UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL ANALYSIS AND CYTOTOXIC EFFECTS OF MANGO (Mangifera indica L) KERNEL ON BREAST CANCER CELL LINES

AL-SHWEYEH HUSSAH ABDULLAH

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By

AL-SHWHYEH HUSA'H ABDULLAH

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

November 2015
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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By

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November 2015

Chairman : Abdulkarim Sabo Mohammed, PhD
Faculty : Food Science and Technology

Breast cancer is a significant cause of morbidity and mortality among women around the world. Currently, anticancer research focuses are on the discovery of alternative therapeutic compounds from natural products. Although numerous study has been done on plants to discover new drugs for the treatment of chronic diseases like cancer, the products of the mango plant species Mangifera indica L. waterlily, particularly the kernel have not adequately investigated. In this study the M. indica waterlily kernel ethanolic extract are investigated to determine its chemical components and anticancer effect. It is postulated that M. indica waterlily kernel extract has antibreast cancer activities through the antioxidant properties of its constituent compounds. Thus, the objective of this study is to determine and characterize the content of the ethanolic M. indica waterlily kernel extract and determine the antibreast cancer effect of the extract. The M. indica waterlily kernel extract was characterized by high performance liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry and its antioxidant potentials determined by 2,2-diphenyl-1-picrylhydrazyl and ferric-reducing antioxidant power assays and determination of its thiobarbituric acid reactive substances and glutathione and reactive oxygen species contents. The cytotoxicity of the extract on breast cancer MDA-MB-231 and MCF-7 cells and normal breast MCF-10A cells was assessed by neutral red uptake, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, and lactate dehydrogenase release assays and morphological studies, using doxorubicin as the control. The effects of the extract on apoptosis-related markers (Bcl-2, Bax, p53 proteins and Caspase-3, -8 and -9) were also assessed to determine mechanism of cell death. The results showed that M. indica waterlily kernel extract has many bioactive compounds especially phenolics such as phenolic acid, flavonoides and xanthones. When chemical components of the extract was test against the breast cancer cell line, it was shown that mangiferin, rutin, chlorogenic acid and myricetin, but not p-coumaric acid or epigallo-catechin, exhibited significant cytotoxic effects against the breast cancer cell only, not the normal cells. It was therefore assumed that it is the mangiferin, rutin, chlorogenic acid and myricetin content of the M. indica kernel extract that contributed towards the cytotoxicity. The extract has high antioxidant potentials, and induced cytotoxicity in MDA-MB-231 and MCF-7 cells lines in a dose- and time-dependent manner, but was not toxic to the normal breast cells, MCF-10A. It is suggested that the anticancer effect of the M. indica extract is via modulation of redox status through its antioxidant constituents and
induction of apoptosis. These findings suggest that *M. indica* kernel extract, with antiproliferative properties towards cancer cells, while relatively innocuous to normal cells, is a good candidate as an alternative to or for supplementation for current chemotherapies in the treatment of breast cancers, without showing the side-effects associated with these anticancer drugs.
mangga *M. indica* adalah dengan mengurangkan tekanan oksidatif melalui kandungan antioksidannya dan mengaruh apoptosis. Penemuan ini menyarankan ekstrak mangga *M. indica* dengan sifat antipemproliferatannya terhadap sel kanser, sambil tidak memudarakan sel normal, adalah calon baik sebagai bahan alternatif dan penambah untuk kemoterapi kini diguna dalam rawatan kanser payudara, tanpa menunjukkan kesan sampingan yang terkait dengan drug antikanser tersebut.
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I certify that a Thesis Examination Committee has met on 6 November 2015 to conduct the final examination of Al-Shwyeh Hussah Abdullah on her thesis entitled "Phytochemical Analysis and Cytotoxic Effects of Mango (*Mangifera indica* L) Kernel on Breast Cancer Cell Lines" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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<td>ATP</td>
<td>adenosine triphosphate</td>
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<tr>
<td>Afap-1</td>
<td>actin Filament Associated Protein 1</td>
</tr>
<tr>
<td>Bak</td>
<td>Bcl-2 antagonistic killer</td>
</tr>
<tr>
<td>Bax</td>
