CYTOTOXIC EFFECTS OF COMBINED CISPLATIN AND CLINACANTHUS NUTANS IN MDA-MB-231 AND MDA-MB-468 TRIPLE NEGATIVE BREAST CANCER CELLS

NUR FITRIYANI AFIQAH BINTI ABU BAKAR

UNIVERSITI TEKNOLOGI MALAYSIA

CYTOTOXIC EFFECTS OF COMBINED CISPLATIN AND CLINACANTHUS NUTANS IN MDA-MB-231 AND MDA-MB-468 TRIPLE NEGATIVE BREAST CANCER CELLS

NUR FITRIYANI AFIQAH BINTI ABU BAKAR

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Philosophy

Faculty of Science
Universiti Teknologi Malaysia

NOVEMBER 2021

DEDICATION

"*Alhamdulillah, thank you Allah, for His favour and blessings for me towards completing this thesis.*"

For my parents, thank you for supporting me throughout the journey.

For my husband and son, thank you for being my greatest strength.

ACKNOWLEDGEMENT

First and foremost, I would like to express my greatest gratitude to my main supervisor, Dr Praseetha Prabhakaran for the encouragement, critics and guidance. I am also very thankful to my co-supervisor, Dr Khairunadwa Jemon for her guidance, advice and motivation. Without their continued support and interest, this thesis would not have been the same as presented here.

I am also highly indebted to my dear housemates and sisters for their support and assistance. My sincere appreciation also extends to all my lab mates who have assisted me on various occasions.

Lastly, I would like to express my utmost appreciation to my husband, Muhammad Hilmi Md Jori and my son, Muhammad Umar Ghazi, for being my biggest strength throughout the whole journey. Besides, I am grateful to my parents and siblings for their never-ending encouragement.

ABSTRACT

The Triple Negative Breast Cancer (TNBC) is the most invasive breast cancer subtype enriched with cancer stem cells (CSCs). The absence of estrogen, progesterone and HER2 receptors make TNBCs difficult to be targeted by existing chemotherapy treatments. This study aimed to identify the effects of cisplatin and *C. nutans* as combined treatment on MDA-MB-231 and MDA-MB-468 breast cancer cells representing the Triple Negative Breast Cancer subtype. The effect of cisplatin, *C. nutans* and their combination were investigated on the viability and proliferative ability of MDA-MB-231 and MDA-MB-468 cells using Cell Titer-Glo® 2.0 and CyQuant NF Proliferation Assays, respectively. Three different treatments were studied, Cisplatin at 0-15.23 µg/mL, *C. nutans* at 0-50 µg/mL and combined treatment of 3.05 µg/mL cisplatin with *C. nutans* at 0-50 µg/mL. Next, a drug interaction study was done using isobologram-combination index analysis. The effect of these treatments to induce apoptosis in both cancer cells was also determined using Caspase-Glo® 3/7. Additionally, cell invasion inhibition of MDA-MB-231 breast cancer cells was examined using Cultrex BME Cell Invasion assays. The expression of cancer stem cells protein (CD49f) and differentiation marker (CK18) was also elucidated using flow cytometry analysis. The results showed that the combined cisplatin-*C. nutans* treatment exhibited potent and synergistic anticancer effects on MDA-MB-231 and MDA-MB-468 breast cancer cells. Cisplatin and *C. nutans* alone reduced MDA-MB-231 cell viability and proliferation in a dose-dependent manner by 6-69% and 1-59% respectively, whereas 13-78% when in combination. Greater cell viability and proliferation inhibition was seen in MDA-MB-468 by cisplatin (11-75%) or *C. nutans* (2-73%) alone or as combined treatment (15-77%). Significant apoptotic induction were exhibited in MDA-MB-231 (2.73%) and MDA-MB-468 cells (3.53%) upon combined treatment in comparison to a negligible apoptotic induction exerted by cisplatin or *C. nutans* alone. Furthermore, the MDA-MB-231 cell invasion ability was significantly reduced to 36% upon combined treatment compared to cisplatin (51%) and *C. nutans* (58%) alone. At protein level, cisplatin and *C. nutans* differentially regulated protein markers associated with cancer stem cells (CD49f) and differentiation (CK18). The expression of CK18 upon cisplatin, *C. nutans* and combined treatments were up-regulated whereas the expression of CD49f was down-regulated upon cisplatin and combined cisplatin-*C. nutans* treatments. Interestingly, *C. nutans* alone caused a negligible change to CD49f expression. Altogether, these findings suggest that cisplatin-*C. nutans* combination is a potent anticancer agent for a targeted therapy against MDA-MB-231 cells and other CSCs-enriched cancers. The up-regulation of CK18 correlates with the induction of CSCs differentiation, on the other hand, down-regulation of CD49f links to cancer stem cells inhibition. The ability of *C. nutans* in sensitizing breast cancer cells against cisplatin was highlighted as a promising strategy to enhance anticancer effect of cisplatin and thus for the treatment and management of TNBC as a whole.

