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Non-toxic antiproliferative effect of *Ficus carica* fruit extracts on estrogen receptor positive breast cancer cell (MCF-7)

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ABSTRACT

Cancer is one of many diseases of global concern due to its high mortality rate with various therapeutic options but most of them are toxic and this compelled many cancer patients to seek alternative and complementary method for treatment. Plants have been recognised as alternative source of remedy for various ailments as they contain many bioactive compounds. The objective of this study was therefore to determine the antiproliferative activity and non-toxic effect of dried preserved fruit extracts of Ficus carica on estrogen/progesterone receptor positive breast cancer cell (MCF-7) and mouse epithelial cell (3T3). The ethyl acetate, ethanol and hexane extracts were prepared by maceration and then concentrated under vacuum at 40^{o}C by rotary evaporator to get the crude extracts. The antiproliferative effect of the extracts was determined using microtitration colorimetric method of 3(4, 5 diphenylmethyl thiazol-2-yl)-2, 5 diphenyltetrazolium bromide assay. The ethyl acetate has low extract yield compared to ethanol and hexane extracts. The ethyl acetate extract was found to have strong and significant antiproliferative activity against estrogen/progesterone receptor positive breast cancer cells (MCF-7) with IC_{50} value of 9.8µg/mL, whereas the ethanol and hexane extracts showed weak antiproliferative activity. Interestingly, all the extracts have non-toxic effect on 3T3 cell line. These finding suggest that crude extracts of Ficus carica fruit have antiproliferative effect on the estrogen/progesterone receptor positive breast cancer cells. These may be attributed to the bioactive compound present in the crude extracts. The study also suggests a promising chemoprotective potential of Ficus carica fruit extracts on estrogen/progesterone receptor positive breast cancer cells. Further studies are required to isolate the bioactive compounds of the fruit extracts and establish their mechanism of actions.

Key words: Non-toxic, antiproliferative, *Ficus carica* fruit, estrogen/progesterone breast cancer cell line (MCF-7).

INTRODUCTION

Cancer is the second leading cause of death in the U.S (1) when ranked within the age groups cancer is one of the five leading causes of death amongst both male and females and single largest cause of death world wide (2). In the year 2012, there were 10.4 million new cancer cases and 8.2 million deaths, according to the global cancer statistics, from which 521,000 cases were breast cancer related and it is expected that this number will be doubled in 2030 (3). The most common cancers among women are breast cancer and cervical cancers. Breast cancer is caused by repeated exposure of breast cells to circulating ovarian hormones (4). Clinical, animal and epidemiological studies have clearly proved that breast cancer is a hormonally mediated disease and several factors that influence hormonal status or are markers of change in hormonal status have been shown to be linked with the risk of breast cancer (5). A variety of risk factors have been revealed such as multipolarity, early onset of menarche, delayed first birth, late menopause and decreased parity. These risk factors point toward endogenous estrogen as likely players in the initiation, progression and promotion of breast cancer (6).

Breast cancer is a notable cause of morbidity and mortality among all forms of cancers in women world wide (7) which has made the prevention and treatment of breast cancer a major health issue and health care goal (8).

Currently, cancer treatment includes surgery, radiotherapy, immunotherapy and chemotherapy. Despite the advancement in cancer treatment, breast cancer remains a tragic disease (9). However, since conventional methods often have undesirable side effects, there is critical need for alternative method for cancer treatments and preventions (10).

Traditionally, plants have been the best sources of medicine for thousands of years and are the best alternatives, as they provide an inexhaustible pool of efficacious agents for treating diseases (11). In this regard, both the scientific community and the general public have recognized the use of many medicinal plants and a tendency to depend on herbal medicine is on the rise. Fruits, vegetables and spices demonstrated ability to suppress cancers (12). It has also been postulated that a high intake of fruits and vegetables could contributes to the prevention of cancer because of their high content of a variety of phytochemicals (13). This has resulted in screening plant products in search of novel therapeutic agents (14). Previous report concerning the nutrients composition of dried *Ficus carica L.*fruits indicated that it has the best nutrients score among the dried fruits, being an important source of minerals and vitamins (15).

