Extracts of Jordanian Date Palm Fruit (*Phoenix Dactylifera* L.)

Inhibit Human Mammary Adenocarcinoma (MCF-7) Cells

*In Vitro* by Inducing Cell Viability

HIBA F. AL-SAYYED*1, LUAY F. ABU-QATOUSEH*2, MOHAMMED MALKAWY,
SUZAN AL-WAWI*2, and MOHAMMAD ALKAFAWEEN*3

1Department of Nutrition, Faculty of Pharmacy and Medical Sciences,
University of Petra, Amman, Jordan.
2Department of Pharmacology and Biomedical Sciences, University of Petra, Amman, Jordan.
3Faculty of Pharmacy, Department of Pharmacy, Al-Zaytoonah University of Jordan, Amman, Jordan.

**Abstract**

World wide, and in Jordan specifically, date palm fruit production has been steadily increasing. Recently, various dates extracts have been used functionally as antioxidants or anticancer agents. In addition, Jordanian date palm fruit of Barhi variety at two maturity stages (*Rutab*) and (*Tamr*) inhibited chemically-induced mammary cancer in animal model. The aim of this study is to strengthen the scientific evidence on the effect of dates on mammary cancer via assessing the effect of different concentrations of water extracts of three varieties of dates grown in Jordan (*Barhi*, *Belle Huwaimil*, and *Medjool*) at two maturity stages (*Rutab* and *Tamr*) on the viability of MCF-7 breast cancer cell line. The effects of different extracts on MCF7 inhibition/proliferation was analyzed using MTT assay. Percentage of inhibition was calculated. The most effective concentration was 100 mg/ml for all varieties. At this concentration, *Medjool* variety at the *Tamr* stage exhibited the highest inhibition. At the 12.5 and 25 mg/ml concentrations of the extracts, the most effective date palm fruit varieties were *Belle Huwaimil* and Barhi at the *Tamr* stage respectively. At lower concentrations (3.125 and 6.25 mg/ml), *Medjool* at the *Tamr* stage and *Belle Huwaimil* respectively were the most effective extracts were *Barhi* at the *Tamr* and *Rutab* stages were the least effective (*P*=0.000**). It is concluded that water extract of palm fruit reduced MCF-7 cell viability.

**Article History**

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**Keywords**

Anticancer; Cell Line; Cell Viability; Date Palm Fruit; Inhibition; Jordan; Mammary Cancer; McF-7.
Introduction
The relationship between food consumption and cancer has been extensively studied. Specifically, the consumption of fruits and vegetables was found to be protective against different types of cancers, especially mammary cancer. By virtue of dietary fiber and antioxidants, fruits and vegetables are thought to improve immunity and inhibit cancerous cell growth.

The date palm (*Phoenix dactylifera* L.) is a monocotyledon of *Palmae* family growing annually. Worldwide, date palm fruit production has been steadily increasing over the last 30 years. Most of the worldwide production of the fruit is in the Arab countries. The last decade showed rapid increase in Jordanian areas cultivated with this plant because it tolerates desert climatic conditions of the Jordanian areas where it is cultivated.

Historically, the plant was used for medicinal purposes such as fever, throat soars, toothaches, liver, abdominal, eye, and respiratory problems. Additionally, it has been mentioned that the fruit contains stimulants that strengthen uterus muscles, and is recommended for breast milk production. Recently, various fruit extracts have been used functionally as antioxidants, and anticancer agents.

The consumption of date palm fruit is related to Islamic values. The fruit consumption reaches the highest levels during the month of Ramadan since eating this fruit is a custom favored by Prophet Mohammad (peace be upon him). Date palm fruit has hundreds of varieties sharing three maturity stages: *Khalal*, *Rutab*, and *Tamr*.

Cancer is a risk factor for mortality; the World Health Organization (WHO) reported 9.5 million deaths from cancer in 2018. Mammary cancer caused 1.5% deaths in 2018. Deaths from cancer worldwide are expected to rise to 11 million in 2030. Breast, and liver cancers cause the most cancer deaths each year. Breast cancer is the second risk factor for cancer deaths in Jordan accounting for 11.3% of total deaths of cancer in 2018. MCF-7 mammary cancer cell line represents the in vitro model for estrogen receptor-positive breast cancer.

Jordanian date palm fruit of *Barhi* variety at the *Rutab* and *Tamr* maturity stages was found to reduce chemically-induced mammary cancer tumor multiplicity, size, and increase tumor latency by inducing the hormone 17-β-estradiol and modulating the glutathione-S-transferase enzyme activity. In addition, the water extracts of the fruit were found to be rich in polyphenols and flavonoids and a potential for antioxidant activity.

In the current study, we attempted to evaluate the effect of several concentrations of water extracts of three varieties of date palm fruit grown in Jordan (*Barhi*, *Belle Huwaimil*, and *Medjool*) at two maturity stages (*Rutab* and *Tamr*) on the viability of MCF-7 breast cancer cell line.

Materials and Methods

**Fruit and Extract Preparation**
Locally grown date palm fruits were provided thankfully by Wardat ElRaml shop. In this study, three fruit varieties were tested: *Barhi*, *Belle Huwaimil*, and *Medjool*. The tested fruits were at the two maturity stages *Rutab* (*Barhi*) and *Tamr* (*Barhi*, *Belle Huwaimil*, and *Medjool* varieties). Then, the water extracts were prepared with deionized water (1:10). Subsequently, serial decimal dilution series of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.563 mg/ml were performed using Dulbecco’s minimal essential medium (DMEM) enriched with 10% fetal bovine serum (FBS).