ABSTRAK

Kanser Payudara Triple Negatif (TNBC) adalah sejenis barah payudara yang paling invasif diperkaya dengan sel stem barah (CSC). Ketiadaan reseptor estrogen, progesteron dan HER2 menyebabkan TNBC sasaran yang sukar bagi rawatan kemoterapi sedia ada. Kajian ini bertujuan untuk mengenal pasti kesan rawatan gabungan cisplatin dan *C. nutans* pada sel barah payudara MDA-MB-231 dan MDA-MB-468 yang mewakili kanser jenis TNBC. Kesan cisplatin, *C. nutans* dan gabungannya telah dikenalpasti ke atas kebolehidupan dan proliferatif sel MDA-MB-231 dan MDA-MB-468 menggunakan Cell Titer-Glo® 2.0 dan CyQuant NF *Proliferation Assays*. Tiga rawatan yang berbeza telah dikaji, Cisplatin pada 0-15.23 µg/mL, *C. nutans* pada 0-50 µg/mL dan gabungan 3.05 µg/mL cisplatin dengan C. *nutans* pada 0-50 µg/mL diuji. Seterusnya, interaksi antara kedua-dua bahan turut dilakukan melalui analisa indeks kombinasi isobologram. Kesan rawatan mendorong apoptosis dalam kedua-dua sel kanser juga telah ditentukan menggunakan Caspase-Glo® 3/7. Selain itu, perencatan penghijrahan sel barah MDA-MB-231 telah diperiksa menggunakan ujian Invasif Kultur Sel BMR. Pengekspresan protein sel stem barah (CD49f) dan penanda pembezaan (CK18) juga ditentukan menggunakan analisa *flow cytometry*. Dalam kajian ini, kombinasi rawatan cisplatin-*C. nutans* jelas menunjukkan kesan antikanser yang ketara dan bersinergi pada sel barah payudara MDA-MB-231 dan MDA-MB-468. Cisplatin dan *C. nutans* masing-masing mengurangkan daya hidup dan proliferasi sel MDA-MB-231 sebanyak 6-69% dan 1-59% manakala 13-78% apabila digabungkan. Perencatan terhadap daya hidup dan proliferasi sel yang lebih besar dilihat dalam MDA-MB-468 oleh cisplatin (11-75%) atau *C. nutans* (2-73%) sahaja atau sebagai rawatan gabungan (15-77%). Induksi apoptosis yang ketara telah dipamerkan di dalam sel MDA-MB-231 (2.73%) dan MDA-MB-468 (3.53%) pada rawatan gabungan berbanding rawatan cisplatin atau *C. nutans* sahaja. Tambahan pula, keupayaan penghijrahan sel MDA-MB-231 telah berkurangan dengan ketara kepada 36% pada rawatan gabungan berbanding cisplatin (51%) dan *C. nutans* (58%) sahaja. Pada tahap protein, rawatan cisplatin dan *C. nutans* menunjukkan perbezaan dalam pengekspresan sel stem barah (CD49f) dan penanda pembezaan (CK18). Ekspresi CK18 menunjukkan peningkatan akibat rawatan cisplatin, C. *nutans* dan rawatan gabungan manakala ekspresi CD49f menunjukkan pengurangan akibat rawatan cisplatin dan kombinasi cisplatin-*C. nutans*. Sebaliknya, rawatan *C. nutans* sahaja tidak menunjukkan sebarang perubahan yang ketara pada ekspresi CD49f. Secara keseluruhannya, penemuan ini mencadangkan bahawa kombinasi cisplatin-*C. nutans* ialah agen antikanser yang kuat untuk terapi yang mensasarkan sel MDA-MB-231 dan kanser lain yang diperkaya dengan CSC. Peningkatan CK18 dikaitkan dengan induksi pembezaan sel stem barah manakala pengurangan CD49f berkait rapat dengan pengurangan sel stem barah. Keupayaan *C. nutans* dalam pemekaan sel barah payudara terhadap cisplatin telah ditonjolkan sebagai strategi yang berpontensi dalam meningkatkan kesan anti-kanser cisplatin dan seterusnya rawatan dan pengurusan TNBC secara keseluruhan.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AM	-	Acetoxymethyl
ANOVA	-	Analysis of Variance
ATCC	-	American Type Cell Collection
ATP	-	Adenosine Triphosphate
BCL-2	-	B-Cell Lymphoma 2
BL1	-	Basal-like 1
BL2	-	Basal-like 2
BME	-	β Mercaptoethanol
BRCA1	-	Breast Cancer Gene 1
BRCA2	-	Breast Cancer Gene 2
C. nutans	-	*Clinacanthus nutans*
CAM	-	Complementary and Alternatives Medicines
CD49f	-	Intregrin Alpha 6
CK18	-	Cytokeratin 18
CSCs	-	Cancer Stem Cells
DMEM	-	Dulbecco's Modified Eagle Medium
DNA	-	Deoxyribonucleic Acid
ECM	-	Extracellular Matrix
EGFR	-	Epidermal Growth Factor Receptor
EMT	-	Epithelial-Mesenchymal-Transition
ER	-	Estrogen Receptor
ESC	-	Embryonic Stem Cell
FBS	-	Fetal Bovine Serum
FDA	-	Food and Drug Administration
FITC	-	Fluorescein Isothiocyanate
G1	-	Growth 1
GSCs	-	Glioblastoma Cancer Stem Cells
HER2	-	Human Epidermal Growth Factor Receptor 2
HMG	-	High Mobility Group
IC_{50}	-	Half Maximal Inhibitory Concentration

