RESEARCH ARTICLE

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Cytotoxic Effect of *Phoenix dactylifera* (Iraqi Date) Leaves and Fruits Extracts against Breast Cancers Cell Lines

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Abstract

Objective: Now a day's cancerous diseases are the most prevalent life threatening that spreading because of the lifestyle. Its due to uncontrolled growth of cell which can be cured if diagnosed in early stage. Treatment of cancer depends on the various internal and external factors causing cancer. The main objective of this study is using herbal based medicine to manage breast cancer, the second most common type of cancer in the world. Methods: In this study, the anticancer effect of two Iraqi date palm part extracts (leaves and fruits) against panel of breast cancer cell lines (AMJ13 و MCF7, MDA-MB-231, CAL51) in vitro to evaluate their possible antitumor effect and their safety on normal cell line (MEF). **Results:** The *Phoenix dactylifera* (dray Zahdi) fresh leave extract showed highly cytotoxic effects in all breast cancer cell lines. The leaves extract was showed concentration dependent cytotoxicity effects after 72 h exposure time. Leave extracts was effective against AMJ13 cell line. The effective concentrations in both cancer cells ranged from 2500-20000 µg/ ml with inhibition percentage against AMJ13 was (66.7, 70.6, 53, and 54%). While the effect against MCF7, MDA-MB, and CAL51 cell lines were less with significant effect only at two concentrations (10000- 20000 µg/ ml) causing 64.3, and 64.3% growth inhibition respectively in MCF7, and 40, and 50% respectively in MDA-MB, and 44.0, and 52.0% respectively in CAL51. The dray date fruit extract has no significant cytotoxicity against all the cancer cells. Both extracts have no effect against normal fibroblast cells. Conclusion: In conclusion, *Phoenix dactylifera* fresh leave extract shows promising anticancer properties while the fruit extract has no direct anticancer effect.

Keywords: AMJ13- breast cancer- cytotoxicity- cell line- Date Palm- CAL51- MCF7- MDA-MB-231

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Introduction

Breast cancer is the second leading cause of cancer deaths among women in the United States and other countries. Cancer is a major public health problem in the world. Therefore, finding natural compounds from plants is an alternative cancer treatment, because chemotherapy is one of the commonly used in breast cancer treatment, but therapy is usually associated with adverse side effects, ranging from nausea to bone marrow failure and development of multidrug resistance (MDR) [1].

Chemotherapy (the use of chemical compounds to fight neoplastic diseases), in addition to surgery, that uses to treated effective in different types of cancers including breast, colorectal, ovarian, and lung cancer but chemotherapeutic agents face the challenge of low selectivity and toxic effects on other non-target tissue. Therefore, the use of alternative and complementary therapies such as herbal medication has bioactive compounds from plants are used to treat various diseases like [2, 3].

Hence, the screening of plants for anticancer effects has been actively pursued on an international scale [2]. This has led to an increased interest and active search for novel anticancer agents from natural products [3]. In chemotherapy field, around 75% of the anticancer agents used nowadays are derived from natural products of different origins, and plants are an important source of new promising therapies (Hanen et al., 2018).

In vitro cell-based assays have been developed to rapidly determine the cytotoxic activity of several compounds. Cell-based assays are also useful in identifying variations in susceptibility of different target cells to several chemotherapeutic agents [4].

Phoenix dactylifera L. (date palm) is an ancient plant used in folk medicine for the treatment of various diseases and disorders (Hanen et al., 2018). Date palm tree, *Phoenix dactylifera* L., is an important plantation crop for many countries extending from North Africa to the Middle East including many states of the Arabian Gulf

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countries especially in Iraq [5]. It's had different chemical substances that responsible for the pharmaceutical properties and activity might be due to the presence of certain compounds within the date extracts such as tannins, flavonoids, phenolic acids, tocopherols, phytosterols, carotenes, phytoestrogens, and tocotrienols [6], also quercetin (a flavonoids glucosides which known for anticancer effect) [7].

