The Anti-proliferative Activity of *Phoenix dactylifera* Seed Extract on MCF-7 Breast Cancer Cell Line

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ABSTRACT

The Aim of the Study is to identify the anti-proliferative activity of *Phoenix dactylifera* seeds extract, (phoenix dactylifera and tamoxifen) against cancer cell line, investigate the possible additive and/or synergistic activity of *Phoenix dactylifera* seeds extract and tamoxifen combination, and identify more probable active constituent to elucidate the mechanism of action. The anti-proliferative activity of the extract tested by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay on breast cancer cell line (MCF7). Dose response relationship was shown. Cytotoxic activity of chloroform extracts have identified on MCF7, the IC₅₀ values for chloroform extract of *Phoenix dactylifera* seeds was 3.18 µg/ml. The IC₅₀ values for chloroform combination with tamoxifen was 7.17 µg/ml. These finding showed that chloroform extracts had have cytotoxic activity against MCF7 cell line. The results showed that the chloroform extract of *Phoenix dactylifera* seeds exerted dose-independent cytotoxic effect on MCF7 cell line after 48 hrs of treatment compared to negative control.

Keywords: anti-proliferative activity, *Phoenix dactylifera* seed extract, MCF-7.

INTRODUCTION

Breast cancer is the most commonly identified life-threatening cancer in women². In the United States, breast cancer represents 29% of all cancers in women, and is second only to lung cancer as a cause of cancer deaths³. The causes of cancer are various, complex, and only partially understood¹. Many things are known to increase the risk of cancer, including: Female sex, increasing age¹, non-Hispanic whites⁵, a positive family history of breast cancer⁶ late age at first pregnancy, null parity, early onset of menses, and late age of menopause⁷, the oral contraceptives (OCs) and hormone replacement therapy (HRT)⁸, diet with of red meat, fat, caffeine, and alcohol⁹, and ionizing radiation¹⁰. Diets that are rich in grains, fruits, vegetables¹¹, calcium/vitamin D¹² low in saturated fats, low in energy (calories), and low in alcohol are the more frequent pattern in less industrialized countries and are thought to be protective against breast cancer¹². *Phoenix dactylifera* Linn (date palm), called the Tree of Life by the Arabs, is considered as one of the oldest cultivated fruit trees¹³ means date palm, and “dactylifera” from a Greek word “daktulos” meaning a finger¹⁴. Date palms and culture are depicted in ancient Assyrian and Babylonian tablets, including the famous Code of Hammurabi¹⁵.

*Phoenix dactylifera* belonging to Palmaeace family and it is differ in shape, size, and weight¹⁶.

MATERIALS AND METHODS

Assessment of Proliferation Inhibition of Breast Cancer Cell Line

The (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) MTT assay was used to identify the cell line proliferation capability. All of the breast cancer cells were between passages 4-7. These cells treated with different concentrations of *Phoenix dactylifera* seeds extract for 48 hrs. The untreated cells received only DMSO as a negative control, while tamoxifen used as a positive control.

MTT prepared by adding 5mg/ml in PBS (phosphate buffer saline). 20 µl of MTT used per well and the plates incubated at 37°C, in 5% CO2 for 4hrs.

The plates removed from the incubator and the supernatant aspirated. DMSO (200µl) added to each well. The plates shook vigorously for one minute at room temperature to dissolve the dark blue crystals.

The absorbance red at 570nm and the reference at 650nm by using micro-plate ELISA reader.

The absorbance of cells cultured in control media took to represent 100% viability. The viability of treated cells was determined as a percentage of that for the untreated control. Each concentration was tested in quadruplicate, and the experiment was repeated twice. The concentration of the cells in each well was 1 x 10⁴. The percentage of cell growth inhibition was determined as a mean ± SD, using the following equation.

The percentage of inhibition =1-(A₀-A₁)/(A₂-A₁)

A₀ = Absorbance of sample
A₁ = Absorbance of blank
A₂ = Absorbance of control

IC₅₀ is the concentration that prevents 50% from the cell growth which calculated by the linear and logarithmic correlation equation.
\[ y = mx + b \]
Where \( y \) is the percentage of inhibition and it set to be 50%, \( m \) is the slope of the standard curve, \( x \) is the concentration of compound tested in \( \mu g/mL \), and \( b \) is the \( y \)-intercept of the line of standard curve\(^{17} \).