The fruits, roots, leaves and latex are used in the native system of medicine in different disorders such as gastrointestinal, respiratory, inflammatory, cardiovascular disorders (16) and offers some other therapeutic effects such as anti-Herpes simplex Virus (17), anthelmintic (18), antimutagenic (19), cytotoxic (20) and antioxidative activities (21).

Presently, information regarding the potential of *Ficus carica L.* fruits as antiproliferative agent on breast cancer cell line (MCF-7) are lacking, only few reports are available regarding antiproliferative study of *Ficus carica L.* fruits extracts on cancer cell line (22). More recent investigations indicated that different parts of *Ficus spp* like fruit, stem and latex posses' antiproliferative, cytotoxic and antioxidant activities (20); (21). Foristance ethanolic extracts of fruit and leaves were found to have cytotoxic activities on human cervical cancer (HeLa) cell line (23). Additionally, *Ficus carica L.* latex inhibited the proliferation of oesophageal cancer cell line (KYSE-30) and stomach cancer cell line, but did not indicate any cytotoxic activity against normal cells *in vitro*(24). To date there is no study to our knowledge that evaluates the *in-vitro* antiproliferative effect of dried preserved fruit extracts (hexane, ethyl acetate and ethanol) obtained from *Ficus carica L.* fruit against estrogen receptor positive breast cancer cell line (MCF-7). Therefore the aims of this study was to evaluate the *in-vitro* antiproliferative effects of the obtained *Ficus carica L.* fruit extracts (hexane, ethyl acetate and ethanol) on estrogen receptor positive breast cancer cell line (MCF-7) and normal cell line (Mouse epithelial cell: 3T3), as positive control cell line.

EXPERIMENTAL SECTION

2.1 Reagents and chemicals

3- (4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulphoxides (DMSO), RPMI-1640, phosphate buffer saline (PBS), fetal bovine serum (FBS), Tryple E, Penicillin/streptomycin (pen/strep) and Hydrogen peroxides (H_2O_2) were all purchased from biodiagnostic ltd. FBS was heat-activated for 30 minutes in a water bath prior to use.

2.2 *Ficus carica L.* fruit (fig fruits)

Commercially dried preserved *Ficus carica L.* fruits (400g) packed by Elmas dried figs Garland, Turkey with best before 07/2015 and lot number 09002085-11-00036 where purchased and collected from Giant Hypermarket Kuala Terengganu, Malaysia and confirm at Department of Plant Science, Faculty of Bioresources UniSZA.

2.3 *Ficus carica L.* fruit Extraction procedure

The method introduced by *Adel et al.,* (2009) was used with slight modifications. The fruits were extracted using different solvents in an increasing order of polarity (hexane, ethyl acetate, and methanol) (25). The fruits were carefully cut into small pieces (0.5 cm × 0.5 cm × 0.5 cm). 100 g of *Ficus carica L.* fruits was carefully macerated using 1L of hexane for 24hours with occasional shaking, and the process was repeated three times. The residue was air dried overnight before it was extracted with ethyl acetate and methanol following the above procedure. Subsequently, all the extracts for each solvent were filtered through whatman no.41filter paper (pore size 20-25 µM) and were then concentrated under vacuum at 40^{o}C by rotary evaporator to get the crude extracts, all the crude extracts were then stored in a refrigerator (-20^{o}C) until they were analysed.

2.4 *Ficus carica L.* fruit extract concentration preparations

To determine the antiproliferative activity of *Ficus carica L.* fruit extracts (hexane, ethyl acetate and ethanol) obtained from the fruits. The extracts were weighed and dissolved in dimethylsulphoxides (DMSO) to an appropriate concentration (20 mg/mL) and stored as a stock solution at -20^{O}C until used. The final concentration of DMSO used was less than 1%, and at this concentration, DMSO does not affect cell viability (26). The stock solutions for the treatment of cell lines were further diluted in Roswell Park Memorial Institute medium (RPMI-1640), enriched with 10% (v/v) fetal bovine serum (FBS) and 1% antibiotic (pen/strep) cocktail to give final concentration of *Ficus carica L.* fruit extracts of 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 and 0.390625 µg/mL each.(27).

