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ANTIPROLIFERATIVE EFFECT ON BREAST CANCER (MCF7) OF *Moringa oleifera* SEED EXTRACTS

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Abstract

Background: *Moringa oleifera* belongs to plant family, Moringaceae and popularly called "wonderful tree", for it is used traditionally to cure many diseases including cancer in Africa and Asia, however, there is limited knowledge on cytotoxic activity of *Moringa oleifera* seeds on MCF7 breast cancer cell. The present study evaluated antiproliferative effect on MCF7 of the seed.
Materials and Methods: Seeds of *Moringa oleifera* were grinded to powder and its phytochemicals were extracted using water and 80% ethanol solvents, part of the ethanolic extract were sequentially partitioned to fractions with four solvents (hexane, dichloromethane, chloroform, and n-butanol). Antiproliferative effects on MCF7 of the samples were determined. Finally, potent samples that significantly inhibited MCF7 growth were tested on MCF 10A.
Results: Crude water extract, hexane and dichloromethane fractions of the seeds inhibited the proliferation of MCF7 with the following IC_{50} values 280 µg/ml, 130 µg/ml and 26 µg/ml respectively, however, of the 3 samples, only hexane fraction had minimal cytotoxic effect on MCF 10A (IC_{50} > 400µg/ml).
Conclusion: *Moringa oleifera* seed has antiproliferative effect on MCF7.

Keywords: *Moringa oleifera* seeds; MCF7; MCF 10A; and Antiproliferative effect.

Abbreviations: CWE-MO, Crude water extracts of *Moringa oleifera* seeds; CEE-MO, Crude ethanolic extract of *Moringa oleifera* seeds; HF-CEE, Hexane fraction of crude ethanolic extract of *Moringa oleifera* seeds; DF-CEE, Dichloromethane fraction of crude ethanolic extract of *Moringa oleifera* seeds; CF-CEE, Chloroform fraction of crude ethanolic extract of *Moringa oleifera* seeds; nBF-CEE, n-Butanol fraction of crude ethanolic extract of *Moringa oleifera* seeds; AR-CEE, Aqueous residue of crude ethanolic extract of *Moringa oleifera* seeds.

Introduction

Herbal medicine has become an alternative remedy to cure, prevent, or manage many diseases such as diabetes, cancer, malaria, and microbial infections because it is effective, affordable, easy and simple to prepare. *Moringa oleifera* belongs to the plant family, Moringaceae and popularly called "wonderful tree", for it is used for many things such as nutritional and medicinal purposes throughout the world especially in Africa and Asia. It is also referred to as horseradish tree or drumstick tree (Verma et al., 2009). *Moringa oleifera* has significant amount of important phytochemicals such as phenolics, flavonoids, alkaloids, vitamins, glycosides, sterols, minerals, and amino acids in seeds, leaves, and fruits (Anwar et al., 2007; Sreelatha et al., 2011). Seeds of *Moringa oleifera* have been found to have diuretic activity (Caceres et al., 1992) as well as antitumor and antimicrobial activities (Guevara et al., 1999). Costa-Lotufo et al. (2005) reported cytotoxic effect of ethanolic extract of *Moringa oleifera* root on leukemia and melanoma cell lines. However, limited knowledge is available on the cytotoxic activity of *Moringa oleifera* seeds against MCF7. MCF 7 is an estrogen positive breast cancer cell line usually employed as an in vitro model in breast cancer therapeutic research(Lee et al., 2015). Breast cancer is a major health concern as it is the most common cancer after lung cancer in both genders. In 2012, GLOBOCAN estimated about 1.67 million new breast cancer cases throughout the world and ranked it one of the most causes of cancer death in developed and less developed regions of the world (Ferlay et al., 2015). Epidemiological studies of breast cancer in Malaysia revealed breast cancer is the commonest type of cancer in Malaysian woman (C. H. Yip et al., 2006). Many chemotherapies capable of supressing breast cancer growth and preventing metastasis are available, however, associated side effects such as immune system compromise, neutropenia, hypercalcemia among others are serious concerns to patients (C. Yip et al., 2014). Hence, effective chemotherapy from natural plant source with less adverse effects and minimal toxicity to normal body systems is desirable. The present study aims to evaluate cytotoxicity on breast adenocarcinoma cells (MCF7) and normal breast cells (MCF10A) of the extracts and fractions from *Moringa oleifera* seeds.

