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. Ther 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($1.040 \pm 0.32229$). 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$(0.074 \pm 0.01542)$ 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 $(19.565 \pm 8.82162)$ 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 pretenses (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 Calicibicans 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 prepared depending on the method of 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]

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°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 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]

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

Table 2: Quality Detection for some active compound for Moringa oleifera

<table>
<thead>
<tr>
<th>Substance</th>
<th>Tannins</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
</tr>
</thead>
<tbody>
<tr>
<td>aqueous Extracts of Moringa oleifera seeds</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Gene Expression of SAP1 Gene Using the Method (2-ΔΔCT Livak Method)

Gene Expression of SAP5 Gene Before and During Infection Yeast Cells Epithelium

shown results in table 3f the high gene expression at a rate(37.156 ±7.66049) treatment T2 With significant difference at the level of the probability P ≤ 0.01 compared with the group c, at a rate(1.040 ± 0.32229).

Effect of Extract Aqueous Seeds of Moringa Oleifera on Gene

Table 3: gene expression for SAP5 gene using the method (2-ΔΔCT Livak method)

<table>
<thead>
<tr>
<th>Treat. Isolation</th>
<th>CT (SAP5 gene)</th>
<th>CT (actin)</th>
<th>Δ CT (Test)</th>
<th>Δ CT (Control)</th>
<th>ΔΔ CT</th>
<th>Fold Change (2^ΔΔ CT)</th>
<th>Mean ± Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>35.26</td>
<td>35.26</td>
<td>0.00</td>
<td>-3.38</td>
<td>3.38</td>
<td>0.096</td>
<td>0.074 ± 0.01542</td>
</tr>
<tr>
<td>T2</td>
<td>35.42</td>
<td>34.61</td>
<td>0.81</td>
<td>-3.38</td>
<td>4.19</td>
<td>0.055</td>
<td>37.156 ±7.66049</td>
</tr>
<tr>
<td>T3</td>
<td>35.52</td>
<td>35.15</td>
<td>0.37</td>
<td>-3.38</td>
<td>3.75</td>
<td>0.074</td>
<td>19.565 ±8.82162</td>
</tr>
<tr>
<td>T4</td>
<td>35.45</td>
<td>35.17</td>
<td>0.28</td>
<td>-3.38</td>
<td>3.66</td>
<td>0.079</td>
<td>1.040 ± 0.32229</td>
</tr>
<tr>
<td>T5</td>
<td>35.31</td>
<td>34.74</td>
<td>0.57</td>
<td>-3.38</td>
<td>3.95</td>
<td>0.065</td>
<td>0.22</td>
</tr>
<tr>
<td>T6</td>
<td>27.13</td>
<td>35.28</td>
<td>-8.15</td>
<td>-3.38</td>
<td>-4.77</td>
<td>27.210</td>
<td>0.859</td>
</tr>
<tr>
<td>T7</td>
<td>26.72</td>
<td>35.53</td>
<td>-8.81</td>
<td>-3.38</td>
<td>-5.43</td>
<td>43.055</td>
<td>1.349</td>
</tr>
<tr>
<td>T8</td>
<td>26.52</td>
<td>35.29</td>
<td>-8.77</td>
<td>-3.38</td>
<td>-5.39</td>
<td>41.957</td>
<td>5.08</td>
</tr>
<tr>
<td>T9</td>
<td>27.15</td>
<td>35.46</td>
<td>-8.31</td>
<td>-3.38</td>
<td>-4.93</td>
<td>30.546</td>
<td>3.93</td>
</tr>
<tr>
<td>T10</td>
<td>26.95</td>
<td>35.76</td>
<td>-8.81</td>
<td>-3.38</td>
<td>-5.43</td>
<td>43.010</td>
<td>15.206</td>
</tr>
<tr>
<td>T11</td>
<td>27.25</td>
<td>35.71</td>
<td>-8.46</td>
<td>-3.38</td>
<td>-5.08</td>
<td>33.844</td>
<td>3.38</td>
</tr>
<tr>
<td>T12</td>
<td>28.11</td>
<td>35.42</td>
<td>-7.31</td>
<td>-3.38</td>
<td>-3.93</td>
<td>15.206</td>
<td>8.82162</td>
</tr>
<tr>
<td>T13</td>
<td>27.72</td>
<td>35.58</td>
<td>-7.86</td>
<td>-3.38</td>
<td>-4.48</td>
<td>22.329</td>
<td>1.406</td>
</tr>
<tr>
<td>T14</td>
<td>26.64</td>
<td>35.70</td>
<td>-7.06</td>
<td>-3.38</td>
<td>-3.68</td>
<td>12.836</td>
<td>1.040 ± 0.32229</td>
</tr>
<tr>
<td>T15</td>
<td>31.44</td>
<td>35.31</td>
<td>-7.15</td>
<td>-3.38</td>
<td>-3.77</td>
<td>13.609</td>
<td>0.12</td>
</tr>
<tr>
<td>T16</td>
<td>31.75</td>
<td>35.56</td>
<td>-3.87</td>
<td>-3.38</td>
<td>-0.43</td>
<td>1.349</td>
<td>0.921</td>
</tr>
<tr>
<td>T17</td>
<td>31.89</td>
<td>35.15</td>
<td>-3.26</td>
<td>-3.38</td>
<td>0.09</td>
<td>0.667</td>
<td>0.59</td>
</tr>
<tr>
<td>T18</td>
<td>31.72</td>
<td>34.51</td>
<td>-2.79</td>
<td>-3.38</td>
<td>0.59</td>
<td>0.667</td>
<td>0.22</td>
</tr>
<tr>
<td>T19</td>
<td>32.22</td>
<td>35.38</td>
<td>-3.16</td>
<td>-3.38</td>
<td>0.22</td>
<td>0.859</td>
<td></td>
</tr>
<tr>
<td>Mean C</td>
<td>31.804</td>
<td>35.18</td>
<td>-3.38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 2shows 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.

Expression in SAP5 gene before and during infection yeast cells Epithelial
Show Results in table 3; figure 1to gene expression for SAP5gene 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 ≤ 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 ≤ 0.01.
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^-ΔΔCT 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.

$$\Delta CT (test) = CT (target, test) – CT (ref, test)$$

$$\Delta CT (Control) = CT (target, Control) – CT (ref, Control)$$

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

$$\Delta\Delta CT = \Delta CT (test) – \Delta CT (Calibrator)$$

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

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 bio films 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 Candida protease 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 immunosuppressant [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 Extraction 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 molecularly clear in terms of type and quantity coupled with other technologies used in the same field to determine the production of an enzyme RNA ase [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 Koelschh [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 Protei nases. 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 with drawal 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].

**Conclusion**

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.

**Acknowledgements**

The authors are thankful to University of Al-Qadisiyah in Iraq for providing all the infrastructural facility to carry out this work.

**References**


Members of the Gene Family Encoding Secretary As party Proteinases in Candida.


