Research Article

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

Noor A. Al-Zubaidy[1], Adeeb A. Al-Zubaidy[2], Hayder B. Sahib*[2]
Pharmacology Department, Al-Nahrain University, College of Medicine[1], Egypt.
Pharmacology Department, Al-Nahrain University, College of Pharmacy[2], Egypt.
***Corresponding author's E-mail:** haider_bahaa@yahoo.com

Accepted on: 12-10-2016; **Finalized on:** 30-11-2016.

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 IC $_{50}$ values for chloroform extract of *phoenix dactylifera* seeds was 3.18 µg/ml. The IC_{50} 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 chloroforme 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 women[1]. In the United States, breast cancer represents of 29% of all cancers in women, and is second only to lung cancer as a cause of cancer deaths[2]. The causes of cancer are various, complex, and only partially understood[3]. Many things are known to increase the risk of cancer, including: Female sex, increasing age[4], non-Hispanic whites[5], a positive family history of breast cancer[6] late age at first pregnancy, null parity, early onset of menses, and late age of menopause[7], the oral contraceptives (OCs) and hormone replacement therapy (HRT)[8], diet with of red meat, fat, caffeine, and alcohol[9], and ionizing radiation[10]. Diets that are rich in grains, fruits, vegetables[12], calcium/vitamin D[11] 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 cancer[12]. *Phoenix dactylifera* Linn (date palm), called the Tree of Life by the Arabs, is considered as one of the oldest cultivated fruit trees[13] means date palm, and "dactylifera" from a Greek word "daktulos" meaning a finger[14]. Date palms and culture are depicted in ancient Assyrian and Babylonian tablets, including the famous Code of Hammurabi[15].

Phoenix dactylifera belonging to Palmaceae family and it is differ in shape, size, and weight[16].

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 dactilefera 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 10^4. 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

IC_{50} 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 µg/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 dactilefera* 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 200µl 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 MCF7cell and incubated for 4 hours earlier to the absorbance measurements at 570nm[17].

RESULTS

Activity of *phoenix dactilefera* 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 = 0.0418ln(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 µg/ml.

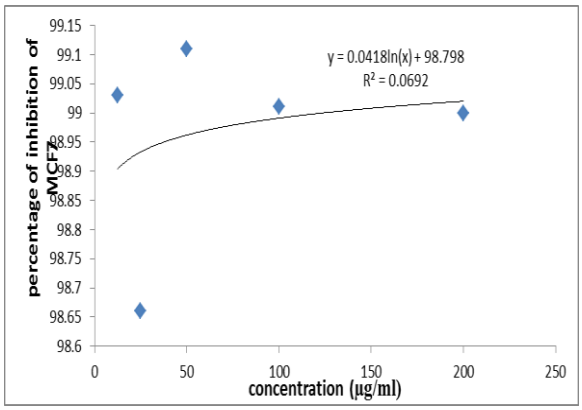

Figure 1: Dose Response Curve of Chloroform Extract of Phoenix Dactylifera Seeds on MCF7 Cancer Cell Line

The IC_{50} values for chloroform combination with tamoxifen was 7.17 µg/ml.

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

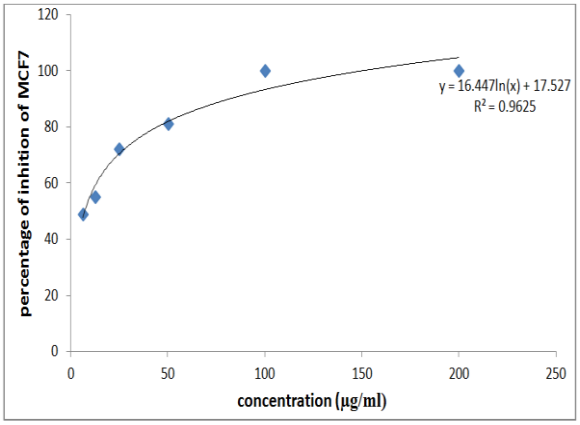

Figure 2: Dose response curve of chloroform extract of *phoenix dactylifera* seeds in combination with tamoxifene on MCF7 cancer cell line