2.5 Cell culture conditions

Human breast cancer cell line (MCF-7) and mouse epithelial cell line (3T3) were obtained from tissue culture research laboratory, Faculty of Bioresources and Food Industry University Sultan Zainal Abidin (UniSZA) Tembila Campus, Besut. The cancer cell line (MCF-7) and normal cell line (3T3) were grown and maintained in the RPMI-1640 medium supplemented with 10% heat activated fetal bovine serum (FBS) in a humidified atmosphere of 5% CO_2 at a temperatures of 37^{O}C and 95% relative humidity. The media was changed twice a week.

2.6 Antiproliferative assay

The antiproliferative activity of the fruit extracts obtained using hexane, ethyl acetate and ethanol was determined using the microtitration colorimetric method of tetrazolium salt 3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide (MTT) reduction assay(28). This assay measured the reduction of yellow MTT by mitochondrial succinate dehydrogenase into an insoluble dark punctate colored formazan product. The insoluble dark formazan products were then solubilized with an organic solvent (DMSO) and the released solubilized formazan were then measured spectrophotometrically. An increased in dark purple color indicated an increase in metabolic activities by the cells.

A total of 100 µL cultured cancer and normal cells suspension with 80-90 % confluence were dispensed in triplicates into each well of 96 well cultured plates at optimized concentrations of 1x10^{5} cells for MCF-7 and 3T3. After 24 hrs of recovery period. Sample extracts were diluted with RPMI-1640 media without serum. The extracts were initially dissolved in DMSO as mentioned earlier with final concentration of DMSO being 0.1% (v/v). Serial dilutions of the samples were prepared in RPMI-1640. Thereafter, various concentrations of the extract samples were plated out in triplicate. Each plate included untreated cells (negative control) and a treatment with 3% hydrogen peroxide (Positive control) which have been implicated as a cause of cellular death (29). After 72 hrs of incubation, 20µL MTT (5 mg/mL) in phosphate buffered saline (PBS) were added in to each well and re-incubated for 4 hours. After re-incubation for 4 hours the medium was removed and formazan crystals were dissolved with 100 µL DMSO. Finally, the absorbance was measured at 570 nm using a micro plate reader (TECAN, INFINTE M2000) and cell proliferation (percentage cell viability) was calculated with the appropriate controls taken into accounts. At least three replicates from each sample were used to determined cell proliferation (percentage cell viability). The relative viability of the treated cells as compared to the control cells was expressed as the percentage cytoviability, using the following formula.

% cytoviability = Absorbance of treated cells / Absorbance of control cells *100%

The inhibitory concentration (IC_{50}) was determined by non-linear regression analysis of the corresponding dose response curve i.e. IC_{50} values were determined from the plot of percentage of cell viability on the Y-axis against extracts concentration on the X-axis.

2.7 Statistical analysis

Data were expressed as mean standard deviation (SD). Statistical analysis was performed with one way ANOVA, using IBM-SPSS v.19.01 to identify the significance ($P<0.05$) of antiproliferative effect of the various extracts.

RESULTS

Antiproliferative activity of *Ficus caricaL.* fruit extracts on estrogen positive receptor breast cancer cell line (MCF-7) and mouse epithelial cell line (3T3)

The *Ficus caricaL.* dried fruit were extracted using three different solvents with their increasing order of polarity. The obtained extracts percentage yields were found to be 18.72%, 0.57% and 0.48% for ethanol, ethyl acetate and hexane respectively**.** The antiproliferative activity of *Ficus carica L.* fruit extracts (ethyl acetate, ethanol and hexane) and the hydrogen peroxide (H_2O_2) (positive control) on estrogen receptor positive breast cancer cells (MCF-7) after 72 hours of incubation are shown (Fig: 1 a & b). Ethyl acetate (EA) extracts of *Ficus carica* fruit had stronger inhibitory ($p<0.05$) activity against the estrogen receptor positive breast cancer cells (MCF-7) with IC_{50}