Date palm fruits have been an important component of the diet in most of the arid and semiarid regions of the world [8]. The fruits are a rich source of carbohydrates, dietary fibers, certain essential vitamins, contain at least six vitamins including a small amount of vitamin C, and vitamins B1 (thiamine), B2 (riboflavin), nicotinic acid (niacin) and vitamin A, and minerals, lipids, and protein [8]. Therefore, dates and their constituents act as potent antioxidant, anti-inflammatory, and antitumoral and provide a suitable alternative therapy in various diseases cure (Hanen et al., 2018). The leaves of *Phoenix dactylifera* show antidiabetic activity and antilipademic activity. Oxidative stress is an imbalance between oxidant production and antioxidant enzymatic and nonenzymatic antioxidant reduce the reactive oxygen species (ROS) induced by oxidation [5]. Also, it contains a biochemical composition and biological activities [9, 10]. Therefore, the *Phoenix dactylifera* has been studied for its potential activity for antioxidant [11], Anti-inflammatory (Hanen et al., 2018), Bioactivity, Pharmacological Potential [12] and as an anticancer effect [3, 13].

Therefore, the aim of this study is evaluated the anticancer effects of the methanolic extract of dry date palm and leaves on breast cancer cells and its safety by testing it on normal cells.

Materials and Methods

This study was carried out on University of Kufa, Najaf, Iraq and Experimental Therapy Department, Iraqi Center of Cancer and Medical Genetics Research (ICCMGR), Mustansiriyah University, Baghdad, Iraq. And the protocols of this work were approved by ethics committee of scientific committee of ICCMGR.

Collection and Extraction of Plant Material 1.0

The *Phoenix dactylifera* fresh leaves, dry fruits were obtained from Kufa cultivar, they were collected during late 2019. The extraction methods were done according to the "quality control methods for herbal materials in scientific committee in Iraqi center for cancer and medical genetics research. The collected material of leave and dry fruit date were washed by tap water, then dried for four weeks in room temperature. The dried leaves were blended to fine powder using a mechanical blender while dry date was cleaned, removed of seed, and dried at room temperature then put in meat grinder to produce date paste, that used for alcoholic extraction.

Alcoholic Extraction 1.1

Hundred grams of date paste or leave powder were added to 1,000 ml of Methanol 70% mixed thoroughly by magnetic stirrer for 24 hours. The extract was filtered used muslin cloth then by Whatman No .1 filter paper and concentrated under reduced pressure at 40˚C, 90 rpm using a rotary evaporator [14]. The yields obtained were kept refrigerator for used. All phytochemical characterization were done according to a previous study [15] according to scientific standardization of ICCMGR laboratory.

Preparation of solutions 2.0

Trypsin versin (T.V.) (US biological, USA) (Amanda and Freshney, 2021) following order: weighed 5.05 gm of trypsin versin powder, add 500 ml triple distal water, add 1gm of Na-bicarbonate when the media without Na-bicarbonat, add antibiotic 0.75 ml, filters T.V by millipore then stirred constantly on a magnetic stirrer at room temperature.

Phosphate buffer saline (PBS) (BDH, UAE) the following materials should be prepared in advance (0.8 gm of NaCl, 0.2 gm of KCl, 1.15 gm of Na_2HPO_4 and 0.2 gm of KH_2PO_4). These materials are dissolved in 500 ml of distilled water, then the volume is completed to 1,000 ml. After adjusting the acidity of the solution to pH= 7.2 then it is sterilized (121^{o}C, 1 bar for 20 minutes) and kept at 4^{o}C [4].

