



UNIVERSITI PUTRA MALAYSIA

**ANTIOXIDANT PROPERTIES AND CYTOTOXICITY OF SUCCESSIVELY
MACERATED *Christia vespertilionis* (L.f) Bakh.f. ROOT AND LEAF
AGAINST BREAST CANCER CELL LINES**

JOANNA JINLING LEE

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By

JOANNA JINLING LEE

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science**

June 2020

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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JOANNA JINLING LEE

June 2020

Chair : Latifah Saiful Yazan, PhD
Institute : Bioscience

Breast cancer is the most diagnosed cancer among women worldwide with substantial after-treatment effects. Even with the constant improvement of current therapeutic strategies, the survival rate of breast cancers continues to decline over the decades. Breast cancer is slowly moving into the category of chronic disease due to various barrier limitations such as poor prognosis, non-specific treatment and silent metastasis. Furthermore, severe side effects from chemotherapy agents were seen to potentially harm healthy cells instead of targeting only cancerous cells. *Christia vespertilionis*, a tropical Southeast Asia Fabaceae, is a medicinal plant known for its anti-cancer and anti-inflammatory properties. The objective of this study was to determine the antioxidant activity and cytotoxicity of the root and leaf of *C. vespertilionis* against breast cancer cell lines. Both the roots and leaves of the plant were extracted using the successive solvent maceration method with hexane, chloroform, ethyl acetate and methanol. All the crude extracts were compared for the best bioactivity and was further fractionated by column chromatography method. Antioxidant activity of all the extracts and fractions were determined using TPC, DPPH and β -carotene bleaching assay. Cytotoxicity of all the extracts and fractions were determined using MTT assay. The potentially active compounds and phytochemical components of the active extract and fraction were tentatively assigned and identified using LC-MS/MS. Based on the results, the root extracts of *C. vespertilionis* showed better bioactivity compared to the leaf extracts. The ethyl acetate root extract showed the highest TPC (TPC = $192.12 \pm 4.41 \mu\text{g GAE/g}$) and antioxidant activity (DPPH $\text{IC}_{50} = 70.16 \pm 1.49 \mu\text{g/mL}$ and lipid peroxidation inhibition = $59.52 \pm 0.57 \%$) compared to others ($p < 0.05$). The extract also

exhibited highly selective cytotoxic effects in a dose-dependent manner against MDA-MB-231 and MCF-7 breast cancer cell lines but not toward the normal 3T3 cells. The IC_{50} of the treated MDA-MB-231, MCF-7 and 3T3 cells were 11.34 ± 1.20 , 44.65 ± 5.78 and 77.38 ± 4.71 $\mu\text{g/mL}$, respectively. Amongst the ethyl acetate root fractions, CV E4 fraction exhibited the highest TPC and lipid peroxidation inhibition (330.92 ± 7.30 $\mu\text{g GAE/g}$ and 83.68 ± 1.31 %, respectively). The highest free radical scavenging activity however was seen in CV E6 fraction. Meanwhile, CV E3 fraction exhibited both significant and selective cytotoxicity in a dose-dependent manner. The fraction's IC_{50} was 5.72 ± 0.99 , 8.98 ± 1.06 and 49.90 ± 8.63 $\mu\text{g/mL}$ against MDA-MB-231, MCF-7 and 3T3 cell lines, respectively. Further qualitative phytochemical characterization using LC-MS/MS on the most active extract and fraction suggested the presence of flavonoids, terpenoids, coumarins and quinones that contributes to the antioxidant and cytotoxic properties. In conclusion, the study suggests *C. vespertilionis* as a potential medicinal plant that is selective toward breast cancers. The phytochemicals present in ethyl acetate root extract and CV E3 fraction demonstrated notable antioxidant activity and cytotoxicity especially toward MDA-MB-231 cell line.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk ijazah Master Sains

**SIFAT ANTIOKSIDAN DAN SITOTOKSISITI MACERASI BERTURUTAN
AKAR DAN DAUN *Christia vespertilionis* (L.f) Bakh.f. TERHADAP JUJUKAN
SEL KANSER PAYUDARA**

