THE CYTOTOXIC EFFECT OF *TYPHONIUM FLAGELLIFORME* AND *CLINACANTHUS NUTANS* ON BREAST CANCER CELL LINE

NADHIRA ATIQAH BTE KHIRU NASIR

UNIVERSITI TEKNOLOGI MALAYSIA
THE CYTOTOXIC EFFECT OF *Typhonium flagelliforme* AND *Clinacanthus nutans* ON BREAST CANCER CELL LINE

NADHIRA ATIQAH BTE KHIRU NASIR

A dissertation submitted in partial fulfilment of the requirements for the award of the degree of

Master of Science (Biotechnology)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

MAY 2015
Special dedication;

To my beloved father and mother;
KHIRU NASIR BIN ROHANI and WAN FARIZAH BT WAN ABDULLAH

My siblings and all my family;
AIMAN, AMIRA, HAZIQAH, HUSNA, ARIFF

Thanks special to;
The person that funded and find out plant sample for this study

The person that always support and motivate me in completing my master journey and all friends
ACKNOWLEDGEMENTS

In the name of Allah, the Gracious, the Merciful.

All praises to Allah for the strengths and His blessing in completing this project. First and foremost, I would like to thank my supervisor Dr. Siti Pauliena binti Mohd Bohari for her guidance throughout my entire research. Her encouragement, advices and daily observation on my work in the laboratory gives me the inspiration to keep on the right direction during my research project.

Very special thanks go to members of Tissue Engineering Lab. All of them were always willing to help and give their best suggestions for me to complete this project. I would like to extend my appreciation to all lecturers in Faculty of Bioscience and Medical Engineering, Universiti Teknologi Malaysia for the valuable advices and laboratory staffs for providing materials and equipments during this study.

Special sincere appreciation goes to my lovely parents for encouragement, blessing, support and their prayer. Very thankful to the person that always encouraged and motivated during I’m in hardship, fell down and less motivation.

Finally, a million thanks for everyone whom I did not mention here but always stay forever in my heart.

Thank you very much and may Allah blessing to them.
ABSTRACT

Cancer is a disease that can cause death and breast cancer is one of its prevalence diseases among women in Asia. Conventional treatments have been used to treat cancer, however, these treatments has been given inefficiency effects and low survival rate. Therefore, most cancer patients began to discover complementary and alternative treatments to treat this disease. Thus, this study is important to identify the cytotoxicity effect of methanolic leaves extract Typhonium flagelliforme and Clinacanthus nutans on breast cancer cells (MDA-MB-231). MTT assay was used to determine the cytotoxicity effect of both plants on MDA-MB-231 cells and Chinese Hamster Ovary (CHO) as the non-cancerous control cells. Results revealed that, T. flagelliforme extract was shown higher cytotoxic effect on MDA-MB-231 cell (IC₅₀: 110 µg/mL) when compared to C. nutans extract (IC₅₀: 170 µg/mL). Then, the IC₅₀ value of T. flagelliforme and C. nutans extracts against CHO cells were showed 100 µg/mL and 240 µg/mL respectively. Based on these IC₅₀ values, T. flagelliforme extract was found higher toxicity effect on CHO cells than MDA-MB-231 cells. Thus, in the future CHO cell can be replaced with the normal breast cell such as HCC1395 (epithelial mammary duct of normal breast cells) to investigate the toxicity effect of T. flagelliforme extract towards the normal breast cell. Moreover, identification of compound that gives inhibition towards MDA-MB-231 cell and normal breast cell is also important for future research.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DECLARATION</td>
<td>ii</td>
<td></td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
<td></td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>iv</td>
<td></td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
<td></td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vi</td>
<td></td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vii</td>
<td></td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xiii</td>
<td></td>
</tr>
<tr>
<td>LIST OF SYMBOLS AND ABBREVIATIONS</td>
<td>xiv</td>
<td></td>
</tr>
</tbody>
</table>

## 1 INTRODUCTION
1.1 Background of study 1
1.2 Problem statement 4
1.3 Objectives 5
1.4 Scope of the study 5
1.5 Significance of the study 6

## 2 LITERATURE REVIEW
2.1 Cancer 7
2.2 Breast cancer 8
2.3 Conventional treatment of breast cancer 10
   2.3.1 Surgery treatment 10
   2.3.2 Radiation therapy 11
2.3.3 Hormonal therapy 11
2.3.4 Chemotherapy 12
2.3.5 Immunotherapy 13

2.4 Alternative complementary treatment 13
  2.4.1 *Typhonium flagelliforme* 14
  2.4.2 Toxicity of *Typhonium flagelliforme* 15
  2.4.3 *Clinacanthus nutans* 16
  2.4.4 Toxicity of *Clinacanthus nutans* 17