Cell maintains growth media were prepared according to tissue culture protocol [16] for each MEM growth medium (US biological, USA) , RPMI-1640 medium (US biological, USA). Methyl thiazolyl tetrazolium (MTT) (Elabscience, USA) (solution prepared as fallow: The stain 3-(Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazoliumbromide (0.024g) is dissolved in 12 ml of PBS in order to prepare a 2 mg/ml concentration of the dye. The solution is then filtered using 0.2 µm Millipore filter to remove any blue formazan product, and stored in sterile, dark, glass bottle covered with foil at 4^{o}C. The solution should be used within no longer than 2 weeks of preparation (Cristina et al., 1998).

Cancer Cell Lines 2.1

Four breast cancer cell lines were used as MDA-MB-231, CAL51, MCF7, AMJ13, and one normal cell line MEF that provided and authenticated by cell bank unit/ experimental therapy department/ ICCMGR, Mustansiriyah University. MDA-MB-231, human breast cancer cell line, derives from a human adenocarcinoma that metastasizes to the pleural fluid. This cell line is one prototype for the study of hormone-independent breast cancer. These cells express high levels of epidermal growth factor (EGF) receptors inducible by ligand binding [17]. CAL-51 cells, obtained under a material transfer agreement [18]. MCF-7, The human breast adenocarcinoma cell line (MCF7) a widely studied epithelial cancer cell line derived from breast adenocarcinoma, has characteristics of differentiated mammary epithelium [19]. AMJ13 as a locally Iraqi established cell line [20]. MEF, the mouse embryonic fibroblast cell line as normal cells, its established in experimental therapy department/ Iraqi center for cancer and medical genetics research for used as control.

All cell lines used in this study was authenticated and mycoplasma was testing by cell bank unit of experimental therapy department/ Iraqi center for cancer and medical genetics research. The MDA-MB-231 and MCF7 cell

lines were growth and maintains in MEM growth medium (US biological, USA) with 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany) 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO2. While each CAL51, AMJ13, and MEF cell lines were cultured and maintained in a RPMI-1640 medium (US biological, USA) supplemented with 10% fetal bovine serum (FBS) (Capricorn- Scientific, Germany), 100 units/mL penicillin, and 100 µg/mL streptomycin at 37°C in a humidified atmosphere of 95% air and 5% CO_2 [16].

Alcoholic extracts dilution 2.2

The stock solutions of *Phoenix dactylifera* fruit and leave of were prepared of 40,000 µg /ml with Serum free media SFM (in media RBMI without serum) then filtered by Millipore 0.2 mm to be ready for serial dilution. One volume of this concentration was taken and added to one volume of SFM to prepared first dilution that continued by duplicate dilution to prepared 40,000, 20,000, 10,000, 5,000, 250, 125, 625 and 312.5 µg /ml.

MTT assay method 3.0

The cancer cell lines MDA-MB-231, CAL-51, MCF-7, AMJ13 and normal cell line MEF as a monolayer attached in tissue culture flask were washed with PBS and add 1ml of trypsin-versine solution with a gentle shaking until the cells were detached, cell suspension was seeded into 96-microtiter well plates. After that, treated with different concentrations of each date and leaves extracts of *Phoenix dactylifera* (40,000, 20,000, 10,000, 5,000, 250, 125, 625, 312.5 µg /ml, and without treatment as negative control) with five replications for each concentration and incubated for 72 hours with 5% CO_2 at 37ºC in a humidified atmosphere. After 72 hrs. MTT reagent 50 µl of fresh culture medium was added to each well for 4-hour incubation at 37ºC under 5% CO_2, the MTT stain was removed from plate after incubation period and added to each well 50µl DMSO according to [21] to dissolve the MTT-formazan crystals. Finally, the plate became ready for reading by ELISA reader at 570 nm. The inhibitory concentration rate was calculated as the drug concentration as fallow:

G.I.%=(Control-Concentration_test)/Control*100

Statistical Analysis

Experiments data of variance was used for data comparison between studied groups (control and samples) using 5 replications for each experiment. was determined using students t- test. A P value ≤ 0.05 was considered statistically significant. For this analysis, we used the GraphPad Prism 8 software (GraphPad Software, Inc. San Diego, California).