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Materials and methods

Plant collection

Dried seeds of *Moringa oleifera* were collected from Herbagus at kepala Batas, Penang, Malaysia in April 2015. The plant seed was authenticated by a biologist at the School of Biological Science, Universiti Sains Malaysia.

Phytochemical extraction and fractionation

The dried seeds were manually de-coated and naked seeds were grinded to powder. 10 g of the powdered seed was soaked in 100 ml of distilled water and 100 g of the powdered seed was soaked in 400 ml 80% ethanol. Mixtures were continuously shaken at 200 rpm for 24 hours. Filtrate was collected by filtration of the mixtures through Whatman #1 filter paper. Exhaustive extraction was done by repeating extraction procedure another two times, and crude extracts were pooled together. Ethanol was evaporated from pooled ethanolic extract using rotary evaporator and the crude water and ethanolic extracts were freeze-dried. For fractionation, 100 g of seed powder was soaked in 80% ethanol, phytochemicals were extracted as explained above and alcoholic solvent was evaporated from the extract. Extract was then fractioned with solvents of different polarities in a sequential manner based on increasing polarity (hexane→ dichloromethane (DCM) → chloroform→ n-butanol) (Figure 1). The fractions were evaporated to remove the solvents and lyophilized. All freeze-dried crude and fractionated extracts were kept at 4˚C till further use.