value of 9.8 µg/mL (Fig:1a & b), while the hydrogen peroxide (H_2O_2) had IC_{50} value of 0.2 µg/mL (Fig: 1a & b). The hexane extracts (HEX) and ethanol extracts (ET) showed weak inhibitory activity towards the proliferation of breast cancer cell line (Fig: 1a & b). A concentration dependent effect was observed for ethyl acetate extract as it was observed for hydrogen peroxide (H_2O_2) (Table 3.1). The antiproliferative activities of *Ficus carica L.* fruit extracts and H_2O_2 on mouse epithelial cells (3T3), having IC_{50} 14 µg/mL was also shown (Fig: 1c & d). Hydrogen peroxide inhibited the growth of normal cell (3T3) whereas the *Ficus carica L.* fruits extract had no inhibitory effects on normal cells.

Table 4.1: Percentage cell viability after 72 hrs of incubation

Samples/Extracts	Concentration in µg/Ml							
	50	25	12.5	6.25	3.125	1.5625	0.78125	0.390625
	Percentage cell viability (%)							
Ethyl acetate extracts	11	17.8	28.5	58	56.5	66.5	87.5	100
Ethanol extracts	86	86	93	82	81	84.5	99	100
Hexane extracts	83	83	94	98	93	65	95	100
H_2O_2 (positive control)	11.8	15.0	15	15	15	13	20	100

