

The relationship between the aquatic extracts of the *Moringa oleifera* seeds and the genetic expression of (SAP5) gene in *Candida albicans* accompanying cancer patients

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Abstract

It has been detected gene expression using the Real - time technique for some Isolations *Candida albicans* before and during infection to cells epithelium , The results be high rate of gene expression (37.156 ± 7.66049) to T2 treatment and significantly difference at the level of the probability $P \leq 0.01$, compared with the C treatment that at a rate (1.040 ± 0.32229). As the use of an extract of the seeds of *Moringa Oleifera* In the concentration of 25% to assess its impact on the gene expression (SAP5) before and during infection yeast cells of epithelial and the results were reduced gene expression treatment T1 at a rate (0.074 ± 0.01542) comparison with the treatment C (1.040 ± 0.32229) and teams significantly at the level of the probability $P \leq 0.01$, As for the T2 treatment was gene expression at a rate (37.156 ± 7.66049) high Compared with T3 treatment in which gene expression dropped at a rate (19.565 ± 8.82162) and with a significant difference at the level of the probability of $P \leq 0.01$.

Keywords: *Moringa Oleifera*, SAP5 gene, *C.albicans*, Candidiasis, Real-time PCR

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 its use is not without of 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 yeast of *C.albicans* opportunistic fungi that invade the body in the case of weakened immunity . It's have to virulent factors , including production of secreted aspartyl proteinases (Saps) It is a major analytical factor which is a cellular exogenous enzyme that works on tissue analysis, which leads to easy penetration and access to the rest of the body [2].The study of molecular genetics of some virulence factors of the yeast *C. albicans* can lead to knowing and understand the role of Virulence factors in the development of candidiasis [3].Human attention has been directed towards the means of relieving pain and disease with compounds with few side effects, using plants and herbs because the drugs used in the treatment of fungal infections have side effects in addition to the therapeutic effects used to it [4] *Moringa Oleifera* is one of the plants that contain a large variety of chemicals that have the capacity for prevention and cure of many diseases and has many medical uses amounts as well as it have nutritional value high, and every part of the *Moringa Oleifera* [5] ,The seeds, for example, its importance in the water purification bacteria positive and negative dye gram, they contain active substances anti-bacterial in addition to being contains a knockdown-fungal fungicidal called terygospermin that working to destroy the cell membrane of the fungus as a result of overlap with bilateral fat layer in the membranes which leads to separation external and internal membranes and swelling of the cell due to the ingress of water at it from the vents thus, a detonation and death [6], in addition to fit on saponins and tannins and Alkaloids and Flavonoids and that many of the studies proved that it has the effect of killer cells fungi [7].This study were conducted in order to achieve our aims, like the isolate and diagnose yeast *C.albicans* infections from oral cancer patients and use seeds

extract *Moringa Oleifera* to assess its effect on gene expression of (SAP5) gene before and during a yeast infection of epithelial cells.

Material and Methods

Samples Collection:

A hundred samples (oral swab) 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 .it was diagnosed relying on [8]

Protease Production Test

It was conducted depending on the method of [9]

Prepare an aqueous Extract of the seeds *Moringa oleifera*

It was Prepare depending on the method Price [10], 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, [11]for the detection of alkaloids while following the method contained in the Shihata [12]for the detection of tannins and saponins, flavonoids have either followed the method contained in the AL-Khazragi [13]for disclosing them Second:Study the inhibitory effect of the Extract of *Moringa oleifera* seeds against *C. albicans* It was following the Sensitivity test depending on the agar well diffusion method of Price[10]

Molecular testes

Experience Design

- **Prepare stuck of yeast:** Yeast stuck attended depending on the method of Clayton [14]
- **Preparation stuck of epithelial cells:** Preparation was done depending on the method Chritchaly and Douglas [15]
- 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)⁰C 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 SAP 5 gene expression as well as the use of gene of (Act1) structured record to calculate gene expression Table 1, has this test was conducted four transactions T1,T2,T3,C depending on the method in Tavanti [16]

Table (1) DNA sequencing primers and their Nitrogenous bases - Bioneer Corporation - South Korea

Primer	Sequence		Amplicon
SAP5	F	5'-TTCCAAAATGGCGTGGTGAC-3'	115bp
	R	5'-ATTTTGGGAAGTGC GGGAAG-3'	
Act1	F	5'-TGTGTAAAGCCGGTTTTGCC-3'	136bp
	R	5'-TTGGATTGGGCTTCATCACC-3'	

Genbank code SAP5: XM_714054.1, and Act1: XM_717232.1.