ITGA6	-	Integrin Alpha 6
JAK-STAT	-	Janus Kinase/Signal Transducer and Activator of Transcription
LAR	-	Luminal Androgen Receptor
M	-	Mesenchymal
MaSCs	-	Mammary Stem Cells
MFI	-	Mean Fluorescence Intensity
mRNA	-	Messenger ribonucleic acid
NCI	-	National Cancer Institute
PBS	-	Phosphate Buffered Saline
pCR	-	Pathologic Complete Response
PFA	-	Paraformaldehyde
PR	-	Progesterone Receptor
RPM	-	Revolutions Per Minute
SD	-	Standard Deviation
SEM	-	Standard Error of The Mean
TGF-β	-	Transforming Growth Factor β
TNBC	-	Triple Negative Breast Cancer
TNF	-	Tumor Necrosis Factor
USM	-	Universiti Sains Malaysia
WHO	-	World Health Organization

LIST OF SYMBOLS

µg	-	microgram
µg/mL	-	microgram per mililiter
µM	-	micromolar
mg/kg	-	milligram per kilogram
mg/mL	-	milligram per mililiter
mL	-	mililiter
mM	-	milimolar
nm	-	nanometer
nM	-	nanomolar
α	-	alpha
β	-	beta
κ	-	kappa
°C	-	Degree Celsius

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Cancer is a non-communicable disease and is one of the leading causes of death at the global level. Recently, the World Health Organization (WHO) revealed that non-communicable diseases make up 7 of the world's top 10 death, according to WHO's 2019 Global Health Estimate. This new data that cover the period from 2000 to 2019 shows an increase from 4 of the 10 in 2000. Being the 6th world's biggest killer, trachea, bronchus and lung cancer death claimed 1.8 million lives, risen from 1.2 million in 2000 (World Health Organization, 2020b). In Malaysia, statistics on cancer incidence shows that 14.8% of Malaysians are at risk of getting cancer and 9.1% risk of dying from cancer before 75 years of age for both sexes with lung and breast cancers as the most frequent cancer in males and females respectively (International Agency for Research on Cancer, 2020). This highlights the urgency of understanding cancer more in the prevention and managing these diseases.

Breast cancer, the most common malignant disease among women (World Health Organization, 2020b), is a complex, biologically and molecularly heterogeneous disease that consists of tumour cells ranging from stem cell-like to more differentiated cells (Feng *et al.,* 2018; Prabhakaran, Hassiotou, Blancafort, & Filgueira, 2013). Multiple studies on gene expression divided breast cancer into five intrinsic molecular subtypes; Luminal A, Luminal B, HER2-enriched, Triple Negative and Normal-like breast cancer (Feng *et al.,* 2018; Prat *et al.,* 2015). This classification is based on the expression of three primary receptors; estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) which are usually become the main target of drug treatment. The Triple Negative Breast Cancer (TNBC) which accounts for approximately 20% of all breast cancer, is characterized by ER negative, PR negative and HER2 negative, making hormonal

therapy non-beneficial towards its treatment (Feng *et al.*, 2018). TNBC tumors often grow rapidly and have a poor prognosis, with high invasiveness, high metastatic potential, high recurrence rate, virtually all relapse within five years and a short interval between recurrence and death (Hwang, Park, & Kwon, 2019; L. Yin, Duan, Bian, & Yu, 2020). Not only because of the heterogeneity of the disease but also due to the absence of well-defined molecular targets, leaving chemotherapy the only option in the therapeutic field (Hwang *et al.*, 2019; L. Yin *et al.*, 2020). TNBC more frequently affects younger patients and are more prevalent in African-American women especially among pre-menopausal individuals (Hwang *et al.*, 2019; Keenan *et al.*, 2015). Risk factors associated with the development of TNBC comprise early menarche, high waist-to-hip ratio, and a lack of breast feeding together with high parity (Feng *et al.*, 2018).

TNBCs are known to be enriched with functional cancer stem-like cells, exhibiting high migration patterns and expressing some specific breast cancer genes, making them the most invasive subtype among breast cancer subtypes (Palomeras, Ruiz-Martínez, & Puig, 2018; L. Yin *et al.*, 2020). Generally, cancer stem cells (CSCs) are a sub-population within the tumor, also known as tumor initiation/propagating cells that possess self-renewal potential, tumor initiation capability, and tumor progression (Palomeras *et al.*, 2018; J. Zhou *et al.*, 2019). The existence of this CSC population with stem cell-like characteristics in TNBC tumors is a significant contributing factor for the development of resistance to treatment and cancer relapses, through their virtue of relative resistance to radiation, cytotoxic chemotherapy and molecular targeted therapy (S.-Y. Park, Choi, & Nam, 2019; J. Zhou *et al.*, 2019). Therefore, patients with TNBC are often challenging to treat and result in lower survival rates than patients with other breast cancer types (Collignon, Lousberg, Schroeder, & Jerusalem, 2016; X. Li *et al.*, 2017; Yeh *et al.*, 2017). In recent years, the TNBCs have shown specific sensitivity towards cisplatin, the first metal-based anti-cancer drug (S. M. Al-Bahlani *et al.*, 2017; Dasari & Tchounwou, 2014; Prabhakaran *et al.*, 2013).

Cisplatin is a metal-based anti-tumour drug that is found to be effective in the treatment of various cancers (S. M. Al-Bahlani *et al.*, 2017; Dasari & Tchounwou, 2014). It is a well-known cytotoxic drug that is claimed to be capable of interfering

with the DNA activity upon entering the nucleus of the cells and preventing the DNA repair process. This event eventually leads to cell death (S. Al-Bahlani *et al.,* 2017). Nevertheless, a previous study had shown that cisplatin may possess other mechanisms of action such as inducing differentiation of cancer cells apart from apoptosis (Prabhakaran *et al.,* 2013). Although previous studies pointed out that cisplatin give rise to some side effects after initial treatment (Czarnomysy *et al.,* 2017; Victoria *et al.,* 2007), accumulated evidence showed that co-treatment of cisplatin with other potential anticancer drugs induces apoptosis or autophagy in various cancer cells (S. M. Al-Bahlani *et al.,* 2017; Baharuddin *et al.,* 2016; Czarnomysy *et al.,* 2017; N. Yu, Xiong, & Wang, 2017). Cisplatin is also very powerful in killing cancer cells with metastatic characteristic (S. Al-Bahlani *et al.,* 2017).