Figure: 1a

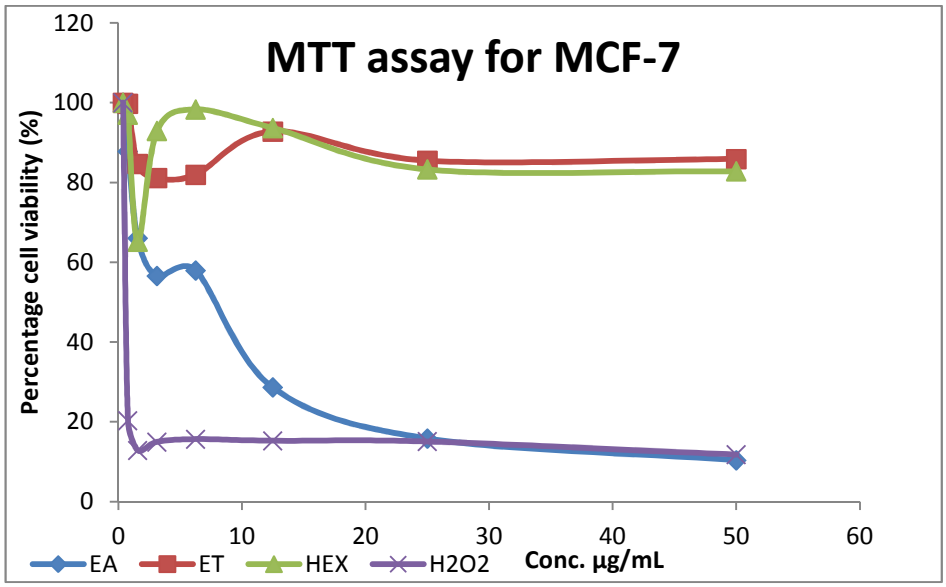

Fig: 1b

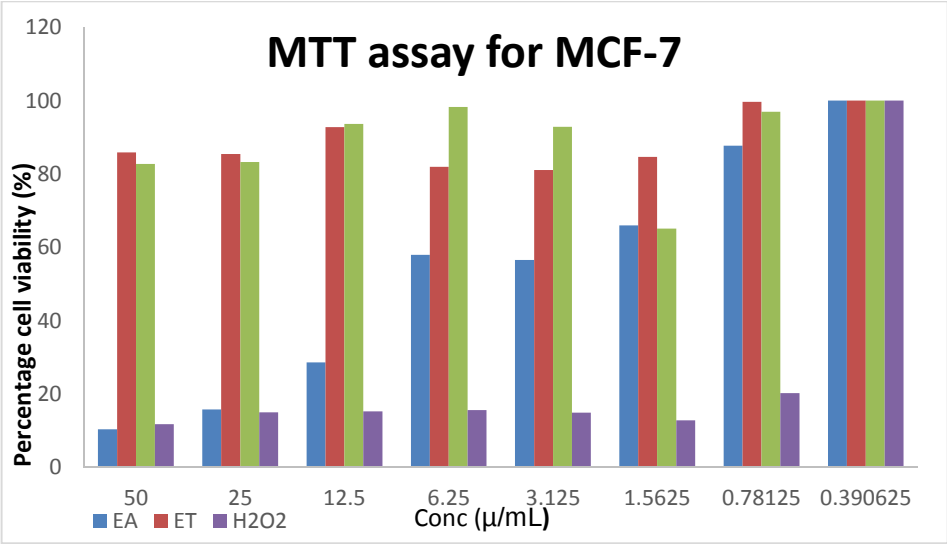

Figure 1a & b: Antiproliferative activity of *Ficus carica L.* fruit extracts (EA, HEX and ET) and standard hydrogen Peroxides (H_2O_2) on estrogen positive receptor breast cancer cell line (MCF-7)

