliniensis* + *Moringa oleifera* seed Extruct ,T2 *Candida dubliniensis* + Epithelial Cell ,T3 *C. dubliniensis* + Epithelial Cell + *Moringa oleifera* seed Extru Ct T1 *C. dubliniensis* + *Moringa oleifera* seed Extru Ct ,T2 *C. dubliniensis* + Epithelial Cell ,T3 *C. dubliniensis* + Epithelial Cell + *Moringa oleifera* seed Extru Ct , C *C. dubliniensis* only , CT: q PCR Threshold Cycle number

(2-ΔΔ CT Livak method) as following: First, the CT of the target gene was normalized to that of the reference (ref) actin gene, for both the test isolates and the Control isolates group.

Δ CT(test) = CT(target, test) – CT(ref, test)

Δ CT(Control) = CT(target, Control) – CT(ref, Control)

Second, the Δ CT of the test isolates were normalized to the Δ CT of the Control:

ΔΔ CT = Δ CT(test) – Δ CT(Calibrator) ,Fold Change of relative gene expression was calculated by following equation = ($2^{-\Delta\Delta CT}$): Normalized expression ratio

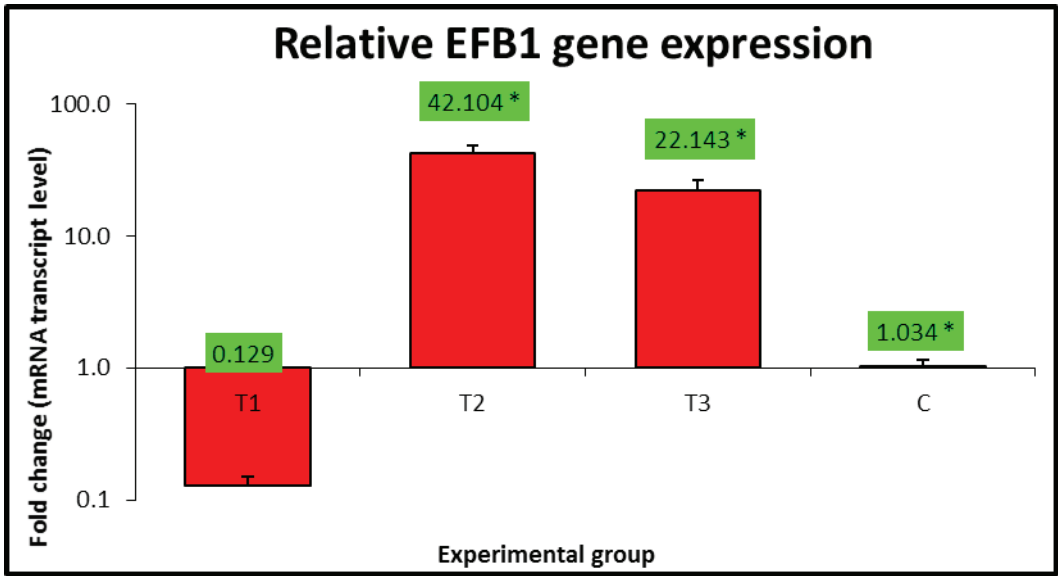

Figure (1): The graph of relative gene expression of the EFB1 gene in the experimental coefficients relative to the control group. *Indicates a significant difference in LSD (mean difference) at P ≤ 0.01.