In contrast, *Clinacanthus nutans (C. nutans)* is a traditional herb that is claimed to be a potential chemoprevention alternative for cancer patients (Fazil, Azzimi, Yahaya, Kamalaldin, & Zubairi, 2016). A study reported that *C. nutans* are the most consumed herbs in complementary and alternative medicines (CAM) among newly diagnosed breast cancer patients in Malaysia (Zulkipli *et al.,* 2018). *C. nutans* extracts contain various types of phytochemical compounds such as phenolic, fatty acids, flavonoids, glycosides, glycoglycerolipids, cerebrosides and monoacylmonogalatosylglycerol with useful biological capabilities (Abd Samat, Ahmad, Awang, Bakar, & Hakiman, 2020; Alam *et al.,* 2016; Ghasemzadeh, Nasiri, Jaafar, Baghdadi, & Ahmad, 2014; Huang, Guo, Gao, Chen, & Olatunji, 2015; Teoh *et al.,* 2017; K. N. Zakaria, Amid, & Jamal, 2017). Natural-derived phytochemical constituents in *C. nutans* extracts also found to exhibit cytotoxicity effects through the induction of apoptosis (Ng *et al.,* 2017; Teoh *et al.,* 2017; Y. Zakaria, Yee, & Nik Hassan, 2017) and antioxidant activity that could reduce the risk of getting cancers (Abd Samat *et al.,* 2020; Ghasemzadeh *et al*., 2014; K. N. Zakaria *et al.,* 2017). Other than that, *C. nutans* is more preferred by the people in common as it is a natural herb that is relatively safe with lesser side effects than conventional drugs (Sarega *et al.,* 2016; Sulaiman *et al.,* 2015). In this study, we demonstrated the potential anticancer effects and mechanism of action of cisplatin, *C. nutans* and combined cisplatin-*C. nutans* at the cellular level on MDA-MB-231 and MDA-MB 468 cells.

1.2 Problem Statement

The triple negative breast cancer (TNBC) subtype is considered the most invasive form of breast cancer and is often difficult to treat as it is known to be enriched with cancer stem cells (CSCs) (O'Conor, Chen, González, Cao, & Peng, 2018; Park, Choi, & Nam, 2019). Triple negative is usually aggressive and more likely to resurface than another breast cancer subtype (Hwang, Park, & Kwon, 2019; L. Yin, Duan, Bian, & Yu, 2020). In TNBC, three main receptors, estrogen, progesterone, and HER2 receptor, are absent, so it does not respond to hormonal therapy, making it difficult to treat (Feng *et al.,* 2018). Cisplatin is the first metal based anticancer drug produced and has been used extensively to treat various difficult to treat cancers for the past 4 decades (Al-Bahlani *et al.,* 2017; Dasari & Tchounwou, 2014).

While cisplatin might be effective in treating TNBC, cisplatin therapy's success is compromised due to dose-limiting toxicity, especially nephrotoxicity as well as resistance by tumor cells to cisplatin (Al-Bahlani *et al.*, 2017; Dilruba & Kalayda, 2016). Therefore, exploring and seeking a new efficient target for TNBC chemotherapy is needed. Combination therapy is one of the efforts to overcome these problems. Numerous studies on combination therapy of cisplatin with other agents on various cancer cells had shown promising results in enhancing anti-cancer efficacy of cisplatin and reducing its cisplatin dose, thus reducing its toxicity (H. Liu, Lee, Lee, Ahn, & Kim, 2019; Rodriguez *et al*., 2021; Sun, Zhang, Zheng, & Feng, 2019; Zhang & Lu, 2021). The widespread consumption of *Clinacanthus nutans* (*C. nutans*) on the other hand has being used as an alternative and complementary approach to cancer treatments with minimal cost and side effects in various cancer types, including breast cancer (Fazil, Azzimi, Yahaya, Kamalaldin, & Zubairi, 2016). Despite its popular consumption among breast cancer patients in Malaysia, to the best of our knowledge, there is no report on combination anticancer effect of combined cisplatin and *C. nutans* on TNBC. Therefore, this study is exploring the potential cytotoxic effect and mechanism of action in combination cisplatin and *C. nutans* treatment in treating TNBC. We hypothesized that *C. nutans* could enhance the anticancer effect of cisplatin treatment in TNBC.

1.3 Research Objectives

To give insight into the mechanisms through which cisplatin-*C. nutans* affecting breast cancer cells in TNBC. Two types of TNBC cell lines, mainly the MDA-MB-468 and MDA-MB-231 will be tested in this study. Followings are the objectives for this study:

(a) To evaluate the effects of combined cisplatin-*C. nutans* extract treatment on the cell viability and proliferation in TNBC cells via Cell Titer-Glo® 2.0 and CyQuant NF Proliferation Assays.

(b) To determine the ability of combined cisplatin-*C. nutans* extract treatment to induce apoptosis in TNBC cells using the Caspase 3/7 Glo Assay.