Each point represents a mean of three determinations

Fig: 1c

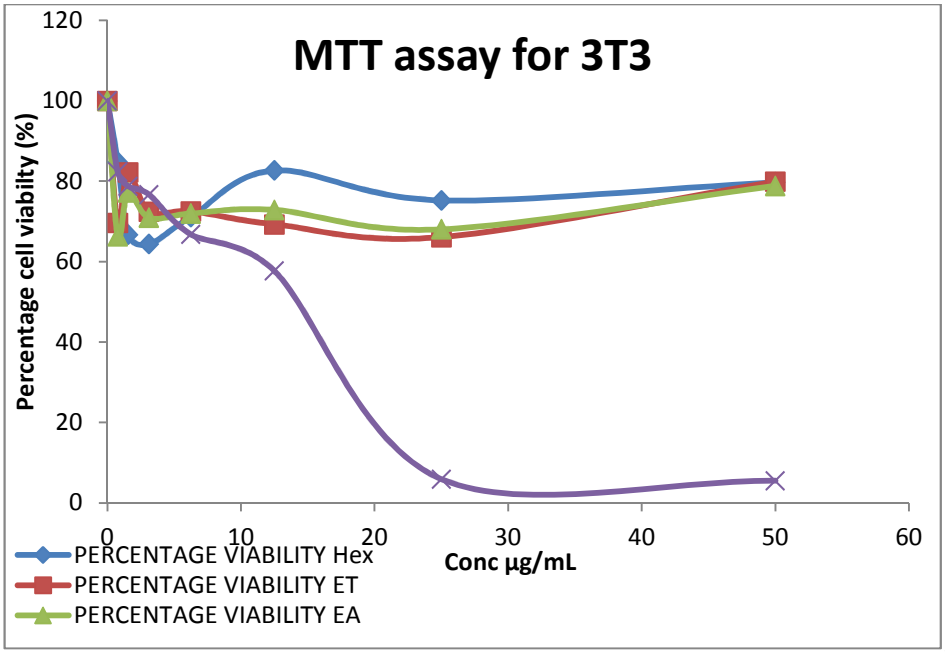

Fig: 1d

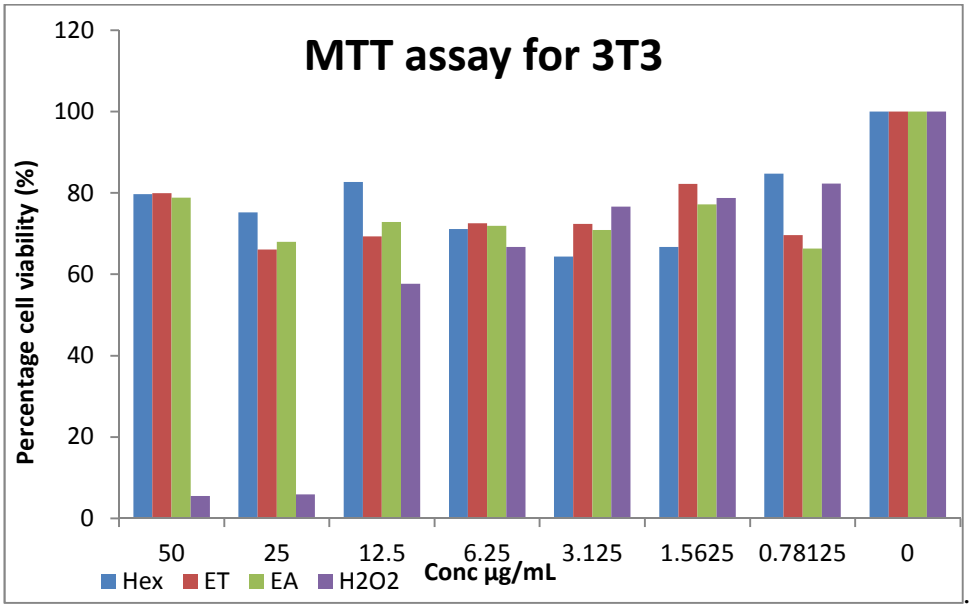

Fig. 1c & d: Antiproliferative activity of *Ficus carica L.* fruit extracts (EA, HEX and Ethanol) and standard hydrogen Peroxides (H_2O_2) on normal cell line (3T3)

Each point represents a mean of three determinations.

DISCUSSION

The antiproliferative activity of *Ficus carica L.* fruit extracts (ethyl acetate, hexane and ethanol) on breast cancer (MCF-7) and mouse epithelial cell lines after 72 hrs of incubation has been shown in figure 3.1a and figure 3.1c respectively. The results showed a dose dependent decrease in percentage viability. The percentage of cell death/ viability due to *Ficus carica* fruit extracts at the respective concentration (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125 and 0.390625µg/ml) was found to be consistent (Table 3.1). This may be as a result of its phytochemical contents. Similar studies were already reported by Jasmine *et al.*, 2015 which supports the present study. Moreover, previous studies reported that crude extracts of *Ficus carica* (leaves, fruits and the latex) showed cytotoxic effect on various cancer cell lines *invitro* (Hashemi *et al.*, 2011; Marrelli *et al.*, 2012; Mostafaie *et al.*, 2011; Rubnov *et al.*, 2001). Additionally, *Ficus carica L.* latex has also been reported to have a greatest effect on growth inhibition of stomach

cancer cell line, esophageal cancer cell line (Hashemi *et al.,* 2011), human cervical cancer cell line (HeLa) (Khodarahmi *et al.,* 2011), and human breast cancer cell line (MCF-7) (Jasmine R *et al.,* 2015). Hashemi *et al.,* (2011) also studied the effect of fresh latex of *Ficus carica* fruits on stomach cancer cell lines where they found that the extracts inhibited the proliferation of stomach cancer cell line but did not indicate any cytotoxic activity against normal cells *in-vitro*.

In the present study, the antiproliferative effect of *Ficus carica L.* fruit extracts against human breast cancer cell line (MCF-7) and mouse epithelial cell line (3T3) were investigated. The ethyl acetate extracts exhibited a significant antiproliferative activity and interestingly possess strong cytotoxic activity towards breast cancer (MCF-7) cells line. It is also relevant to test the same extracts against normal cell line, as it is known that most antiproliferative agents do not greatly differentiate between cancer and normal cell lines, which could lead to systemic cytotoxicity. Therefore, in this study the selectivity effects of the fruit extracts towards normal cell line (mouse epithelial cell; 3T3) were also determined. It was however, observed that *Ficus carica* fruit extracts had no considerable antiproliferative and cytotoxic effects on the normal cell line (3T3) Even at higher concentrations, the normal cell line was considerably viable (Table 3.1).

CONCLUSION

The present result together with previous studies suggest that the dried preserved *Ficus carica L.* fruits extracts could be a candidate as a potential agent for the for the inhibition of estrogen positive receptor breast cancer cell (MCF-7) proliferation *in vitro*, and might have the same effect on clinical studies.

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