Data analysis of Real-Time PCR:

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, [17]

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

The genus *Candida* was diagnosed in patients with cancer *C. albicans* 41 (41 %)

Protease production by *C. albicans*

Results shows the protein production by *C. albicans* isolated from patients the number of positive samples to test was 40 (97.5%) while the negative samples were 1 (2.4%),

Qualitative detection of some of the active compounds of aqueous Extracts of the seeds of *Moringa oleifera*

The table 2 shows 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 <i>Moringa oleifera</i> seeds	+	-	+	+

Gene expression of SAP1 gene using the method (2- $\Delta\Delta$ CT Livak method)

Gene expression of SAP5 gene before and during infection yeast cells epithelium: shown results in table 3 of the high gene expression at a rate (37.156 ± 7.66049) treatment T2 With significant difference at the level of the probability $P \leq 0.01$ compared with the group c, at a rate (1.040 ± 0.32229).

Effect of Extract aqueous seeds of *Moringa oleifera* on gene expression in SAP5 gene before and during infection yeast cells epithelial : show Results in table 3 ; figure 1 to gene expression for SAP5 gene was in the transaction C, at a rate (1.040 ± 0.32229) , while the value dropped to (0.074 ± 0.01542) treatment T1 with significant difference at the level of the probability $P \leq 0.01$, either in terms of high T2 treatment was worthless gene expression at a rate (37.156 ± 7.66049) compared with the treatment T3 where gene expression dropped at a rate (19.565 ± 8.82162) with a significant difference at the level of the probability of $P \leq 0.01$.

Table 3: gene expression for SAP5 gene using the method (2- $\Delta\Delta$ CT Livak method)

Treatment Isolation	CT (SAP5 gene)	CT (actin)	Δ CT (Test)	Δ CT (Control)	$\Delta\Delta$ CT	Fold Change ($2^{\Delta\Delta$ CT)	Mean + Std.Error
T1	35.26	35.26	0.00	-3.38	3.38	0.096	0.074 $\pm$ 0.01542
T1	35.42	34.61	0.81	-3.38	4.19	0.055	
T1	35.52	35.15	0.37	-3.38	3.75	0.074	
T1	35.45	35.17	0.28	-3.38	3.66	0.079	
T1	35.31	34.74	0.57	-3.38	3.95	0.065	
T2	27.13	35.28	-8.15	-3.38	-4.77	27.210	37.156 $\pm$ 7.66049
T2	26.72	35.53	-8.81	-3.38	-5.43	43.055	
T2	26.52	35.29	-8.77	-3.38	-5.39	41.957	
T2	27.15	35.46	-8.31	-3.38	-4.93	30.546	
T2	26.95	35.76	-8.81	-3.38	-5.43	43.010	
T3	27.25	35.71	-8.46	-3.38	-5.08	33.844	19.565 $\pm$ 8.82162
T3	28.11	35.42	-7.31	-3.38	-3.93	15.206	
T3	27.72	35.58	-7.86	-3.38	-4.48	22.329	
T3	28.64	35.70	-7.06	-3.38	-3.68	12.836	
T3	27.25	34.40	-7.15	-3.38	-3.77	13.609	
C	31.44	35.31	-3.87	-3.38	-0.49	1.406	1.040 $\pm$ 0.32229
C	31.75	35.56	-3.81	-3.38	-0.43	1.349	
C	31.89	35.15	-3.26	-3.38	0.12	0.921	
C	31.72	34.51	-2.79	-3.38	0.59	0.667	
C	32.22	35.38	-3.16	-3.38	0.22	0.859	
Mean C	31.804	35.18	-3.38				

T1 *C.albicans* + *Moringa oleifera* seed Extract ,T2 *C.albicans* + Epithelial Cell ,T3 *C.albicans* + Epithelial Cell + *Moringa oleifera* seed Extract , C *C.albicans* only , CT: q PCR Threshold Cycle number (2- $\Delta\Delta$ 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.

$$\Delta \text{CT}(\text{test}) = \text{CT}(\text{target, test}) - \text{CT}(\text{ref, test})$$

$$\Delta \text{CT}(\text{Control}) = \text{CT}(\text{target, Control}) - \text{CT}(\text{ref, Control})$$