(c) To elucidate the effects of combined cisplatin-*C. nutans* extract treatment on the cell invasion activity in TNBC cells via the Cultrex® BME Cell Invasion Assay.

(d) To analyze the effects of combined cisplatin-*C. nutans* extract treatment on the protein expression of CD49f and CK18 in TNBC cells via flow cytometry analysis.

1.4 Scope of Study

The goal of this research is to study the effect of cisplatin, *C. nutans* and combined cisplatin-*C. nutans* treatment in TNBC cells representative by MDA-MB-231 and MDA-MB-468 on cell viability and proliferation via Cell Titer-Glo® 2.0 and CyQuant NF Proliferation Assays respectively. The effect of these treatments was also tested on the ability to induce apoptosis in both cell lines using Caspase-Glo® 3/7. Besides, the ability to inhibit cancer cell invasion in MDA-MB-231 was investigated via Cultrex BME Cell Invasion assays. In addition, the protein expression of cancer stem cell (CCD49f) and differentiation markers (CK18) in MDA-MB-231 via flow cytometry analysis was also elucidated in this study.

1.5 Significant of Study

Years of study have failed to demonstrate a single unifying alteration that is targetable in TNBC. Recently, treatment of breast cancer is selected according to the specific subtype, moving closer to the goal of ‘’tailor made therapy’’. Treatment strategies for breast cancer are no longer similar for different subtypes (Feng *et al*., 2018; Jézéquel *et al*., 2019; Yagata, Kajiura, & Yamauchi, 2011). Besides, severe side effects and development of resistance in standard therapeutic chemotherapy becoming significant problems in the treatment of TNBC. Therefore, much effort has been devoted to developing a novel strategy to overcome these issues. In this study, we investigate the potential anticancer effect of cisplatin, *C. nutans* and combined cisplatin-*C. nutans* treatment in TNBC cells. This research provides insight into the mechanism of single cisplatin, *C. nutans* and combination treatment in TNBC, thus treating them in the future.

REFERENCES

Al-Bahlani, S. M., Al-Bulushi, K. H., Al-Alawi, Z. M., Al-Abri, N. Y., Al-Hadidi, Z. R., & Al-Rawahi, S. S. (2017). Cisplatin Induces Apoptosis Through the Endoplasmic Reticulum-mediated, Calpain 1 Pathway in Triple-negative Breast Cancer Cells. *Clinical Breast Cancer, 17*(3), e103-e112. doi:10.1016/j.clbc.2016.12.001

Alam, M. A., Zaidul, I. S. M., Ghafoor, K., Ferdosh, S., Ali, M. E., Mirhosseini, H., . . . Khatib, A. (2017). Identification of bioactive compounds with GC–Q-TOF–MS in the extracts from Clinacanthus nutans using subcritical carbon dioxide extraction. *Separation Science and Technology, 52*(5), 852-863. doi:10.1080/01496395.2016.1271342

Arullappan, S., Rajamanickam, P., Thevar, N., & Kodimani, C. C. (2013). In Vitro Screening of Cytotoxic, Antimicrobial and Antioxidant Activities of Clinacanthus nutans (Acanthaceae) leaf extracts. *Tropical Journal of Pharmaceutical Research, 13*(9), 7.

Azizah AM, H. B., Nirmal K, Siti Zubaidah AR, Puteri NA, Nabihah A, Sukumaran R, . . . AA, A. (2019). *Malaysia National Cancer Registry Report 2012-2016*. Retrieved from http://nci.moh.gov.my/index.php/ms/main-menu-2/laporan

Baharuddin, P., Satar, N., Fakiruddin, K. S., Zakaria, N., Lim, M. N., Yusoff, N. M., . . . Yahaya, B. H. (2016). Curcumin improves the efficacy of cisplatin by targeting cancer stem-like cells through p21 and cyclin D1-mediated tumour cell inhibition in non-small cell lung cancer cell lines. *Oncology reports, 35*(1), 13-25. doi:10.3892/or.2015.4371

Bareche, Y., Venet, D., Ignatiadis, M., Aftimos, P., Piccart, M., Rothe, F., & Sotiriou, C. (2018). Unravelling triple-negative breast cancer molecular heterogeneity using an integrative multiomic analysis. *Ann Oncol, 29*(4), 895-902. doi:10.1093/annonc/mdy024

Basu, A., & Krishnamurthy, S. (2010). Cellular responses to Cisplatin-induced DNA damage. *J Nucleic Acids, 2010*. doi:10.4061/2010/201367

Beck, B., & Blanpain, C. (2013). Unravelling cancer stem cell potential. *Nature Reviews Cancer, 13*(10), 727-738. doi:10.1038/nrc3597

Bhola, N. E., Balko, J. M., Dugger, T. C., Kuba, M. G., Sánchez, V., Sanders, M., . . . Arteaga, C. L. (2013). TGF-β inhibition enhances chemotherapy action against triple-negative breast cancer. *The Journal of clinical investigation, 123*(3), 1348-1358. doi:10.1172/JCI65416

Blows, F. M., Driver, K. E., Schmidt, M. K., Broeks, A., van Leeuwen, F. E., Wesseling, J., . . . Huntsman, D. (2010). Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. *PLoS medicine, 7*(5), e1000279-e1000279. doi:10.1371/journal.pmed.1000279

Bosch, A., Eroles, P., Zaragoza, R., Vina, J. R., & Lluch, A. (2010). Triple-negative breast cancer: molecular features, pathogenesis, treatment and current lines of research. *Cancer Treat Rev, 36*(3), 206-215. doi:10.1016/j.ctrv.2009.12.002

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin, 68*(6), 394-424. doi:https://doi.org/10.3322/caac.21492

Campbell, V., & Copland, M. (2015). Hedgehog signaling in cancer stem cells: a focus on hematological cancers. *Stem cells and cloning : advances and applications, 8*, 27-38. doi:10.2147/SCCAA.S58613

Castellaro, A. M., Rodriguez-Baili, M. C., Di Tada, C. E., & Gil, G. A. (2019). Tumor-Associated Macrophages Induce Endocrine Therapy Resistance in ER+ Breast Cancer Cells. *Cancers, 11*(2), 189.