Results

The date palm (fruit and leaves) extracts of *Phoenix dactylifera* were tested against four breast cell lines, MCF7, MDA-MB-231, CAL-1, AMJ13 and testing its safety against normal cell line MEF. The results showed the extracts activity against breast cancers and the normal cell line as listed in the following Tables (1 and 2) and Figure 1 (A-D).

Table 1. showed there were a cytotoxic effect of leaves extracts on all breast cancer cell lines used in this study with concentration dependent. AMJ13 cell line showed highest significant effect (P< 0.05) on four concentrations (from 2500 - 20000) µg/ ml with growth inhibition percentage (GI) with (66.7, 70.6, 53, and 54 %) respectively in AMJ13 cell line. While the effect against MCF7, MDA-MB, and CAL51 cell lines were showed low growth inhibition with the highest significant effect (P< 0.05) at two concentrations (10000- 20000 µg/ ml) causing growth inhibition (64.3, and 64.3 %) respectively in MCF7, and (40.0, and 50.0 %) respectively in MDA-MB, and (44.0, and 52.0 %) respectively in CAL51. While the normal MEF cell line showed no appearance for any cytotoxic effect in all concentration of leaves extract (causing increase the proliferation rate in all concentration).

While, Table 2. showed slightly or no cytotoxic effects on the dray date fruit extracts in all breast cell lines significantly (P< 0.05). All concentration of the all-cell lines showed no or slight growth inhibition with the highest effect on 40000 µg/ml in MCF7 cell line with growth inhibition percentage (50.0%). While normal MEF cell line showed no appearance any effect in all concentration of extract dray date fruit.

Table 1. Growth Inhibition Effect of Leaves Date Palm Extract in Different Concentrations of Breast Cancers and Normal Cell Lines after 72 hours Exposure Time.

Concentration of leaves date palm (µg /ml)	Growth Inhibition %				
	MCF7	AMJ13	MDA-MB	CAL51	MEF
312.5	0.0	41.18	0.0	0.0	0.0
625	14.4	46.7	0.0	0.0	0.0
1250	21.4	47.1	0.0	0.0	0.0
2500	40.0	66.7	0.0	10.0	0.0
5000	40.0	70.6*	0.0	16.0	0.0
10000	64.3*	53.0	40.0	44.0	0.0
20000	64.3*	54.0	50.0*	52.0*	0.0
40000	57.1	53.3	50.0*	50.0	0.0