**Cell Lines**

Breast cancer cell line (MCF-7) was used in this study. The entire cell lines were maintained in its specific medium; in Dulbeccos Modified Eagle medium. 10% fetal bovine serum (HIFCS) and 1% pen/strep (Penicillin/streptomycin) added to those medium to prepare complete growth medium. The cells were used for the experiment was between passage 4 and 7. Serial dilutions from *phoenix dactylefera* seeds extract made by dissolving the samples in DMSO then diluting it with the medium that used for breast cancer cell line. The final DMSO concentration in the medium was 1%; the control wells got 200\( \mu \)l from the medium with the final DMSO concentration, while the samples added to the well in quadruplicate, and then incubated in the incubator at 37°C with 5% CO2 for 48hrs. MTT added on the MCF7 cell and incubated for 4 hours earlier to the absorbance measurements at 570nm\(^{17} \).

**RESULTS**

**Activity of phoenix dactylefera seeds Extracts on MCF7 Hormonal Dependent Breast Cancer Cell Line**

*In vitro* screening of *Phoenix dactylifera* seeds extracts on MCF7 hormonal dependent breast cancer cell line, which were in passage between 4-7. The results showed a dose-dependent inhibition on the cell growth after 48hr. The extracts concentrations used were 200, 100, 50, 25, and 12.5. Each concentration was tested in quadruplicate, and the experiment was repeated twice. The data is represented as the mean ± SD. Tamoxifen was used as a positive control.

The \( IC_{50} \) value was deduced from the graph for chloroform extract of *phoenix dactylifera* seeds by using the following linear regression equations: for chloroform \( y = 0.0418\ln(x) + 98.798 \). Where \( Y= \) the percentage of inhibition and \( X= \) concentration. The \( IC_{50} \) values for chloroform extract of *phoenix dactylifera* seeds was 3.18 \( \mu g/ml \).

![Figure 1: Dose Response Curve of Chloroform Extract of Phoenix Dactylifera Seeds on MCF7 Cancer Cell Line](image)

The \( IC_{50} \) values for chloroform combination with tamoxifen was 7.17 \( \mu g/ml \).

Figure 2 shows the dose response curve of chloroform extract of *phoenix dactylifera* seeds in combination with tamoxifen on MCF7 cancer cell line.

![Figure 2: Dose response curve of chloroform extract of phoenix dactylifera seeds in combination with tamoxifen on MCF7 cancer cell line](image)

**Table 1: Peaks Absorbance and their Functional Groups for Chloroform Extract of Phoenix dactylifera Seeds**

<table>
<thead>
<tr>
<th>No</th>
<th>Peak Absorbance</th>
<th>Functional Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>592-623</td>
<td>C-Br Stretch (alkyl halide)</td>
</tr>
<tr>
<td>2</td>
<td>721</td>
<td>C-H Rock (alkanes)</td>
</tr>
<tr>
<td>3</td>
<td>765-823</td>
<td>C-Cl Stretch (alkyl halide)</td>
</tr>
<tr>
<td>5</td>
<td>871</td>
<td>C-H (aromatic)</td>
</tr>
<tr>
<td>6</td>
<td>1068</td>
<td>C-O Stretch (alcohols, carboxylic acids, esters, ethers)</td>
</tr>
<tr>
<td>7</td>
<td>1159-1242</td>
<td>C-N Stretch (aliphatic amine)</td>
</tr>
<tr>
<td>8</td>
<td>1375</td>
<td>C-H Rock (alkanes)</td>
</tr>
<tr>
<td>9</td>
<td>1458</td>
<td>C-C in ring (aromatics)</td>
</tr>
<tr>
<td>10</td>
<td>1518</td>
<td>N-O Asymmetric stretch (nitro compound)</td>
</tr>
<tr>
<td>11</td>
<td>1743</td>
<td>C=O Stretch (esters, saturated aliphatics, carboxylic acids, carbonyls)</td>
</tr>
<tr>
<td>12</td>
<td>2854-2924</td>
<td>C-H Stretch (alkanes)</td>
</tr>
<tr>
<td>13</td>
<td>3354</td>
<td>O-H Stretch (alcohols, phenols)</td>
</tr>
</tbody>
</table>
DISCUSSION

Chloroform extract of *Phoenix dactylifera* seeds displayed significant anti-angiogenesis activity against the rat aorta\(^{18}\); and the anti-angiogenic agents may have antitumor activity. The majority of the clinically used anti-tumor agents have significant cytotoxic activity in cell culture systems\(^{19}\). In the present study, effect of CE extracts of *Phoenix dactylifera* seeds were evaluated with MCF7 cell line to investigate if these compounds have any cytotoxicity against breast cancer cell lines. Selective cytotoxicity is a desired feature of a new candidate anticancer agent. The cytotoxicity for CE was tested by the MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide assay. The MTT assay is a colorimetric assay for evaluating cell viability. NAD (P) H-dependent cellular oxidoreductase enzymes may reflect the number of viable cells present\(^{20}\). These enzymes are able of reducing the tetrazolium dye MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide to its insoluble formazan, that has a purple color\(^{21}\).