Cobain, E. F., Milliron, K. J., & Merajver, S. D. (2016). Updates on breast cancer genetics: Clinical implications of detecting syndromes of inherited increased susceptibility to breast cancer. *Seminars in Oncology, 43*(5), 528-535. doi:https://doi.org/10.1053/j.seminoncol.2016.10.001

Collignon, J., Lousberg, L., Schroeder, H., & Jerusalem, G. (2016). Triple-negative breast cancer: treatment challenges and solutions. *Breast cancer (Dove Medical Press), 8*, 93-107. doi:10.2147/BCTT.S69488

Dai, X., Cheng, H., Bai, Z., & Li, J. (2017). Breast Cancer Cell Line Classification and Its Relevance with Breast Tumor Subtyping. *Journal of Cancer, 8*(16), 3131-3141. doi:10.7150/jca.18457

Dai, X., Li, T., Bai, Z., Yang, Y., Liu, X., Zhan, J., & Shi, B. (2015). Breast cancer intrinsic subtype classification, clinical use and future trends. *American journal of cancer research, 5*(10), 2929-2943.

Dai, X., Xiang, L., Li, T., & Bai, Z. (2016). Cancer Hallmarks, Biomarkers and Breast Cancer Molecular Subtypes. *Journal of Cancer, 7*(10), 1281-1294. doi:10.7150/jca.13141

Dasari, S., & Tchounwou, P. B. (2014). Cisplatin in cancer therapy: molecular mechanisms of action. *European journal of pharmacology, 0*, 364-378. doi:10.1016/j.ejphar.2014.07.025

Demiray, M., Ulukaya, E. E., Arslan, M., Gokgoz, S., Saraydaroglu, O., Ercan, I., . . . Manavoglu, O. (2006). Response to neoadjuvant chemotherapy in breast cancer could be predictable by measuring a novel serum apoptosis product, caspase-cleaved cytokeratin 18: a prospective pilot study. *Cancer Invest, 24*(7), 669-676. doi:10.1080/07357900600981307

Denkert, C., Liedtke, C., Tutt, A., & von Minckwitz, G. (2017). Molecular alterations in triple-negative breast cancer—the road to new treatment strategies. *The Lancet, 389*(10087), 2430-2442. doi:10.1016/S0140-6736(16)32454-0

Dhale, D. A., & Mogle, U. P. (2011). Phytochemical Screening and Antibacterial Activity of Phyllanthus emblica (L.). *Science Research Reporter, 1*(3), 5.

Dilruba, S., & Kalayda, G. V. (2016). Platinum-based drugs: past, present and future. *Cancer Chemotherapy and Pharmacology, 77*(6), 1103-1124. doi:10.1007/s00280-016-2976-z

Doherty, M. R., Cheon, H., Junk, D. J., Vinayak, S., Varadan, V., Telli, M. L., . . . Jackson, M. W. (2017). Interferon-beta represses cancer stem cell properties in triple-negative breast cancer. *Proceedings of the National Academy of Sciences of the United States of America, 114*(52), 13792-13797. doi:10.1073/pnas.1713728114

Farooqui, M., Hassali, M. A., Shatar, A. K. A., Farooqui, M. A., Saleem, F., Haq, N. u., & Othman, C. N. (2016). Use of complementary and alternative medicines among Malaysian cancer patients: A descriptive study. *Journal of Traditional and Complementary Medicine, 6*(4), 321-326. doi:https://doi.org/10.1016/j.jtcme.2014.12.008

Fazil, F. N. M., Azzimi, N. S. M., Yahaya, B. H., Kamalaldin, N. A., & Zubairi, S. I. (2016). Kinetics Extraction Modelling and Antiproliferative Activity of Clinacanthus nutans Water Extract. *The Scientific World Journal, 2016*.

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., . . . Ren, G. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & diseases, 5*(2), 77-106. doi:10.1016/j.gendis.2018.05.001

Fillmore, C. M., & Kuperwasser, C. (2008). Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. *Breast Cancer Res, 10*(2), R25-R25. doi:10.1186/bcr1982

Flobak, Å., Niederdorfer, B., Nakstad, V. T., Thommesen, L., Klinkenberg, G., & Lægreid, A. (2019). A high-throughput drug combination screen of targeted small molecule inhibitors in cancer cell lines. *Scientific Data, 6*(1), 237. doi:10.1038/s41597-019-0255-7

Fong, S. Y. (2015). *Genetic, phytochemical and bioactivity studies of Clinacanthus nutans (Burm. f.) Lindau (Acanthaceae).* (Doctor of Philosophy