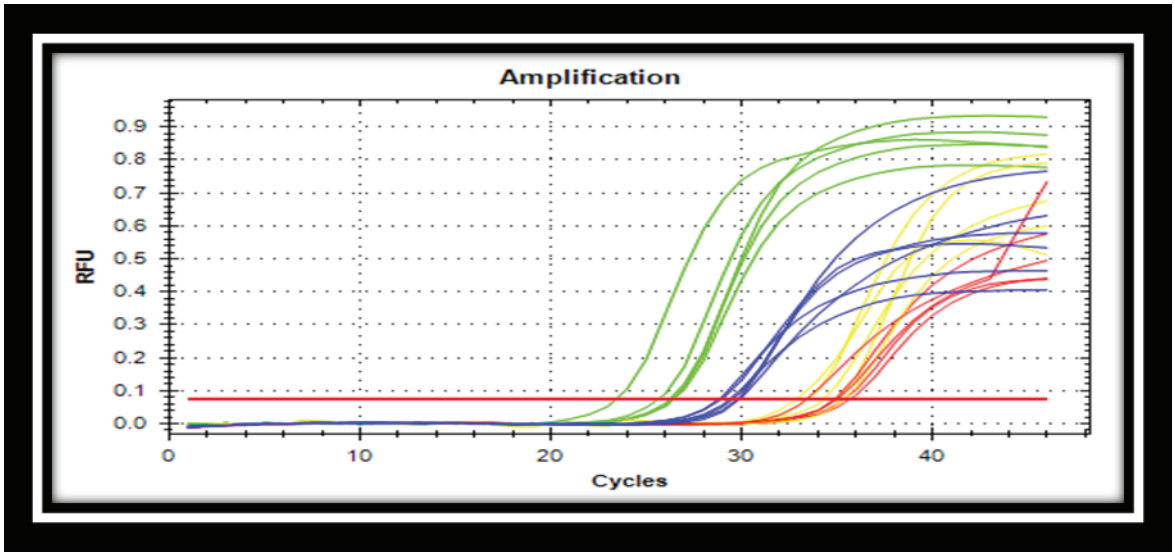

Figure (2) :amplification curve of the interaction of Real-Time PCR and private EFB1 gene in *Candida dubliniensis* , accounting for curves as follows:

1. curves with a yellow color control group (C).

2. with red *Candida dubliniensis* group curves with *Moringa oleifera* seeds extract T1.

3. Curves-green *Candida dubliniensis* group with epithelial cells T2.

4. curves of blue *C. dubliniensis* group with *Moringa oleifera* seed extract and epithelial cells T3

Discussion

Out of the 100 sample under study, 30 (30. %) samples were identified as C. dubliniensis, The results of this study were related to the results of Abdelghani et al., [12] *C. dubliniensis* appears to be a minor component of the normal oral flora of humans. Fewer than 10% of germ tube-positive yeasts in culture collections or from the oral cavities of healthy individuals have been identified as *C. dubliniensis* [13]. However, as with other yeasts, immunosuppression and the use of antimicrobials permit *C. dubliniensis* to increase in numbers and eventually to cause oral candidiasis. Approximately 25% of HIV-infected patients may be colonized with the yeast, and *C. dubliniensis* has been isolated from the oral cavity of 30% of patients with AIDS and oral candidiasis [14]. *C. dubliniensis* was implicated as a pathogen in linear gingival erythema in an HIV-infected child [15]. Although several independent reports have described the recovery of *C. dubliniensis* from patients with HIV infection and from those without since 1995, it was not until recently that invasive disease was reported. In 1999, *C. dubliniensis* was reported as a cause of fungemia in 2 recipients of bone marrow transplantations and in 1 patient with chemotherapy-induced neutropenia [16].

We note the high genetic production of expression for the treatment of T2 rate of the EFB1 gene for treatment C and team's morale high at the level of the probability $P \leq 0.01$ reasons for the rise could be due to the fact that epithelial cells are considered the center of a vital contain her assistant For their growth and reproduction and existence is a catalyst for the production of enzymes in the pathogenesis of C. dubliniensis and Which EFB1 gene contributes to its construction[17] While noting low the genetic production of expression for the treatment of T1 rate of the EFB1 gene for treatment C high spirits and differences at the level of the probability $P \leq 0.01$ may be due to contain the extracted substances that reduce or disable the gene regulation in the cell Such as tannins that have the ability to link with cell proteins by bonds, hydrogen or covalent bonds and the formation of complexes with Thus, working to disable the enzymes and protein carrier in the cell, and its concentrations few of them are working on narrowing micro tubule in the cell wall and thereby prevent the entry of materials into and out of the cell and Cell abstraction of minerals such as iron and magnesium [18] The withdrawal of magnesium from the cell leads to an effect on the work of RNA polymerase, which is involved in the process of cloning mRNA of DNA, the first step to build protein because the catalyst helps to add the nucleotides and thus loss reduces gene expression [19]

Conclusions

Aqueous extracts of the seeds of *Moringa oleifera* contain effective compounds that has an effective role in reducing the rate of gene expression of EFB1 gene in *Candida dubliniensis* .

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Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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