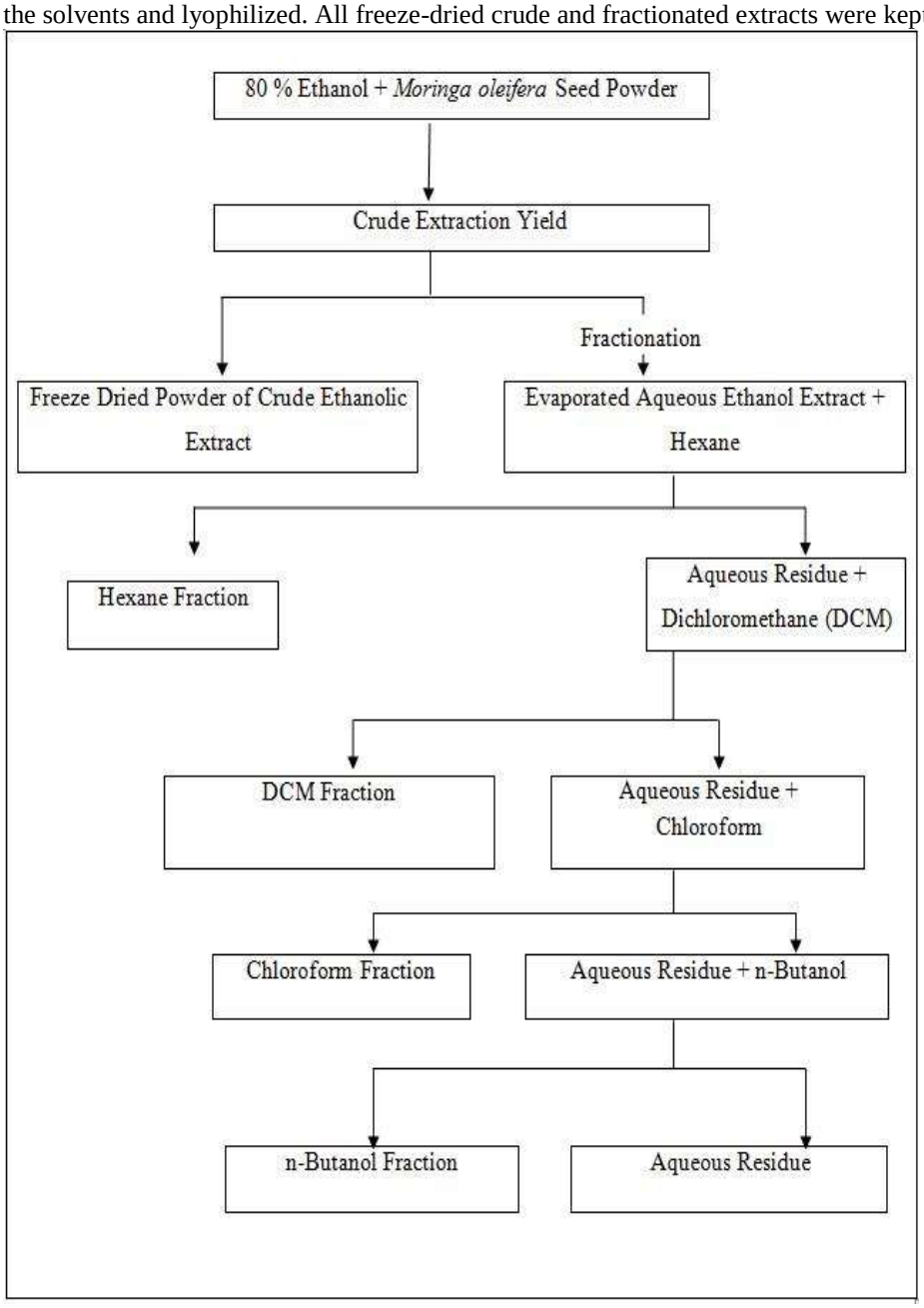

Figure 1: Schematic Diagram showing Extraction and Partitioning of *Moringa oleifera* seeds

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Cell culture

MCF7 and MCF 10A cells were purchased from American Type Culture Collection (ATCC). MCF7 cells were cultured and maintained in RPMI 1640 medium supplemented with 10% (v/v) Foetal Bovine Serum (FBS) and 1% antibiotic, penicillin/streptomycin (PenStrep). MCF 10A cells were grown and maintained in DMEM medium supplemented with 5% (v/v) horse serum, 0.2% EGF (20 mg/ml), 0.5 mg/ml hydrocortisone, 10 µg/ml insulin and 1% PenStrep. Cells were usually sub-cultured after 4-5 days when it had reached about 80 % confluence.

Cell viability assay

For this assay, all samples were dissolved in DMSO (0.3% highest final concentration) except crude water extract and aqueous residue that were dissolved in media. MCF7 cells were harvested from an exponentially growing cell culture that has reached 80% confluence. Cell counting was made and a concentration of 5000 cells/ well (5×10^4 cells/ml) in 100 µl was seeded to each well of 96 well-plates with exception of few rows for 24 hours in a CO_2 saturated incubator. The media in the wells were removed and replaced with media containing sample of different concentrations (0, 50, 100, 200, 300, and 500µg/ml) dissolved in dimethyl sulfoxide (DMSO) or media. Other wells were filled with DMSO (0.3%) containing media or media only as controls. To eliminate absorbance effect of the media and DMSO, an empty cell free row in the plate was filled with media containing DMSO or media only. The plates were incubated for 72 hours. MTS assay was used for cell viability assessment according to manufacturer's instruction, 20 µl of MTS solution was added to each well and plates were incubated in 5% CO_2 atmosphere for 3-4 hours. Absorbance values at 490 nm were taken after incubation using plate reader. Thereafter, anti-proliferation of potent samples (CWE-MO, HF-CEE, and DF-CEE) on MCF 10A were evaluated using the same methodology described above. Percentage cell viability was calculated for different extracts' concentrations using the following formula;

Percentage cell viability = $\{(A_E - A_{DM}) \div (A_C - A_{DM})\} \times 100$

A_E = Absorbance of treated cell culture with extract, A_{DM} = Absorbance of media/ media with 0.3% DMSO (blank), A_C= Absorbance of untreated cell culture (control).

Statistical Analysis

Numerical values were generated in triplicates for each experiment and data were expressed as mean ± standard deviation. Confidence limit of $p < 0.05$ was considered significant.

Results and Discussion

Extraction of phytochemical compounds

Extraction of *Moringa oleifera* seeds was done using water (aqueous) and 80% ethanol solvents. The crude ethanolic extract was further fractioned with different solvents (hexane, dichloromethane, chloroform and n-butanol) based on their polarity to various fractions. Water was employed for the extraction because of its universal solubility of polar compounds and choice of ethanol was due to its higher solubility, strong extraction ability of plant phytochemicals and its tendency of yielding relevant compounds as it has been reported in previous studies on *Moringa oleifera* (Guevara et al., 1999). Part of crude ethanolic extract was fractionated by different solvents to concentrate and enhance the purity of active compounds and remove unwanted interferences (Dai & Mumper, 2010) . CWE-MO yield was 25% of the dry weight seed powder sample and freeze-dried CEE-MO yielded 11% of the weight of dry seed powder sample while the yield of the fractions varied from 0.50 g to 6.66 g according to the following ascending order; AR-CEE (6.66g) > n-BF-CEE (2.13 g) > HF-CEE (1.00 g) > DF-CEE (0.50 g) > CF-CEE (nil) (Table 1.). CEE-MO found to be insoluble in chloroform may be due to the unsuitability of the solvent or that compounds soluble in chloroform had been solubilised by the previous solvents used (hexane and dichloromethane) while the butanol fraction had the highest yield amount.