(Applied Biology and Biotechnology)), RMIT University,

Fong, S. Y., Piva, T., Dekiwadia, C., Urban, S., & Huynh, T. (2016). Comparison of cytotoxicity between extracts of Clinacanthus nutans (Burm. f.) Lindau leaves from different locations and the induction of apoptosis by the crude methanol leaf extract in D24 human melanoma cells. *BMC Complement Altern Med, 16*(1), 368. doi:10.1186/s12906-016-1348-x

Fougner, C., Bergholtz, H., Norum, J. H., & Sørlie, T. (2020). Re-definition of claudin-low as a breast cancer phenotype. *Nature Communications, 11*(1), 1787. doi:10.1038/s41467-020-15574-5

Gan, G. G., Leong, Y. C., Bee, P. C., Chin, E., & Teh, A. K. H. (2015). Complementary and alternative medicine use in patients with hematological cancers in Malaysia. *Support Care Cancer*, 8. doi:DOI 10.1007/s00520-015-2614-z

Garcia-Mayea, Y., Mir, C., Masson, F., Paciucci, R., & Lleonart, M. E. (2020). Insights into new mechanisms and models of cancer stem cell multidrug resistance. *Seminars in Cancer Biology, 60*, 166-180. doi:https://doi.org/10.1016/j.semcancer.2019.07.022

Ghasemzadeh, A., Nasiri, A., Jaafar, H. Z., Baghdadi, A., & Ahmad, I. (2014). Changes in phytochemical synthesis, chalcone synthase activity and pharmaceutical qualities of sabah snake grass (Clinacanthus nutans L.) in relation to plant age. *Molecules, 19*(11), 17632-17648. doi:10.3390/molecules191117632

Goel, H. L., Pursell, B., Chang, C., Shaw, L. M., Mao, J., Simin, K., . . . Mercurio, A. M. (2013). GLI1 regulates a novel neuropilin-2/α6β1 integrin based autocrine pathway that contributes to breast cancer initiation. *EMBO molecular medicine, 5*(4), 488-508. doi:10.1002/emmm.201202078

Ha, S.-A., Lee, Y. S., Kim, H. K., Yoo, J., Kim, S., Gong, G.-H., . . . Kim, J. W. (2012). The prognostic potential of keratin 18 in breast cancer associated with tumor dedifferentiation, and the loss of estrogen and progesterone receptors. *Cancer Biomarkers, 10*, 219-231. doi:10.3233/CBM-2012-0250

Hamid, H. A., & Yahaya, I. H. (2016). CYTOTOXICITY OF CLINACANTHUS NUTANS EXTRACTS ON HUMAN HEPATOMA (HepG2) CELL LINE. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(10), 293-295. doi:10.22159/ijpps.2016v8i10.13626

Hashmi, A. A., Naz, S., Hashmi, S. K., Hussain, Z. F., Irfan, M., Bakar, S. M. A., . . . Edhi, M. M. (2018). Cytokeratin 5/6 and cytokeratin 8/18 expression in triple negative breast cancers: clinicopathologic significance in South-Asian population. *BMC Research Notes, 11*(1), 372. doi:10.1186/s13104-018-3477-4

Hastak, K., Alli, E., & Ford, J. M. (2010). Synergistic chemosensitivity of triple-negative breast cancer cell lines to poly(ADP-Ribose) polymerase inhibition, gemcitabine, and cisplatin. *Cancer Res, 70*(20), 7970-7980. doi:10.1158/0008-5472.can-09-4521

Hatina, J. (2012). The dynamics of cancer stem cells. *Neoplasma, 59*(6), 700-707. doi:10.4149/neo_2012_092

Hill, D. P., Harper, A., Malcolm, J., McAndrews, M. S., Mockus, S. M., Patterson, S. E., . . . Blake, J. A. (2019). Cisplatin-resistant triple-negative breast cancer subtypes: multiple mechanisms of resistance. *BMC Cancer, 19*(1), 1039. doi:10.1186/s12885-019-6278-9

Hilliard, T., Miklossy, G., Chock, C., Yue, P., Williams, P., & Turkson, J. (2017). 15∝-methoxypuupehenol induces antitumor effects in vitro and in vivo against

human glioblastoma and breast cancer models. *Molecular cancer therapeutics*, molcanther. 0291.2016.

Hwang, S.-Y., Park, S., & Kwon, Y. (2019). Recent therapeutic trends and promising targets in triple negative breast cancer. *Pharmacology & Therapeutics, 199*, 30-57. doi:https://doi.org/10.1016/j.pharmthera.2019.02.006

Intan, S. C. S., Mahiran, B., Kim, W. C., Siti, E. A., Hamid, R. F. M., & Maznah, I. (2015). In vitro antioxidant, cytotoxic and phytochemical studies of Clinacanthus nutans Lindau leaf extracts. *African Journal of Pharmacy and Pharmacology*, *9*(34), 861-874. doi:10.5897/ajpp2015.4396

Islam, S. S., Al-Sharif, I., Sultan, A., Al-Mazrou, A., Remmal, A., & Aboussekhra, A. (2018). Eugenol potentiates cisplatin anti-cancer activity through inhibition of ALDH-positive breast cancer stem cells and the NF-κB signaling pathway. *Molecular Carcinogenesis, 57*(3), 333-346. doi:https://doi.org/10.1002/mc.22758

Ismail, N. Z., Md Toha, Z., Muhamad, M., Nik Mohamed Kamal, N. N. S., Mohamad Zain, N. N., & Arsad, H. (2020). Antioxidant Effects, Antiproliferative Effects, and Molecular Docking of Clinacanthus nutans Leaf Extracts. *Molecules, 25*(9), 2067.