*, Significant effects at P< 0.05

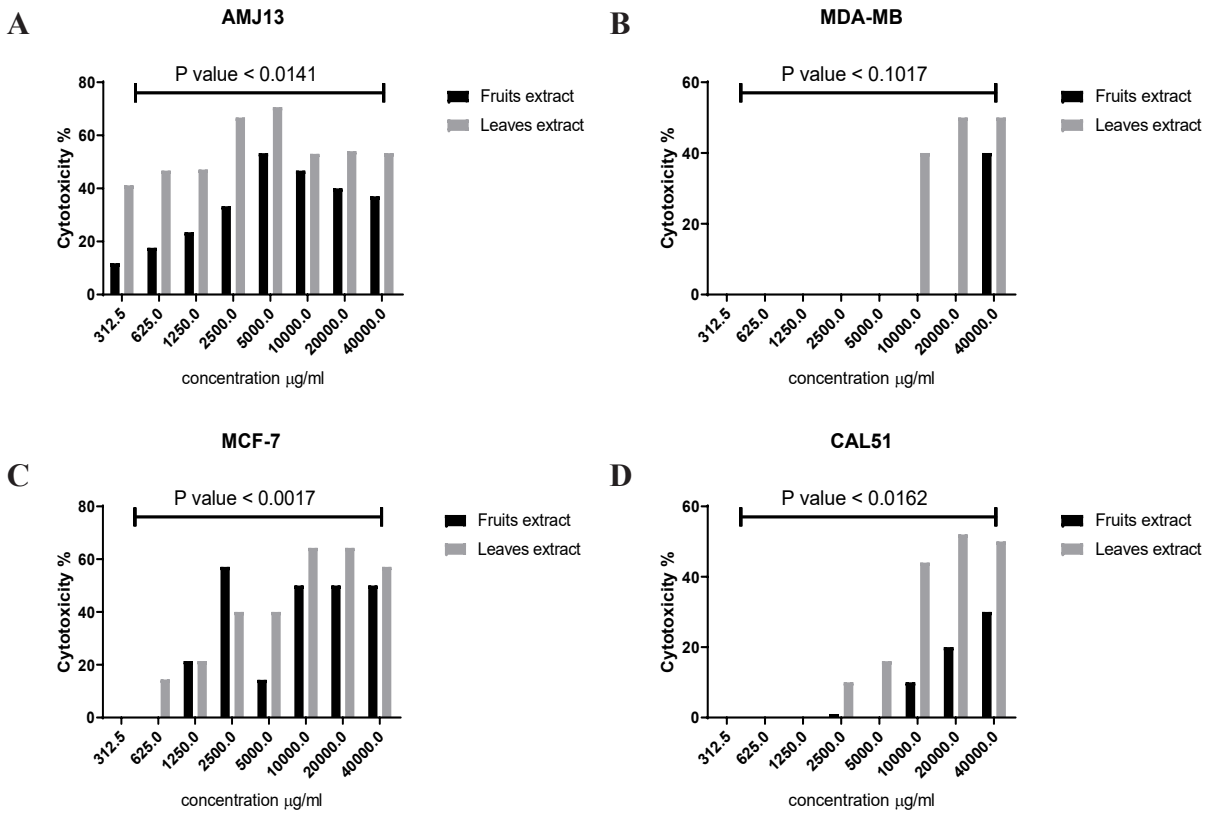

Figure 1. Comparation between Fruit and Leaves Extract in Growth Inhibition Percentage in Different Concentrations of Breast Cancers (A-D) after 72 hours exposure time which showed that fruit extract was the highest effect with significant effect at P >0.05.

Discussion

This study aimed to investigate the possible growth inhibition effect of both dray date fruits and leaves of *Phoenix dactylifera* against breast cancer cells. This study investigated both dray date fruits and leaves extracts of *Phoenix dactylifera* on panel of breast cancer cell lines on AMJ13, and MCF-7, CAL-51 and MDA-MB with no effect on normal cells MEF. The results indicate weak or no effect for the fruit extract while the leaves extract showed higher killing activity (Figure 1), this may be due to the fact that the fruits extract considered a food substance, as it does contain many essential nutritional compounds [22], these facts compatible with Al-Snafi et al that showed leaves dates extract has cytotoxic effect more than fruits extract [23].

A Previous studies revealed that polyphenol compounds in date palm reduce cancer cell growth by inducing apoptosis in many cell lines of mammary cancer [24, 25], also the activity of date extracts may due to present of variety compound contains such as ascorbic acid, phenolic compounds [26], gallic acid [27], p-hydroxybenzoic acid, vanillic, caffeic, synergic, and ferulic acids [28, 11].

The results of our study showed mild cytotoxic effect of the Iraqi date palm fruit on AMJ13, and less in MCF-7 breast cancer cell lines, this results were different from other studies such as reported by Fazal et al. [3] which demonstrated that the methanolic extract of Ajwa date,

Table 2. Growth Inhibition Percentage Effect of Dray Date Palm Fruit Extract in Different Concentrations of Breast Cancers and Normal Cell Lines after 72 hours Exposure Time

Concentration of fruit date palm (µg /ml)	Growth Inhibition %				
	MCF7	AMJ13	MDA-MB	CAL51	MEF
312.5	0	11.8	0	0	0
625	0	17.6	0	0	0
1250	21.4	23.5	0	0	0
2500	57.1*	33.3	0	1	0
5000	14.3	53.3*	0	0	0
10000	50	46.7	0	10	0
20000	50	40	0	20	0
40000	50	37	40	30	0