Table 1: Crude extraction and partitioning yield of *Moringa oleifera* seeds

Extract	Initial weight of sample (g)	Extraction yield (g)	% yield
CEE-MO	100	11.04	11
CWE-MO	10	2.47	25
Partitioning of Crude 80% ethanol extract			
Fraction		Yield (g)	% yield
HF-CEE		1.00	1
DF-CEE		0.50	1
CF-CEE		-	-
nBF-CEE		2.13	2
AR-CEE		6.66	7

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(-) = nil

Antiproliferative assay on MCF7 and MCF 10A

The crude extracts and fractions of *Moringa oleifera* seeds were tested on MCF7 breast cancer cell to determine their inhibitory effects on the cell proliferation (Fig. 4). As reported by Al-Asmari et al. (2015), CEE-MO had insignificant antiproliferative effect on the cells, however, upon partitioning, HF-CEE and DF-CEE had inhibitory effects on the cell proliferation in a dose dependent manner. CWE-MO also inhibited MCF7 proliferation. The half inhibitory concentration (IC_{50}) of CWE-MO, HF-CEE, and DF-CEE were 280 µg/ml, 130 µg/ml and 26 µg/ml respectively. Hence, DF-CEE was most effective and had relatively highest cytotoxicity on the cells with least IC50.

Inhibition of breast cancer cell (MCF7) growth by CWE-MO, HF-CEE and DF-CEE may be due to the antioxidant activities of the phenolics the samples contain. Our observations are consistent with several past works that have linked the antioxidant activities of phenolic compounds to their antiproliferative effects on various cancer cells (Diogo et al., 2009; Goel et al., 2008; Sa et al., 2010; Saeidnia & Abdollahi, 2013). Moreover, antiproliferative effects of the analysed samples may also be attributed to the non-phenolic compound, hexadecanoic acid (palmitic acid) in the samples as identified by GC-MS analyses, since it had been shown to have selective *in vitro* cytotoxicity against leukemic cells and *in vivo* antitumor in mice (Al-Asmari et al., 2015; Harada et al., 2001).

The samples (CWE-MO, HF-CEE and DF-CEE) that showed anticancer activity on MCF7 were tested on non-tumour breast cell (MCF 10A) and their antiproliferative effects on MCF7 and MCF 10A are shown in Fig. 3. IC50 values of the CWE-MO, HF-CEE and DF-CEE were 70 µg/ml, >400 µg/ml, and 25 µg/ml respectively. The HF-CEE was found to have least inhibitory effect on MCF 10A with higher IC_{50} when compared with its effect on MCF7 cancer cell. CWE-MO was found to have higher antiproliferative effect on normal cell MCF 10A compared to MCF7 while DF-CEE inhibited both MCF 10A and MCF7 proliferation at almost the same rate with approximately equal IC_{50} values. The HF-CEE was found to be most adequate as it contains potent anticancer agent against MCF7 cell with limited cytotoxicity to normal breast cell.