<td>Bcl-2-like protein 4</td>
</tr>
<tr>
<td>Bcl-xL</td>
<td>B-cell lymphoma-extra large</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylatedhydroxytoluene</td>
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<tr>
<td>Bid</td>
<td>BH3 interacting-domain death agonist</td>
</tr>
<tr>
<td>BRCA1</td>
<td>breast cancer 1-early onset</td>
</tr>
<tr>
<td>BRCA2</td>
<td>breast cancer 2-early onset</td>
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<td>c-FLIP</td>
<td>Cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein</td>
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<td>DAD</td>
<td>diode array detector</td>
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<td>DMEM</td>
<td>Dulbecco’s modified Eagle medium</td>
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<td>DMSO</td>
<td>Dimethylsulphoxide</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>2,2-diphenyl-1-picrylhydrazyl</td>
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<tr>
<td>DRI</td>
<td>dietary reference intake</td>
</tr>
<tr>
<td>E2</td>
<td>17-β estradiol</td>
</tr>
<tr>
<td>EGC</td>
<td>epigallocatechin</td>
</tr>
<tr>
<td>EGCG</td>
<td>Epigallocatechin gallate</td>
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<td>ER-</td>
<td>Estrogen receptor negative</td>
</tr>
<tr>
<td>ER+</td>
<td>Estrogen receptor positive</td>
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<td>ESI-MS</td>
<td>Electrospray ionization mass spectrometric</td>
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<td>FADD</td>
<td>Fas-Associated protein with Death Domain</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<td>FAS</td>
<td>Fas ligand</td>
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<tr>
<td>FIC</td>
<td>Ferrous ion chelating</td>
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<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
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<td>FRAP</td>
<td>Ferric reducing acid potential</td>
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<td>FSH</td>
<td>Follicle stimulating hormone</td>
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<td>GCMS</td>
<td>Gas chromatography–mass spectrometry</td>
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<td>GSH</td>
<td>Glutathione</td>
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<td>HER2</td>
<td>Human epidermal growth factor receptor 2</td>
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<td>HPLC</td>
<td>High performance liquid chromatography</td>
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<td>IAPs</td>
<td>Inhibitors of apoptosis</td>
</tr>
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<td>IHC</td>
<td>Immunohistochemistry</td>
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<tr>
<td>LCMS</td>
<td>Liquid chromatography–mass spectrometry</td>
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<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<td>M. indica</td>
<td><em>Mangifera indica</em></td>
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<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>MTT</td>
<td>Thiazoyl blue tetrazolium bromide</td>
</tr>
<tr>
<td>MW</td>
<td>Molecular weight</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide</td>
</tr>
<tr>
<td>NR</td>
<td>Neutral red</td>
</tr>
<tr>
<td>p53</td>
<td>p53 tumor suppressor</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PR</td>
<td>Progesterone receptor</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RT</td>
<td>Retention time</td>
</tr>
<tr>
<td>TEP</td>
<td>Total extractable polyphenols</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive species</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<td>TNF</td>
<td>tumor necrosis factor</td>
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<td>TPC</td>
<td>Total phenolic content</td>
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<td>TRAIL</td>
<td>TNF-related apoptosis-inducing ligand</td>
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<td>WHO</td>
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</table>
Breast cancer is the most common cancer among women and causes the highest mortality among cancers. In the past, breast cancer burden was most prevalent in the developed countries but in recent years, the incidence in developing countries is also rising. Over 1 million people have been diagnosed with breast cancer while over 400,000 die from the disease every year [Coughlin & Ekwueme, 2009; Ferlay et al., 2004; Jemal et al., 2011]. Although there are many factors involved in the pathogenesis and progression of breast cancer, oxidative stress is thought to play a significant role [Hakkak et al., 2013; Reuter et al., 2010]. Estrogen treatment is implicated in the causation of increased oxidative stress in breast cancer [Sastre-Serra et al., 2010].