Oleh

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Kanser payudara ialah kanser yang paling kerap ditemui dalam kalangan wanita di seluruh dunia dengan kesan sampingan selepas rawatan yang membimbangkan. Kadar kehidupan makin merosot walaupun dengan kemajuan strategi rawatan kanser. Pelbagai faktor halangan seperti prognosis serta susulan terapi yang kurang tepat dan kebarangkalian metastasis menjadi penyebab utama kanser payudara menuju ke arah kategori penyakit kronik. Kesan sampingan dari agen kimoterapi yang parah dilihat berpotensi untuk merosakkan sel sihat selain menumpu kepada sel kanser. *Christia vespertilionis* merupakan sejenis pokok ubatan tropika Asia Tenggara daripada keluarga Fabaceae yang dikenali berdasarkan ciri-ciri anti kanser dan anti-keradangan. Objektif penyelidikan ini adalah untuk menentukan ciri-ciri antioksidan dan sifat sitotoksik *C. vespertilionis* keatas sel kanser payudara. Akar dan daun tumbuhan jujukan ini telah diekstrak dalam turutan menggunakan kaedah macerasi dengan heksana, klorofom, etil asetat dan metanol. Kesemua ekstrak mentah dibandingkan untuk bioaktiviti yang terbaik dan kemudian difraksikan melalui teknik kromatografi kolum. Ciri-ciri antioksidan kesemua ekstrak dan fraksi ditentukan menggunakan asai TPC, DPPH dan pelunturan β -karotena-linoleat. Kajian sitotoksik kesemua ekstrak dan fraksi ditentukan menggunakan asai MTT. Sebatian yang berpotensi aktif dan komponen fitokimia daripada ekstrak dan fraksi yang aktif ditentukan melalui kaedah LC-MS/MS. Berdasarkan keputusan kajian, ekstrak akar *C. vespertilionis* menunjukkan bioaktiviti yang lebih baik berbanding ekstrak daun. Kandungan fenolik dan aktiviti antioksidan yang paling tinggi didapati pada ekstrak etil asetat (TPC = $192.12 \pm 4.41 \mu\text{g GAE/mg}$, DPPH $\text{IC}_{50} = 70.16 \pm 1.49 \mu\text{g/mL}$ dan penghambatan peroksidasi lipid

= 59.52 ± 0.57 %) berbanding yang lain ($p < 0.05$). Ekstrak tersebut memaparkan kesan sitotoksik selektif yang tinggi ke atas jujukan sel kanser payudara MDA-MB-231 dan MCF-7 bergantung kepada dos tetapi tidak toksik pada sel normal 3T3. Nilai IC_{50} ekstrak tersebut ke atas sel MDA-MB-231, MCF-7 dan 3T3 ialah 11.34 ± 1.20 , 44.65 ± 5.78 and 77.38 ± 4.71 $\mu\text{g/mL}$, masing-masing. Dalam kalangan fraksi ekstrak akar etil asetat, CV E4 menunjukkan TPC dan perencatan peroksidasi lipid tertinggi (330.92 ± 7.30 $\mu\text{g GAE/g}$ and 83.68 ± 1.31 % masing-masing). Aktiviti pemulangan radikal bebas paling tinggi bagaimanapun dilihat dalam CV E6. CV E3 menunjukkan kesan sitotoksik yang paling ketara and selektif yang bersandar kepada dos. Nilai IC_{50} frakti tersebut ke atas sel MDA-MB-231, MCF-7 dan 3T3 ialah 5.72 ± 0.99 , 8.98 ± 1.06 and 49.90 ± 8.63 $\mu\text{g/mL}$ masing-masing. Pencirian kualitatif fitokimia seterusnya menggunakan LC-MS/MS kepada ekstrak dan fraksi aktif mencadangkan kehadiran flavonoid, terpenoid, komarin dan kuinon yang menyumbang kepada sitotoksik dan aktiviti antioksidan. Kesimpulannya, penyelidikan ini mencadangkan *C. vespertilionis* sebagai tumbuhan ubatan yang berpotensi terhadap kanser payudara. Fitokimia yang terkandung dalam ekstrak akar etil asetat dan pecahan CV E3 menunjukkan sifat antioksidan dan sitotoksik yang ketara terutamanya pada sel MDA-MB-231.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