2.5 Breast cancer cell line (MDA-MB-231) 18
2.6 Chinese Hamster Ovary (CHO) 19

3 MATERIALS AND METHODS 20
  3.1 General flow of research methodology 20
  3.2 Materials 21
  3.3 Mammalian cell lines 21
  3.4 Plant collection 22
  3.5 Plant extraction 22
  3.6 Preparation media for growth cell lines and MTT assay 23
    3.6.1 Preparation of cell culture media 23
    3.6.2 Preparation of phosphate buffered saline 23
    3.6.3 Preparation of MTT solution 24
    3.6.4 Preparation of acid-isopropanol 24
  3.7 Growth cell line 25
  3.8 MTT assay 25
  3.9 Statistical analysis 26

4 RESULT AND DISCUSSION 27
  4.1 Cytotoxicity effect of *T. flagelliforme* and *C. nutans* methanolic leaves extract on CHO cells 27
  4.2 Antiproliferative effects of *T. flagelliforme* and *C. nutans* methanolic leave extract against MDA-MB-231 at IC$_{50}$ value 31
5 CONCLUSION AND RECOMMENDATION 36
5.1 Conclusion 36
5.2 Recommendation 36

REFERENCES 38
Appendices A-C 51-59
# List of Tables

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Phosphate Buffer Saline (PBS) compositions</td>
<td>23</td>
</tr>
<tr>
<td>4.2</td>
<td>In vitro cytotoxic activity of plant extracts tested against MDA-MB-231 and CHO cells for exposure of 72 hours</td>
<td>33</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE NO.</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Anatomy of a normal breast organ (front side)</td>
<td>9</td>
</tr>
<tr>
<td>2.2</td>
<td>Anatomy of a normal breast organ (cross side)</td>
<td>9</td>
</tr>
<tr>
<td>2.3</td>
<td><em>T. flagelliforme</em> plant from Taman Botani, Perak</td>
<td>14</td>
</tr>
<tr>
<td>2.4</td>
<td><em>C. nutans</em> plant collection from Institut Pertanian, Air Hitam, Johor</td>
<td>16</td>
</tr>
<tr>
<td>2.5</td>
<td>MTT reaction from yellow is reduced by enzyme forming purple formazan</td>
<td>19</td>
</tr>
<tr>
<td>3.1</td>
<td>Flowchart of an MTT assay procedure</td>
<td>20</td>
</tr>
<tr>
<td>4.1</td>
<td>Comparison morphology of CHO and MDA-MB-321 cells (treated and untreated cells with <em>T. flagelliforme</em> methanolic leave extract)</td>
<td>28</td>
</tr>
<tr>
<td>4.2</td>
<td>Comparison morphology of CHO and MDA-MB-321 cells (treated and untreated cells with <em>C. nutans</em> methanolic leave extract)</td>
<td>29</td>
</tr>
<tr>
<td>4.3</td>
<td>MTT assay for effect of <em>T. flagelliforme</em> extract on MDA-MB-231 and CHO cells after 72 hours incubation</td>
<td>32</td>
</tr>
<tr>
<td>4.4</td>
<td>MTT assay for effect of <em>C. nutans</em> extract on MDA-MB-231 and CHO cells after 72 hours incubation</td>
<td>32</td>
</tr>
</tbody>
</table>
**LIST OF SYMBOLS AND ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>°C</td>
<td>Degree Celcius (centigrade)</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CHO</td>
<td>Chinese Hamster Ovary Cell Line</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>dH₂O</td>
<td>Distilled water</td>
</tr>
<tr>
<td>DMEM</td>
<td>Dulbecco’s modified eagle medium</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>FBS</td>
<td>Fetal Bovine Serum</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>KCl</td>
<td>Potassium chloride</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>Dihydrogen phosphate</td>
</tr>
<tr>
<td>µl</td>
<td>Micro liter</td>
</tr>
<tr>
<td>mL</td>
<td>Milili liter</td>
</tr>
<tr>
<td>mg</td>
<td>Milli gram</td>
</tr>
<tr>
<td>mM</td>
<td>Milili molar</td>
</tr>
<tr>
<td>M</td>
<td>Molar</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>Breast Cancer Cell Line</td>
</tr>
<tr>
<td>MTT</td>
<td>3-[4,5-dimethylthiazol-2-yl]-2,5-</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>Phosphate dibasic</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium dioxide</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffer Saline</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------------------------------------</td>
</tr>
<tr>
<td>Pen/Strep</td>
<td>Penicillin-Streptomycin</td>
</tr>
<tr>
<td>RMPI 1640</td>
<td>Roswell Park Memorial Institute Medium</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
</tbody>
</table>
## LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>Cell counting using Haemocytometer</td>
<td>64</td>
</tr>
<tr>
<td>A-2</td>
<td>Volume of aliquot sample in 96 well plate (VI)</td>
<td>65</td>
</tr>
<tr>
<td>A-3</td>
<td>Serial dilution of drug treatment</td>
<td>66</td>
</tr>
<tr>
<td>B-1</td>
<td>Data analysis of MTT</td>
<td>67</td>
</tr>
<tr>
<td>C-1</td>
<td>Statistical analysis (SPSS)</td>
<td>72</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Background of study

Cancer is defined as an abnormal cell (malignant cell) that growth without control in the body (Cameron, 1956). Based on previous researches, one million cancer cases were reported with 400, 000 deaths within a year worldwide (Yip, 2006; Lopez et al., 2006). The World Health Organization (WHO) claimed that cancer death rate will rise to the number of twelve million deaths per year in 2030 if there is no prevention (Hosmane et al., 2012; Farooqui et al., 2013). Recently, breast cancer has emerged as the most common female malignancy in majority of Asian countries (Agarwal et al., 2007). In Malaysia, one out of twenty women will suffer from breast cancer during their lifetime and worst fatal always be presented to them at the late stages (Yip, 2006; Farooqui et al., 2013).

Breast cancer is the most common cancer among Malaysian women (Abdullah et al., 2013). It can be originated growth from either glands or duct of breast (Haagensen et al., 1978; Erbas et al., 2006). If the cancer is originated from the glands it is called lobular carcinoma. This lobules parts are special milk-producing glands which connected by thin tubes, called ducts that allow milk to flow
into the nipple (Tanis et al., 2001; Giuliano et al., 2011). When cancer occurs in the duct of the breast it is known as ductal carcinoma (Giuliano et al., 2011). Meanwhile, the extension of breast cancer beyond its immediate tissue surrounding it is known as infiltrating or invasive cancer; the common neighbouring tissue that will be invaded are lung, liver, bone and brain (Wolfgang, 2005; Society, 2009).

There were several symptoms that can indicate the extension of breast cancer. It can be recognized by changes of shape or size of breast or nipple, nipple discharge, breast and bone pain, as well as swelling of the armpit (Agarwal et al., 2007). Nevertheless, these symptoms could not hard to be identified in the early stage during the initial diagnosis which is known as carcinoma in situ (Ertong et al., 1996). Carcinoma in situ is a tumour of non-invade or no penetrate epithelial to surrounding of the normal tissue (Haagensen et al., 1978; Albert, 1932). Specifically, there are two types of carcinoma in situ which are ductal carcinoma in situ and lobular carcinoma in situ. Ductal carcinoma in situ is a tumour that grows inside the milk ducts while lobular carcinoma in situ is a static tumour that grows in the lobules (Erbas et al., 2006; Zengel et al., 2013). However, only lobular carcinoma in situ is likely increase the risk of the invasive breast cancer development (Erbas et al., 2006; Zengel et al., 2013).

Women afflicted with breast cancer are caused by several factors including unhealthy lifestyle, reproductory problem and have a history of breast cancer disease in her family (Stephen, 2010). For women that neglect healthy daily meal intake such as alcoholism, less antioxidative food intake; fruits and vegetables, and smoking prior to pregnancy will face such problem (Susan, 2003; Stephen, 2010). Breast cancer also occurs to the person who lacks regular exercises that will cause overweight at early age or postmenopausal patient. Besides that, most of married women are susceptible to breast cancer when they are infertile, late age at first delivery (age 40 and above) and late age at menopause (after age 54 and above) (Helmrich et al., 1983; McPherson et al., 2000; Stephen, 2010). Lastly, breast cancer patient have possibility of incurring this disease again when their family have prominent history of breast, colon and ovarian cancer. This breast cancer disease will
be inherited by transferring mutated gene (BRCA 1 and BRCA 2) in the tumour suppressor genes from one family to other family members (Olivier, 1999; Stephen, 2010). In relation to that, all women were advised to undergo breast screening exams by either using mammography, clinical breast examination or breast self-examination (Yip et al., 2006). In fact, most of the treatments during these stages were successful compared to the numerous cases of breast cancer at later stages. Research proved that many cases treated during the third and fourth stages faces less survival rate (Warner, 2011).

The modern medicine was extensively developed due to various types of diseases that have caused fatality among less survival cancer patient (Hosmane et al., 2012; Society, 2014). Common treatments have been used to treat breast cancer including radiation therapy, surgery treatment, and chemotherapeutic agents; although they were found to be less effective with low survival rate of breast cancer patient and potential of long-term negative side effects (Rates, 2001; Moongkarndi et al., 2004; Lyons, 2007). Because of the shortcomings, they are looking into alternative therapies for their primary health care which by using natural product derived from plants (Rates, 2001). The plant parts were found to contain bioactive components that can cure and treat many diseases including cancer (Doughari, 2012). Therefore, plant herbs are widely used as an alternative remedy and as a main choice for scientists to find out new discovery of cancer disease remedy.