There are many treatment modalities for breast cancer including surgery, chemotherapy and radiation that pose very heavy financial burdens on patients and health authorities [Radice & Redaelli, 2003]. Multidisciplinary approach is recommended for treatment of breast cancers, although there are serious side-effects associated with these treatment options [Saini et al., 2011]. Recent studies have indicated that the use of plant bioresources for the management of breast cancer may offer relatively safer alternatives in comparison with currently available chemotherapy agents. Additionally, the considerations of the high financial burden related to chemotherapy agents is driving the search for cheaper alternatives [Mukherjee et al., 2001]. The need for alternatives is further underscored by the fact that chemotherapy agents can cause serious and debilitating long term health effects. Already, fruits and vegetable consumption has been linked with reduced risks of chronic diseases including cancers [Willett, 2010]. The effect of these diets is mostly attributable to their antioxidants properties [Borek, 2004]. These observations are driving the search for newer alternative therapies for cancers that are safer and more cost-effective.

Mango (Mangifera indica L.), a member of the family Anacardiaceae. M. indica, has become naturalized and adapted to the environment of the subtropics and tropics [Rocha Ribeiro et al., 2007]. There are over 500 classified M. indica varieties throughout the world. The genus of Mangifera consists of 69 species and mostly restricted to tropical Asia [Ramanatha Rao & Mal 2002; Yonemori et al., 2002]. Malaysia is a tropical country with heavy precipitation, high temperatures, and high humidity that favors M. indica vegetation. Malaysia, particularly the peninsular area, has variety of mangoes; the better known cultivars are Golek (MA 162), Masmuda (MA 204), Maha 65 (MA 165) and Chok Anan (MA 224) [Yonemori et al., 2002]. Generally, Chok Anan is very suitable for the export market as it has desirable color and sweetness and good flavor. Mangoes are rich in vitamins, minerals, anti-oxidants and other bioactive compounds [Ribeiro et al., 2008; Soong & Barlow, 2004; Soong & Barlow, 2006] and these are heavily consumed causing accumulation of waste. Similarly, M. indica seed kernels are usually wasted during processing although recent studies have indicated that they contain useful bioactive compounds that may potentially confer functional effect [Fowomola, 2010; Nkizou et al., 2010]. Thus, with appropriate treatment, and understanding of the chemical and functional characteristics of the kernel, the mango waste can be used as a food ingredient and in nutraceutical or pharmaceutical applications. It is in view of the current drive to find cheaper and safer alternatives to chemotherapy agents and the rich bioactive composition of M. indica seed kernel that the present study was conceived to study the potential anti-breast...
cancer effects of *M. indica* kernel extract. Moreover, *M. indica* is reported to be antioxidant-rich, and foods with potent antioxidant potentials have been shown to regulate cancer cell growth via modulating the redox status of the cells and inducing apoptosis [Ghasemzadeh & Ghasemzadeh, 2011]. Thus, the phytochemical composition and antioxidant potentials of *M. indica* kernel extract, and its potential anticancer effects were evaluated *in vitro* on breast cancer cell lines.

The study aims to evaluate the cytotoxic effect of the extract from the kernel of *M. indica* towards breast cancer cell lines to determine its utility as complementary or alternative anti-breast cancer compound.

**Study Hypothesis:**

It is hypothesized that the solvent extract of *M. indica* kernel

1. contains anti-oxidative compounds.
2. has cytotoxic effects towards breast cancer cell lines.
3. has minimal cytotoxicity towards normal breast cell lines.

**General objective:**

The main objective of the study is to determine the composition of bioactive compounds in *M. indica* kernels and their anti-breast cancer cell properties.

**Specific objectives:**

**The specific objectives are to:**

1. obtain a solvent extract of *M. indica* kernel and determine its compositional analysis.
2. determine the antioxidant properties of *M. indica* solvent extract.
3. determine the *in vitro* cytotoxicity effect of *M. indica* solvent extract on MCF-7 and MDA-MB-231 breast cancer cell lines.
4. determine the anticancer mechanism of *M. indica* solvent extract.
REFERENCES


Soong, Y.Y and Barlow, P.J. (2006). Quantification of gallic acid and ellagic acid from longan (Dimocarpus longan Lour.) seed and mango (Mangifera indica L.) kernel and their effects on antioxidant activity. *Food chemistry* 97: 524–530.