Jézéquel, P., Kerdraon, O., Hondermarck, H., Guérin-Charbonnel, C., Lasla, H., Gouraud, W., . . . Campone, M. (2019). Identification of three subtypes of triple-negative breast cancer with potential therapeutic implications. *Breast Cancer Research, 21*(1), 65. doi:10.1186/s13058-019-1148-6

Jin, H., Lee, W. S., Eun, S. Y., Jung, J. H., Park, H.-S., Kim, G., . . . Kim, H. J. (2014). Morin, a flavonoid from Moraceae, suppresses growth and invasion of the highly metastatic breast cancer cell line MDA-MB‐231 partly through suppression of the Akt pathway. *Int J Oncol, 45*(4), 1629-1637. doi:10.3892/ijo.2014.2535

Khoo, L. W., Audrey Kow, S., Lee, M. T., Tan, C. P., Shaari, K., Tham, C. L., & Abas, F. (2018). A Comprehensive Review on Phytochemistry and Pharmacological Activities of Clinacanthus nutans (Burm.f.) Lindau. *Evidence-Based Complementary and Alternative Medicine, 2018*, 9276260. doi:10.1155/2018/9276260

Kligys, K. R., Wu, Y., Hopkinson, S. B., Kaur, S., Platanias, L. C., & Jones, J. C. R. (2012). α6β4 integrin, a master regulator of expression of integrins in human

keratinocytes. *The Journal of biological chemistry, 287*(22), 17975-17984. doi:10.1074/jbc.m111.310458

Krebsbach, P. H., & Villa-Diaz, L. G. (2017). The Role of Integrin α6 (CD49f) in Stem Cells: More than a Conserved Biomarker. *Stem cells and development, 26*(15), 1090-1099. doi:10.1089/scd.2016.0319

Lathia, J. D., Gallagher, J., Heddleston, J. M., Wang, J., Eyler, C. E., Macswords, J., . . . Rich, J. N. (2010). Integrin alpha 6 regulates glioblastoma stem cells. *Cell stem cell, 6*(5), 421-432. doi:10.1016/j.stem.2010.02.018

Lee, C., Ryu, H. W., Kim, S., Kim, M., Oh, S.-R., Ahn, K.-S., & Park, J. (2020). Verminoside from Pseudolysimachion rotundum var. subintegrum sensitizes cisplatin-resistant cancer cells and suppresses metastatic growth of human breast cancer. *Scientific Reports, 10*(1), 20337. doi:10.1038/s41598-020-77401-7

Lee, J. O., Kang, M. J., Byun, W. S., Kim, S. A., Seo, I. H., Han, J. A., . . . Kim, H. S. (2019). Metformin overcomes resistance to cisplatin in triple-negative breast cancer (TNBC) cells by targeting RAD51. *Breast Cancer Res, 21*(1), 115. Retrieved from http://europepmc.org/abstract/MED/31640742 doi:10.1186/s13058-019-1204-2

Lee, K.-L., Kuo, Y.-C., Ho, Y.-S., & Huang, Y.-H. (2019). Triple-Negative Breast Cancer: Current Understanding and Future Therapeutic Breakthrough Targeting Cancer Stemness. *Cancers, 11*(9), 1334.

Lehmann, B. D., Jovanović, B., Chen, X., Estrada, M. V., Johnson, K. N., Shyr, Y., . . . Pietenpol, J. A. (2016). Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. *PLoS One, 11*(6), e0157368. doi:10.1371/journal.pone.0157368

Leng, Y. Z. (2018). *Effect of combined cisplatin and Clinacanthus nutans on gene expression of MDA-MB-231 breast cancer cells.* (Bachelor of Science (Industrial Biology)), Universiti Teknologi Malaysia,

Li, S., Shen, X.-Y., Ouyang, T., Qu, Y., Luo, T., & Wang, H.-Q. (2017). Synergistic anticancer effect of combined crocetin and cisplatin on KYSE-150 cells via p53/p21 pathway. *Cancer Cell International, 17*(1), 98. doi:10.1186/s12935-017-0468-9

Li, Y.-W., Xu, J., Zhu, G.-Y., Huang, Z.-J., Lu, Y., Li, X.-Q., . . . Zhang, F.-X. (2018). Apigenin suppresses the stem cell-like properties of triple-negative breast

cancer cells by inhibiting YAP/TAZ activity. *Cell death discovery, 4*, 105-105. doi:10.1038/s41420-018-0124-8

Liu, H., Lee, G., Lee, J. I., Ahn, T. G., & Kim, S. A. (2019). Effects of genistein on anti-tumor activity of cisplatin in human cervical cancer cell lines. *Obstet Gynecol Sci, 62*(5), 322-328. doi:10.5468/ogs.2019.62.5.322

Liu, Y., Choi, D. S., Sheng, J., Ensor, J. E., Liang, D. H., Rodriguez-Aguayo, C., . . . Chang, J. C. (2018). HN1L Promotes Triple-Negative Breast Cancer Stem Cells through LEPR-STAT3 Pathway. *Stem Cell Reports, 10*(1), 212-227. doi:https://doi.org/10.1016/j.stemcr.2017.11.010

Livasy, C. A., Karaca, G., Nanda, R., Tretiakova, M. S., Olopade, O. I., Moore, D. T., & Perou, C. M. (2006). Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. *Modern Pathology, 19*(2), 264-271. doi:10.1038/modpathol.3800528

Lo, P. K., