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

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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;
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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-231cell (IC_{50}: 110 µg/mL) when compared to *C.nutans* extract (IC_{50}: 170 µg/mL). Then, the IC_{50} value of *T. flagelliforme* and *C. nutans* extracts against CHO cells were showed 100 µg/mL and 240 µg/mL respectively. Based on these IC_{50} 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.

ABSTRAK

Kanser adalah penyakit yang boleh menyebabkan kematian dan kanser payudara merupakan salah satu penyakit yang biasa terjadi di kalangan wanita di Asia. Rawatan konvensional telah digunakan bagi merawat penyakit kanser, tetapi rawatan ini kurang berkesan dan kadar pesakit yang hidup adalah kurang. Oleh itu, kebanyakan pesakit kanser mula mencari alternatif rawatan yang lain bagi merawat penyakit ini. Maka, kajian ini adalah penting untuk mengenalpasti kesan ketoksikan ekstrak daun methanol bagi *Typhonium flagelliforme* dan *Clinacanthus nutans* terhadap sel kanser payudara (MDA-MB-231). MTT assay telah digunakan bagi mengukur kesan ketoksikan kedua-dua pokok ini kepada MDA-MB-231 dan CHO iaitu sel kawalan yang bukan kanser. Hasil analisis dapatan kajian, *T. flagelliforme* ekstrak telah menunjukkan kesan sitotoksik yang lebih tinggi kepada sel MDA-MB-231 (IC_{50}: 110 µg/mL) berbanding *C. nutans* ekstrak (IC_{50}: 170 µg/mL). Kemudian, IC_{50} bagi *T. flagelliforme* dan *C.nutans* ekstrak terhapat CHO telah menunjukkan nilai masing-masing adalah 100 µg/mL dan 240 µg/mL. Oleh itu, nilai IC_{50}, *T. flagelliforme* telah menunjukkan kesan sitotoksik yang tinggi kepada sel CHO berbanding sel MDA-MB-231. Maka, penambahbaikan kajian pada masa akan datang adalah menggantikan sel CHO kepada sel payudara yang normal seperti HCC1395 (duktus susu epithelium bagi sel normal payudara) dan mengenalpasti kesan toksik ekstrak *T. flagelliforme* terhadap sel normal payudara. Selain itu, sebatian yang memberikan perencatan terhadap sel MDA-MB-231 dan sel payudara yang normal juga perlu dikenalpasti pada penyelidikan akan datang.

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LIST OF SYMBOLS AND ABBREVIATIONS

BSA	-	Bovine Serum Albumin
^0C	-	Degree Celcius (centrigrade)
CO_2	-	Carbon dioxide
CHO	-	Chinese Hamster Ovary Cell Line
cm	-	Centimeter
dH_2O	-	Distilled water
DMEM	-	Dulbecco`s modified eagle medium
DMSO	-	Dimethylsulfoxide
FBS	-	Fetal Bovine Serum
HCl	-	Hydrochloric acid
KCl	-	Potassium chloride
KH_2PO_4	-	Dihydrogen phosphate
µl	-	Micro liter
mL	-	Mili liter
mg	-	Mili gram
mM	-	Mili molar
M	-	Molar
MDA-MB-231	-	Breast Cancer Cell Line
MTT	-	3-[4,5-dimethylthiazol-2-yl] -2,5-tetrazoliumbromidediphenyl
Na_2HPO_4	-	Phosphate dibasic
NaCl	-	Sodium chloride
NaOH	-	Sodium dioxide
nm	-	Nanometer
PBS	-	Phosphate Buffer Saline

Pen/Strep	-	Penicillin-Streptomycin
RMPI 1640	-	Roswell Park Memorial Institute Medium
WHO	-	World Health Organization

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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* (Erton *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 (Pieroni *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 (Wirotesangthong *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.

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