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

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

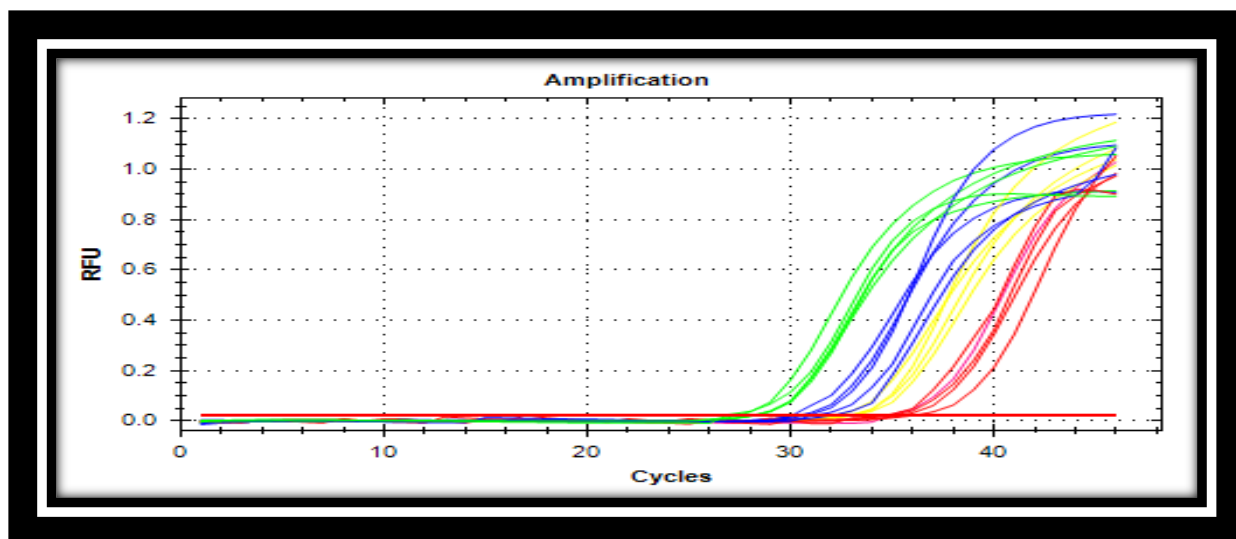


Figure 1 amplification curve of the interaction of Real-Time PCR and private SAP5 gene in the yeast *C. albicans*, accounting for curves as follows:

1. curves with a yellow color control group (C).
2. with red *C.albicans* group curves with *Moringa oleifera* seeds Extract T1.
3. Curves-green *C.albicans* group with epithelial cells T2.
4. curves of blue *C.albicans* group with *Moringa oleifera* seed Extract and epithelial cells T3

Discussion

C. albicans came ranked first and by 41 (41%) of the total samples that this result identical with many of the results of studies Mokaddas [18] and de Sousa [19] who pointed out that this kind poses the highest rate of Infect of oral candidiasis in cancer patients for being opportunistic fungus that exploits the weakness of the body's immunity and possession of a number of virulence factors such as adhesion and the formation of biofilms and secretion of enzymes hydrolysis and transformation as well as secreted toxins [20]

With respect to Protease production :that the number of samples *C .albicans* from patients that gave the result is positive is 40 (97.5%) which is consistent with Calderone and Fonzi[21]the cause may be due to the lack of production of protease difference between type strains or to the lack of activity or inactivity SAP gene is responsible for the production of protease which is one of the basic structures in the genes of Candidaprotease works in yeast *C. albicans* on analysis, protein barriers in the host body , as well as the working of these enzymes to provide nutrients for the yeast Candida within the body of an organism to penetrate host cells and invasion as well as immunosuppression [22] as well as linked to the presence of these enzymes configure mycelium lying in the yeast Candida, and helps in the adhesion of yeast in the host cells in the process, in addition to that, these enzymes use the proteins present in the composition of the host cells as the basis of work by leading to the decomposition of the cell wall and then her death [23]

Qualitative detection of some of the active compounds of aqueous extracts of the seeds of *Moringa oleifera* .The results indicated the presence of saponins and flavonoids and tannins, but not the presence of alkaloids, and agreed results disclosure of material in the active participation in the aqueous Extract of the seeds of *Moringa oleifera* with Sahar [24] and that the cause may be due to the saponins and flavonoids and tannins is because it has the ability to blend into the water can be obtained in method aqueous Extraction of alkaloids are either non-polar compounds insoluble in water [25]

The technique enzyme chain polymerization in real-time reaction (qRT-PCR) characterized by accuracy and sensitivity, especially when measuring to a microbial gene expression, as it gives these technical expressions of molecularlyclear in terms of type and quantity coupled with other technologies used in the same field to determine the production of an enzyme RNAase [26]

The key to understanding the mechanism of occurrence of candidiasis oral comes from our study of the gene (SAP5) which is produced by *C. albicans* factors ferocity to colonize parts of the mouth, inflammation, and the results of the study are consistent with a study Koelshch [27] who pointed out that the isolates of *C. albicans* isolated from the mouth has SAP5 gene responsible for the production of an enzyme Secreted Aspartyl Proteinases. We also note the high genetic production of expression for the treatment of T2 rate of the gene (SAP5) for treatment C and teams morale high at the level of the probability $P \leq 0.01$ reason 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 for the pathogenesis of Candida , As increased activity in the yeast works on the secretion of tissue lysis enzymes, including the Secreted aspartyl proteinases encoded by Sap family genes that helps the yeast to analyze and penetrate tissue and invade other parts of the body and provide nutrients [28] While noting down the genetic production of expression for the treatment of T1 rate of the gene (SAP5) 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 it's 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 [29] 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 [30]

Conclusions

Aqueous extracts of the seeds of *Moringa oleifera* contain effective compounds has an effective role in reducing the rate of gene expression of Sap5 gene .

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