3T3	Normal mouse fibroblast cell line
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CI	Combination index
DPPH	2,2-diphenyl-1-picrylhydrazyl
ER	Estrogen receptor
GAE	Gallic acid equivalent
GCC	Gravitational chromatography
HER2	Human Epidermal growth factor Receptor 2
IC ₅₀	Half maximal inhibitory concentration
LC-MS/MS	Liquid chromatography – tandem mass spectrometer
MCF-7	Hormone dependent breast adenocarcinoma cell line
MDA-MB-231	Basal triple negative breast adenocarcinoma cell line
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PR	Progesterone receptor
SI	Selectivity index
TLC	Thin layer chromatography
TN	Triple negative
TNM	Tumor, node, metastasis
TPC	Total phenolic content

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Through centuries, cancer remains as one of the leading causes of death worldwide. It is characterized by the uncontrolled proliferation of mutated cells that invade and spread via the lymphatic system to other parts of the body. Despite countless discoveries in research advancement for a promising cure, cancer occurrence continues to peak with the increasing population and substantiate risk factors (Torre et al., 2015). According to World Health Organization (WHO), cancer was responsible for nearly 1 in 6 deaths globally with a sum of 9.6 million deaths in 2018. In the cause of cancer death, statistics revealed about 33.33 % of deaths were due to the present urbanized lifestyle such as obesity, low dietary fiber intake, inactiveness, excessive tobacco and alcohol consumption (World Health Organization, 2018). As such, the world is expected to see an increase in mortality due to various types of cancer as the new global cancer incidence continues to increase yearly.

Breast cancer ranked as the second most occurring cancer in the world among women having an estimate of 25 % of the total new cancer cases among hundred cancer types in 2011. Frequently diagnosed worldwide in both developed and developing countries, breast cancer was responsible for the death in women up to approximately 1.67 million cases with 522,000 deaths (Torre et al., 2015). In the present, these figures for breast cancer cases have increased drastically to 2.09 million cases with 627,000 deaths (Bray et al., 2018). In fact, breast cancer was deemed as the second leading cause of cancer death among women after lung cancer (Desantis et al., 2011).

Through decades of endless studies and research, breast cancer treatment has been a tremendous forward progress resulting in millions of women surviving breast cancer. However, this advancement also brings along new barriers, posing more questions and solid challenges. Current therapeutic strategies employed for breast cancer patients vary from risk assessment, prevention and screening to surgery, radiation, chemotherapy, endocrine therapy and warding off metastasis. In many cases, breast cancer is slowly moving into the category of chronic disease as vast majorities who succumb to death by this disease are women with metastatic breast cancers (Fawcett, 2017).

While it remains true that the advancement of technologies developed for medical application has greatly aided physicians to identify areas and severity of the cancer. Imaging scans such as the positron emission tomography-computed

tomography (PET-CT) scan and magnetic resonance imaging (MRI) scan enable the outline of risk areas slice by slice so that suitable therapy can be administered. The research and improvements of chemotherapy drug coupled with other breast cancer targeting therapies are continuous and many saw promising results toward breast cancer recovery.

The current therapeutic strategies are good but not enough as these therapies greatly compromise healthy cells. These approaches are mostly associated with possible therapeutic resistance, high toxicity, low efficacy and multiple side effects. Cancer treatments are bound to bring along mild to severe side effects at any given dosage; although physicians will try to keep it at its minimum as chemotherapy is at its best to prevent cancer recurrence and metastasis (American Cancer Society, 2016). However, they do not eliminate the risk for cancer recurrence or future metastasis. Even with multiple success stories, some may find the therapy side effects outweighing the potential benefit while the number of fatalities from breast cancer continues to climb.

This is because not all breast cancers are the same. The fuel for each type of breast cancer varies between progesterone, estrogen, HER2 protein and none at all. Different type of breast cancer requires specific treatment plans and chemotherapy drug not forgetting the different response from each individual greatly affects the recovery outcome (Fawcett, 2017). This leads to the need to explore new methods for breast cancer treatment through natural products. Alternative medicine and chemoprevention research using medicinal plants are a novel proposal toward discovering a safer alternative to cancer control and treatment.