**Cell Line and Culture Conditions**
The human breast adenocarcinoma cell line (MCF7) was purchased from American Type Culture Collection (MCF7 ATCC® HTB-22). The MCF7 cells were maintained DMEM, DMEM High Glucose (4.5 g/l), with L-Glutamine supplemented with 10% FBS, 1% penicillin-streptomycin and 0.25 mg/ml amphotericin B (Capricorn-scientific, Germany) at 37°C in a humidified atmosphere of 95% air and 5% CO₂. The cell line that has been used in this assay was sourced from the ATCC and all the STR profile and data are available on the ATCC website. The authors would like to mention that cell lines in our labs are regularly checked for any microbial contamination and strict rules has been followed to discard any cell line or flask with possible contamination.
**Cell Proliferation and Viability Assay**

Different extract effects on MCF7 proliferation/inhibition was analyzed by MTT assay cell proliferation and viability after 24-hour incubation. Initially, $2 \times 10^4$ cells/well were transferred into 96-well plates and let to attach overnight. Initially, 20000 cells/well were seeded into 96-well plates and let to attach overnight. After that, the cells were treated with different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, 6.25 mg/mL, 3.125 mg/mL or 1.56 mg/mL) of each fruit extract and incubated for 24 hours with 5% CO$_2$ at 37ºC in a humidified atmosphere. The wells with cells only was used as a positive control. After this treatment, MTT reagent (5 mg/ml) in 100 μl of fresh culture medium was added to each well for 4-hour incubation. The formazan crystals formed with MTT treatment were solubilized with acidic isopropanol and the optical density was then measured at 570 nm wavelength using a microplate reader (GloMax, promega). The assay controls were used to specify the cytotoxicity of the extract and to exclude the effect of the vehicle. Furthermore, IC50 was estimated using Prism software. The procedure was performed in triplicate.

**Statistical Analysis**

To detect the interaction effect of date palm fruit variety (factor 1), maturity stage (factor 2), as well as concentration (factor 3), data of the duplicate readings of percentage inhibition as well as percentage of viable cells were analyzed by multivariate analysis of variance (MANOVA) using the statistical package for social sciences (SPSS) analysis system. Data in the figures was extracted from mean ± standard deviation values for the inhibition and viable cell percentages $P<0.05$ was set for significance.

**Results**

Results of inhibition of cell line are shown in Figure 1. All of date palm fruit concentrations showed inhibition effect on cell line (Figures 1 and 2). The statistical analysis of data showed that there is no interaction effect between date palm fruit variety, maturity stage, and extract concentration in terms of inhibition percentage, neither viable cell percentage ($P>0.05$). However, there are significant ($P<0.01$) differences between different extracts in terms of the interaction effect between date palm variety and extract concentration.

![Fig.1: Inhibition percentage of different concentrations of the studied dates varieties](image)

Results show that the inhibition percentage is dose-dependent. The effect of the tested concentrations (1.563 to 100 mg of dates extract (dry)/mL) on the viability of MCF-7 cells. There is insignificant ($P>0.05$) inhibition of the growth of cells observed at (1.563-100 mg/mL). However, results showed
that the cytotoxic effect appeared at higher concentrations (50 and 100 mg/mL) where cell viability reduced by ~44% and 78%, respectively (Figure 2). MCF-7 cells growth inhibition as well as cytotoxicity were not significant until a concentration of 25 mg/mL (80% of cell viability). Interestingly, after 24 hours of treatment with date palm fruit extracts, MCF-7 cell viability decreased sharply and dose-dependently with IC50 values of 12 ± 0.02 mg/mL (P< 0.01) (Figure 2).
A study by El-sharkawy (2017)\textsuperscript{28} reported that the inhibition of cell proliferation was 42\% at 15 mg/ml concentration of extract. Inhibition values found in this research ranged from 9\% to 92\% and the results were close to those found by Odeh et al. (2014)\textsuperscript{30} who found inhibition value of 50 mg/ml extracts of Barhi variety at both maturity stages.\textsuperscript{32}

Figure 3 shows summarization of the experimental procedure as well as suggested mechanisms and functional compounds that are thought to protect against mammary cancer.

The various compounds in the date palm fruit render it capable of having an antioxidant capacity. This property has been proved at the \textit{in vitro}\textsuperscript{33–35} and the \textit{in vivo} levels.\textsuperscript{36–38} A previous study by Vayalil (2002)\textsuperscript{14} showed the antioxidant and antimutagenic activity of the water extract of date palm fruit at the Tamr stage. The author found that the extract scavenged superoxide and hydroxyl radicals in a dose-dependent manner.\textsuperscript{14}

The compounds that are thought to add the (functional) antioxidant property to the date palm fruit are metals such as Se, Cu,\textsuperscript{39} Zn, and Mn, phenolic acids,\textsuperscript{38} enzymes such as: phytase, invertase,\textsuperscript{40} polyphenols specially flavonoids\textsuperscript{12} such as catechins\textsuperscript{38} and rutin,\textsuperscript{34} antioxidant vitamins such as the vitamins C and E.\textsuperscript{41}
Conclusion
This study revealed that the date palm fruit extract from Barhi and Belle Huwaimil varieties of Jordan provide a strong activity against MCF-7 cancer cell line proliferation. Given the folk claims of anticancer properties of Barhi and Belle Huwaimil date extract and our results on MCF-7 cells, the date fruits could be used as a supplemental food for chemoprotective effects against breast cancer and possibly other lesions. Results of this research supported the results of previous reports regarding Medjool variety. However, results of this study are limited by the type of extract, maturity stage, the indicators tested for anticarcinogenicity, and the lack of using control cell line like the MCF7-A10 to exclude general cytotoxicity effect on other cell line.

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Conflict of Interest
The author(s) declares no conflict of interest.

References