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

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

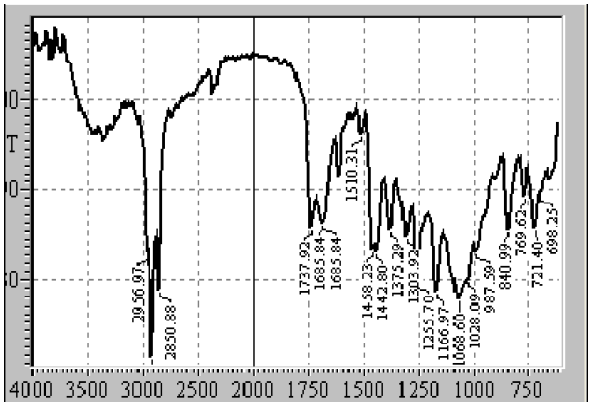

Figure 3: FT – IR peaks Absorbance Chart for Chloroform Extract of *Phoenix dactylifera S*eeds

DISCUSSION

Chloroform extract of *phoenix dactylifera* seeds displayed significant anti-angiogenesis activity against the rat aorta[18]; and the anti-angiogenic agents may have anti-tumor 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].

MTT 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 extract 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 dactylifere* 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 tumoriogenesis, attributable to functioning as an antioxidant in addition to displaying some anti-carcinogenic potential[24], this finding agreed with the present study. In Habib and coworker (2014)[25] 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 dactylifere* seeds which inhibit the initiation, progression and spread of cancers in vitro and in animals *in vivo*. This results support a very promising anticancer 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[31].* Sylvei and colleagues, 2014[33] 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[36]. 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[34,35]. 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[34,35].

REFERENCES

1. Torre L. A., Bray F., Siegel R. L., Ferlay J., Lortet-Tieulent J., & Jemal A. Global cancer statistics, 2012. *CA: a cancer journal for clinicians*, 65(2), 2015, 87-108.
2. Siegel R. L., Miller K. D., & Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians, 65(1), 2015, 5-29.
3. Anand P., Kunnumakara A. B., Sundaram C., Harikumar K. B., Tharakan S. T., Lai O. S., & Agarwal, B. B. Cancer is a preventable disease that requires major lifestyle changes. Pharmaceutical research, 25(9), 2008, 2097-2116.
4. Loscalzo J. *The Brigham Intensive Review of Internal Medicine*. Oxford University Press, USA. 2012.
5. Alteri R., Bandi P., Brinton L., Casares C., Cokkinides V., & Gansler T. Breast cancer facts & figures 2011-2012. American Cancer Society, Atlanta, GA., USA, 2011, 1-36.
6. Parmigiani G., Chen S., Iversen E. S., Friebel T. M., Finkelstein D. M., Anton-Culver H. & Daling, J. R. Validity of models for predicting BRCA1 and BRCA2 mutations. Annals of internal medicine, 147(7), 2007, 441-450.
7. Colditz G. A., Rosner B. A., Chen W. Y., Holmes M. D., & Hankinson S. E. Risk factors for breast cancer according to estrogen and progesterone receptor status. Journal of the National Cancer Institute, 96(3), 2004, 218-228.
8. Garbe E., Levesque L., & Suissa S. Variability of breast cancer risk in observational studies of hormone replacement therapy: a meta-regression analysis. *Maturitas*, 47(3), 2004, 175-183.
9. Press D. J., & Pharoah P. Risk factors for breast cancer: a reanalysis of two case-control studies from 1926 and 1931. Epidemiology, 21(4), 2010, 566-572.
10. Carmichael A., Sami A. S., & Dixon J. M. Breast cancer risk among the survivors of atomic bomb and patients exposed to therapeutic ionising radiation. European Journal of Surgical Oncology (EJSO), 29(5), 2003, 475-479.
11. Maughan K. L., Lutterbie M. A., & Ham P. S. Treatment of breast cancer. Am Fam Physician, 81(11), 2010, 1339-1346.
12. Holmes, M. D. & Willett W. C. Does diet affect breast cancer risk? Breast Cancer Research, 6(4), 2004, 1.
13. Phillipson J. D. Phytochemistry and medicinal plants. Phytochemistry, 56(3), 2001, 237-243.
14. Zaid A and DeWet PF (2002). Date palm cultivation. Chapter I: Botanical and systematic description of the date palm. FAO Plant Production and Protection Paper 156 Rev. 1.
15. Nixon R. W. The date palm— "Tree of Life" in the subtropical deserts. Economic Botany, 5(3), 1951, 274-301.
16. Chao CT and Robert RK. The Date Palm (Phoenix dactylifera L.): Overview of Biology, Uses, and Cultivation. Hortscience, 42(5), (2007), 1077-1082.
17. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol Methods, 16, 1983, 55-63.
18. Al-zubaidy, A. A. Hayder B Sahib, & Hussein Z. A. The Anti–Angiogenic Activity of Phoenix Dactylifera Seeds Extracts. International Journal of Pharmacy and Pharmaceutical Sciences, 8(1), 2015, 311-315.
19. Ajith T.A. & Janardha K.K. Cytotoxic and antitumor activities of a polypore macrofungus, Phellinus rimosus (Berk) Pilat. Journal of Ethnopharmacology, 84, 2003, 157-162.
20. Berridge MV, Herst PM, and Tan AS. Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnology Annual Review, 11, 2005, 127-152.
21. Bernas T, Dobrucki J. "Mitochondrial and non mitochondrial reduction of MTT: interaction of MTT with TMRE, JC-1, and NAO mitochondrial fluorescent probes". Cytometry, 47(4), 2002, 236–242.
22. Tan M.L., S.F. Sulaiman N. Najimuddin, Samian M.R. & Muhammed T.S. Methanolic extract of Pereskia bleo (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma T47-D cell line. Journal of ethinopharmacology, 96, 2005, 287-294.
23. Sahib H. B., Adeeb A., AL-zubaidy S. M. H., & Jassim G. A. The antiangiogenic activity of Vitex agnus-castus leaves extracts. International Journal of Pharmacy and Pharmaceutical Sciences, 6(1), 2014, 863-869.
24. Waly M. I., Al–Ghafri B. R., Guizani N., & Rahman M. S. Phytonutrients effect of date pit extract against azoxymethane–induced oxidative stress in rat colon. Asian Pacific J. Cancer Prev. (2013).
25. Habib H., Platat C., AlMaqbali F., & Ibrahim W. Date seed (Phoenix dactylifera) extract reduces the proliferation of pancreatic cancer cells, DNA damage and superoxide-dependent iron release from ferritin *in vitro*. The FASEB Journal, 28(1 Supplement), 2014, 829-20.
26. Rice-Evans C. A., Miller N. J., & Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free radical biology and medicine*, 20(7), 1996, 933-956.
27. Rice-evans C. A., Miller N. J., Bolwell P. G., Bramley P. M., & Pridham J. B. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free radical research*, 22(4), 1995, 375-383.
28. Hodek P., Trefil P., & Stiborová M. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chemico-biological interactions, 139(1), 2002, 1-21.
29. Galati G., Teng S., Moridani M. Y., Chan T. S., & O'Brien P. J. Cancer chemoprevention and apoptosis mechanisms induced by dietary polyphenolics. *Drug metabolism and drug interactions*, 17(1-4), 2000, 311-350.
30. Webster R. P., Gawde M. D., & Bhattacharya R. K. Protective effect of rutin, a flavonol glycoside, on the carcinogen-induced DNA damage and repair enzymes in rats. Cancer letters, 109(1), 1996, 185-191.