*, Significant effects at P< 0.05

a variety of *Phoenix dactylifera*, inhibited human breast adenocarcinoma (MCF7) cells in vitro by inducing apoptosis and cell cycle arrest, also showed up-regulation of p53, Bax, Fas, and FasL and down-regulation of Bcl-2 [29, 3]. Also, other study showed cytotoxic effects for MCF7 and two other cell line (Lung Cancer A54, Human cervical carcinoma HeLa) by Shakkeela et al. [30] when using of *Phoenix dactylifera*, dates fruit early-stage hababuk for phytochemical screening, antioxidant, and cytotoxic potentials.

Another study on other part which is seeds of the fruit, conducted by Khan et al. [31] found that the seed extract of *Phoenix dactylifera* exhibited anticancer potential in human breast cancer MDA-MB-231 and MCF-7 cells, as well as liver cancer HepG2 cells, its revealed that the extract inhibited cell proliferation, induced loss of mitochondrial membrane potential (MMP), and caused the formation of apoptotic bodies. It also activated the enzymatic activity of cleaved caspase-3, an apoptosis executioner protein, and caused the cleavage of poly-ADB ribose polymerase (PARP) protein [31]. Furthermore, a study on Medjool dates, another variety of *Phoenix dactylifera*, also indicated potential anticancer on breast adenocarcinoma (MCF-7) cells and antioxidant effects [11]. Another study showed *Phoenix dactylifera* extract has anti-proliferative effect on MCF-7 and MDA-MB-231cancer cell lines (Cristina et al., 1998; Hanen, et al., 2018). While no studies showed its activity on the Iraqi cell line AMJ13 which indicated that this is the first study showed activity on date palm extracts against Iraqi patient derived cancer cells.

Therefore, our results propose that *Phoenix dactylifera* leaves extract may have promising role as cancer therapy, particularly against breast cancer. However, more research is needed to fully understand its anticancer potential and to evaluate its safety and efficacy.

While the results showed slightly or no cytotoxic effects on MDA-MB-231 and CAL51 cell lines in both leaves and fruit extracts, it may due to that both these cell lines were a model of triple-negative breast cancer and its ER, PR, and E-cadherin negative and expresses mutated p53 so its chemo resistant cell lines [32-34] compared with MCF7 and AMJ13 which was an Invasive breast carcinoma cell lines which have wild type P53 [20, 35].

In conclusion, the Iraqi *Phoenix dactylifera*, or the date palm, has shown promising potential as an anticancer agent, particularly against breast cancer. Our study has demonstrated that leaves extract can inhibit to certain level cell proliferation of breast cancer with high safety on normal cells. Its suggest that *Phoenix dactylifera* may have a role in cancer therapy, particularly against breast cancer, it is important to note that more research is needed to fully understand its anticancer potential and efficacy.

Author Contribution Statement

AL-Zeiny, Saadia Saleh Mehdy; Mohammad, Maeda Hussain; Almzaien, Aous Kahtan; Al-Shammari, Ahmed Majeed performed the experimental tests. Cell culture was carried out by Ahmed, Ayser Ayed; Shaker, Hiba Karem. AL-Zeiny, Saadia Saleh Mehdy; Mohammad, Maeda Hussain; Al-Shammari, Ahmed Majeed performed the statistical analysis. AL-Zeiny, Saadia Saleh Mehdy; Mohammad, Maeda Hussain; Almzaien, Aous Kahtan; Al-Shammari, Ahmed Majeed wrote the article.

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Consent for publication

All authors have given consent for publication.

Approval

This work approved by the scientific committee of Iraqi Center for cancer and Medical Genetics Research, Mustansiriyah University.

Conflict of interest

The authors declare no potential conflict of interest.

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