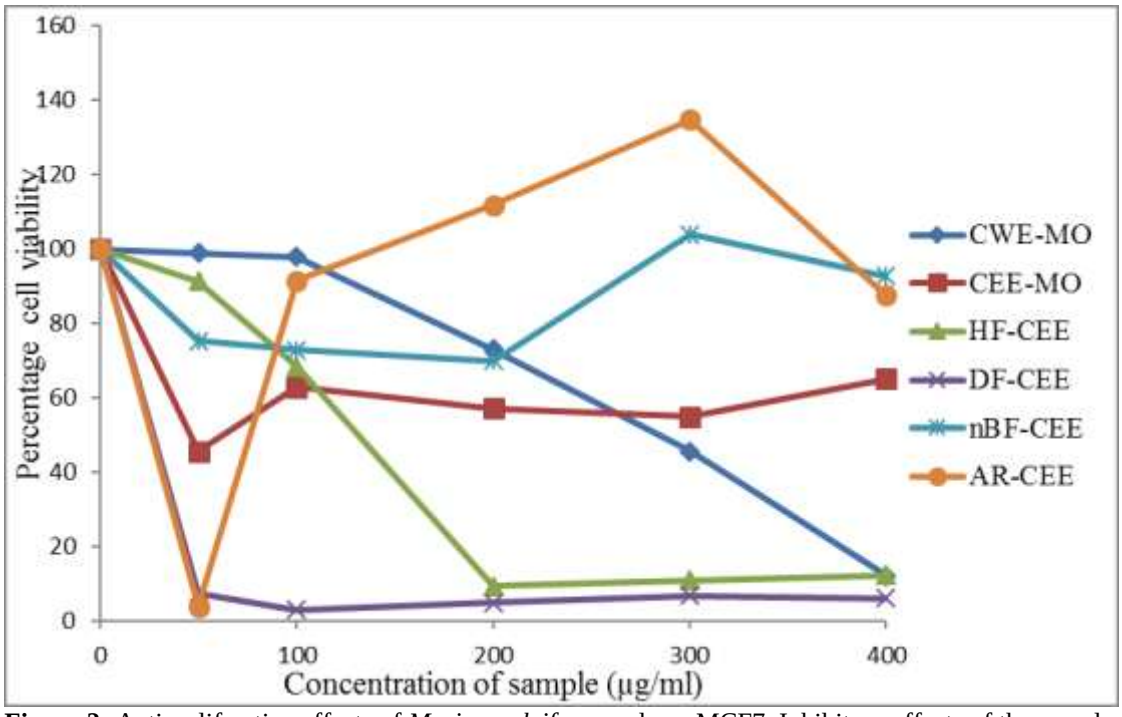

Figure 2: Antiproliferative effects of *Moringa oleifera* seeds on MCF7**.** Inhibitory effects of the samples on MCF7 cell proliferation were determined by MTS assay. Crude water extract, hexane and dichloromethane fractions significantly suppress MCF7 proliferation.

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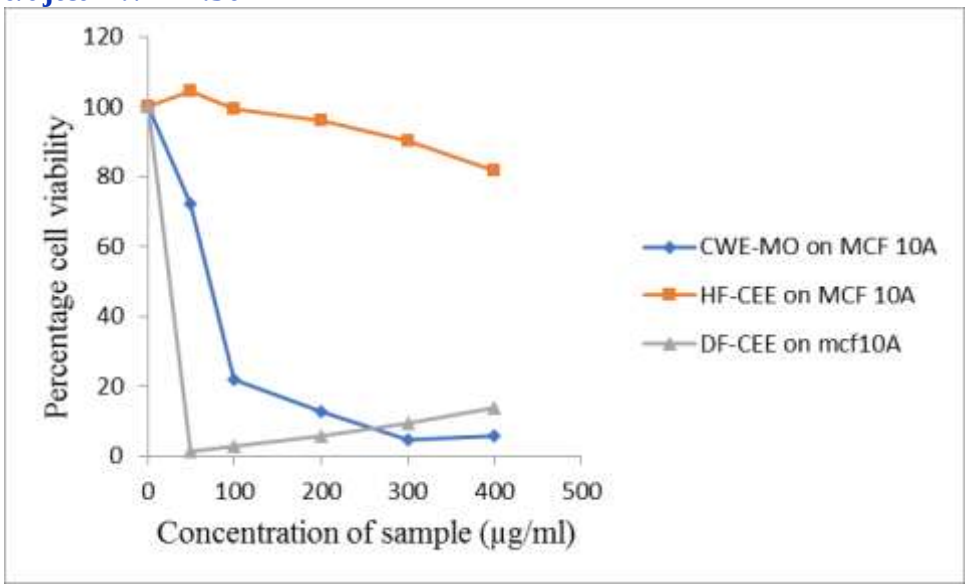

Figure 3: Antiproliferative effects of *Moringa oleifera* seeds on MCF 10A

Conclusion

In this study, we extracted phytochemicals from *Moringa oleifera* seeds, fractionated ethanolic extract and cytotoxicity of the extracts and fractions on MCF7 and MCF 10A were determined, our result showed that HF-CEE (IC_{50} = 130µg/ml) has antiproliferative effect on MCF7 breast cancer cell with insignificant cytotoxicity to MCF 10A normal breast cell, however, molecular analyses of action mechanism of the fraction along cancer signalling pathways are required.

Conflict of interest: The authors declare that there is no conflict of interest

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