31. Ramos, S. Cancer chemoprevention and chemotherapy: dietary polyphenols and signalling pathways. Molecular nutrition & food research, 52(5), 2008, 507-526.

32. Mojzis J., Varinska L., Mojzisova G., Kostova I., & Mirossay L. Antiangiogenic effects of flavonoids and chalcones. Pharmacological research, 57(4), 2008, 259-265.

33. Sylvie L, Amira O, Richard B and Richard RD. Olive oil compounds inhibit vascular endothelial growth factor receptor-2 phosphorylation. Experimental Cell Research, 322(1), 2014, 89–98.

34. Sivasubramanian R and Brindha P. *In vitro* cytotoxic, antioxidant and GC-MS studies on centratherum punctatum Cass. International Journal of Pharmacy and Pharmaceutical Sciences, 5(3), 2013, 364-367.

35. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, and Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Research, 22, 2002, 2587−2590.

36. Jeong W. C., Kim K. J., Ju H. W., Back H. K., Kim H. K., Im S. Y., & Lee H. K. Cytoplasmic phospholipase A2 metabolites play a critical role in pulmonary tumor metastasis in mice. Anticancer research, 30(9), 2010, 3421-3427.

37. Aparna V., Dileep K. V., Mandal P. K., Karthe P., Sadasivan C., & Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chemical biology & drug design, 80(3), 2012, 434-439.

38. Sermakkani M., & Thangapandian V. GC-MS analysis of Cassia italica leaf methanol extract. Asian journal of pharmaceutical and clinical research, 5(2), 2012, 90-94.

39. Gopalakrishnan K., & Udayakumar R. GC-MS Analysis of Phytocompounds of Leaf and Stem of Marsilea quadrifolia (L.). International Journal of Biochemistry Research & Review, 4(6), 2014, 517.

Source of Support: Nil, **Conflict of Interest:** None.