Plant herbs are mostly used as a traditional medicine due to the presence of natural drug content like flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides (Craig, 1999). These compounds have potential as anticancer, antivirus, antiparasitics, lipid control agents, and immunosuppressant (Li et. al., 2009). Other than that, herbal products were reported to have potential in enhancing the function of the immune system and repairing the inflammatory cell inside the human body (Craig, 1999). In mostly, there are a lot of medical plants for cancer treatment (Rates, 2001; Choo et al., 2001a; Doughari, 2012; Yong et al., 2013). Among them are Typhonium flagelliforme and Clinacanthus nutans (Choo et al., 2001a; Yong et al., 2013). Both
of these herbs (T. flagelliforme and C. nutans) have been selected in this study to compare which plants have higher potential to inhibit proliferation of breast cancer cell.

_T. flagelliforme_ is commonly known as rodent tuber or Keladi Tikus in Malaysia and this medicinal herb belongs to the Araceae family (Lai _et al._, 2008). It is characterized by its oblong, whitish tuber, triangular leaves and a spathe which is dilated and rounded at the base enclosing the yellowish spadix (Ridley, 1967; Lai _et al._, 2008). _T. flagelliforme_ has been categorized as toxic, warming, and phlegm resolving plant and have potential to soothe swelling, coughing and more predominantly for the treatment of cancer (Teo _et al._, 1999). Previous study reported that _T. flagelliforme_ extracts inhibit the proliferation of _in vitro_ cancer such as P388 murine leukaemia, human lung carcinoma and breast carcinoma cell lines (Choo _et al._, 2001b; Chan _et al._, 2005; Lai _et al._, 2008).

_C. nutans_ which comes from Acanthaceae family is growing widely in tropical Asia and also known as Sabah Snake Grass or Belalai Gajah (Chin _et al._, 2012). This plant can be identified by their characteristic on cylindric shaped stems and peculiar leaves which are opposite, simple and slightly serrated (Pieri _et al._, 2007). This herb is not only accepted as remedy in neutralizing venomous insect and snake bites but it also has potential to treat Herpes Simplex Virus infection, minimize inflammations and to reduce _in vitro_ carcinogenic effects (Wiroteangthong _et al._, 2006; Yong _et al._, 2013).

### 1.2 Problem statement

Cancer is considered as a silent killer and it’s very dangerous if not treated sooner. Cancer cell is distinguishable from normal cell due to its abnormal
characteristic. The cancer cell is recognized by its rapid and uncontrolled growth. Furthermore, cancer cell is invasive to the normal cell. Thus, it needs to be removed from the normal body system. There are many treatment can be used or chosen by the patient either conventional or alternative treatment. Currently, advance treatments that commonly used in Malaysia are not satisfactory such as surgery, radiotherapy, and chemotherapy. This treatment give negative side effect in long-term to the patient. Therefore, patient’s is demand for alternative complementary treatments. However, these alternative complementary treatments still understudy. This experiment emphasizes the use of *T. flagelliforme* and *C. nutans* as herbs that will be used to screen cancer cell. Screening is carried out to evaluate the plant’s potential to inhibit breast cancer cell (MDA-MB-231) in *in vitro* culture.

1.3 Objectives of the study

1) To test the cytotoxic activity of two traditional medicinal plants on breast cancer cell (MDA-MB-231)

2) To analyse which medicinal plants (*T. flagelliforme* or *C. nutans*) has the highest or better activity to inhibit the growth of breast cancer cell (MDA-MB-231) but less or no effect on normal cells (CHO).

1.4 Scope of the study

The crude extracts from these two plants (*T. flagelliforme* and *C. nutans*) were tested for its cytotoxic effect on breast cancer cell (MDA-MB-231) and CHO (normal cells) as the non-cancerous control cells. Then, the cytotoxic activity of
these plants will be compared to identify which plant has the higher inhibitory effect on breast cancer cells, but no or less effect on normal cells.

1.5 Significance of the study

Since breast cancer is the common cancer in women that leads to death throughout the world, the findings of this study are important in order to determine the potential *T. flagelliforme* and/or *C. nutans* as an alternative complementary remedy in treating breast cancer.
REFERENCES


Nordin, N. H. (2012). 3’, 4’, 7-trihydroxyelavone bioactivities and cytotoxicity effects on Chinese hamster ovary (CHO) cells (Doctoral dissertation, Universiti Teknologi Malaysia, Faculty of Bioscience and Bioengineering).