MITT assays are commonly done in the dark because the MTT reagent is sensitive to light whereby the reduction of MTT gives information about mitochondrial function throughout the activity of succinate dehydrogenase\(^{17}\). The CE extract demonstrated as a potent anti-proliferative compound that decreases the viability of MCF7 which are hormonal dependent breast cancer cell line. To consider any agent as cytotoxic against cell lines, its IC\(_{50}\) should be less than 20µg/ml\(^{22}\). This is a well-recognized criterion for a compound to be judged as cytotoxic or not, as defined by the National Cancer Institute (NCI)\(^{22}\). The IC\(_{50}\) values for CE were 3.18µg/ml, so the CE extract have cytotoxic activity against MCF7 cell line. The results showed that the CE extracts of *Phoenix dactylifera* seeds exerted dose-independent cytotoxic effect on MCF7 cell line after 48 hrs. of treatment compared to negative control. The IC\(_{50}\) values for CE combination with tamoxifen were 7.17 µg/ml. However the IC\(_{50}\) of CE extract combination exhibited higher value than the IC\(_{50}\) value of tamoxifen alone. This mean the CE combination may be have antagonized effect, this may be due to the presence of chemical compounds in extract may antagonized the active compounds\(^{23}\). Inspite of these results, the CE combination remained cytotoxic on the MCF7 cell line. P>0.05, so there is no significant difference between the use of extract alone and the combination of extract with tamoxifen on MCF7. Al-zubaidy and coworker (2015)\(^{18}\) reported that CE extract of *Phoenix dactylifera* seeds demonstrated the most significant anti-angiogenesis activity as well as a significant free radical scavenging activity than other extracts. In present study we found that the CE extract showed high percent of inhibition for MCF7 cell line due to the direct cytotoxicity on breast cancer cell line. Therefore the finding of the present study agreed with Al-zubaidy and coworker (2015) study\(^{18}\). The date seed extract intake might afford protection against colon tumorigenesis, attributable to functioning as an antioxidant in addition to displaying some anticarcinogenic potential\(^{20}\), this finding agreed with the present study. In Habib and coworker (2014)\(^{24}\) showed that date seed extract impeded the proliferation of pancreatic cancer cells line Mia-Pa-Ca-2 in a dose-dependent manner due to polyphenolic compounds that present in the *Phoenix dactylifera* seeds which inhibit the initiation, progression and spread of cancers in vitro and in animals *in vivo*. This results support a very promising antioxidant potential of date seed extracts. The proposed mechanism of anti – proliferative activity of chloroform extract of *Phoenix dactylifera* seeds on MCF7, It may be related to the presence of variety of phytochemicals in the CE a like phenolic compounds, terpenes, fatty acids, aliphatic alcohols, flavonoid, and others. Phenolics have been regarded as powerful antioxidants *in vitro* and proved to be more potent antioxidants than Vitamin C and E and carotenoids\(^{26,27}\). In the initiation stage, phenolics may prevent activation of procarcinogens by impeding phase I metabolizing enzymes, such as cytochrome P450\(^{28}\) and also facilitate detoxifying and removal of the carcinogens by induction of phase II metabolizing enzymes such as glutathione S-transferase (GST)\(^{29}\). They may also reduce the formation of the initiated cells by stimulating DNA repair\(^{30}\). Phenolics may prevent the formation and growth of tumors by induction of cell cycle arrest and apoptosis\(^{31}\). Furthermore, phenolic compounds possess anti-angiogenesis effects\(^{32}\). Furthermore, phenolic compounds possess anti-angiogenesis effects\(^{32}\), which is an important feature in the inhibition of tumor growth, invasion and metastasis\(^{32}\). Sylvei and colleagues, 2014\(^{13}\) reported that oleic acid produced a potent anti – angiogenesis activity. The other major compounds identified in the CE extracts analysed by GC-MS were: palmitic acid has been reported to have anti-inflammatory, antioxidant and antitumor activity\(^{38,39}\). It was revealed that palmitic acid exerts anti-inflammatory activity through competitive inhibition of phospholipase A2\(^{37}\). Phospholipase A2 has a critical role in angiogenesis, tumorigenesis, and tumor metastasis\(^{38}\). As for palmitic acid, it was found that it owns antitumor activity *in vivo* in mice and *in vitro* cytotoxic activity.
against human leukemic cells and human gastric cancer cells. As for palmitic acid, it was found that it owns antitumor activity in vivo and in vitro cytotoxic activity against human leukemic cells and human gastric cancer cells.

REFERENCES


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