1.2 Research Justification

Medicinal plants have long been used as traditional and complementary medicine in many areas especially cancer. In the recent research trend, a handful of medicinal plants are being subjected to scientific study with an aim to discover or isolate secondary metabolites as an alternative to breast cancer treatment (Syed Najmuddin et al., 2016). Countless of naturally occurring bioactive compounds in diverse medicinal plants have been reported to show potential anti-proliferative effects and inhibition of cancer through various signaling pathways (Bishayee and Sethi, 2016). It was deduced that the anti-cancer properties of a plant depend on the content of phytochemicals present particularly terpenes, phenolics, alkaloid and flavonoids (Asadi-samani et al., 2016).

Christia vespertilionis (L.f) Bakh.f., locally known as 'butterfly wing' or 'Daun rerama' is typical to the tropical Southeast Asia. This ornamental medicinal plant from the Fabaceae family has recently gained numerous attentions with claims that its herbal preparation demonstrated anti-proliferative and anti-cancer effects (Bunawan et al., 2015). Traditionally, *C. vespertilionis* has been known for its

ayurvedic remedies against respiratory related diseases and other external injuries with beneficial health properties (Dash, 2016; Upadhyay et al., 2013; Whiting, 2007).

Previously, this plant has also been reported to exhibit anti-plasmodial properties (Upadhyay et al., 2013; Nguyen-Pouplin et al., 2007) and anti-cancer effects against neuroendocrine tumors in both *in-vitro* and *in-vivo* (Hofer et al., 2013). Recently, a simple evaluation on the subacute oral toxicity of the plant was conducted (Nurul et al., 2018) and antioxidant activity of the plant was reported to contain the classes of compounds that attribute toward its bioactivity (Jusoh et al., 2019). The extraction methods of *C. vespertilionis* reported thus far include the solvent maceration and supercritical fluid extraction method depending on the aim of the study (Mohd Ariff et al., 2018; Nurul et al., 2018; Osman et al., 2017; Hofer et al., 2013)

However, the existing literature regarding the pharmacology and phytochemical aspect of this plant are not evident to pose *C. vespertilionis* as a plant with high medicinal benefits but low side effects. The lack of strong scientific evidence regarding its safety, effectiveness and toxicity raises a concern especially with the increased consumption among Malaysians. The evaluation of the efficacy and risk of the plant is very crucial to educate the people consuming it for both health and medical benefits. Besides, there is a need to further understand *C. vespertilionis* in regard to the relation between its antioxidant strength and effectiveness in specific cancer types, the extraction methods employed leading to the classes of compounds being isolated and the phytochemicals that gave rise to the bioactivity studied. This shows that there are still plenty of unexplored methods to be conducted on this plant in order to completely eliminate the long-term risk associated with the use of *C. vespertilionis* extracts as a natural source for alternative medicine.

1.3 Objectives and Hypothesis

The general objective of this study was to determine the antioxidant properties of the root and leaf of *Christia vespertilionis* and their cytotoxicity specifically against breast cancer cell lines.

The specific objectives of this study were:

- (1) To evaluate and compare the antioxidant properties and cytotoxicity of the root and leaf crude extracts of *C. vespertilionis* extracted from different solvents.
- (2) To fractionate the most active crude extract and evaluate the antioxidant properties and cytotoxicity of the fraction of *C. vespertilionis*.

- (3) To tentatively identify the chemical constituents present in the most active extract and fraction of *C. vespertilionis* that may have caused their antioxidant properties and cytotoxicity.

It is hypothesized that the ethyl acetate extracts of *C. vespertilionis* will exhibit antioxidant capabilities and cytotoxicity against breast cancer cell lines.



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LIST OF PUBLICATIONS

Journals

Lee, J. J., Yazan, L. S., Kassim, N. K., Che Abdullah, C. A., Esa, N., Lim, P. C., & Tan, D. C. (2020). Cytotoxic activity of *Christia vespertilionis* root and leaf extracts and fractions against breast cancer cell lines. *Molecules*, 25, 11, 2610.

Lee, J. J., Yazan, L. S., & Che Abdullah, C. A. (2017). A review on current nanomaterials and their drug conjugate for targeted breast cancer treatment. *International Journal of Nanomedicine*, 12, 2373 – 2384.



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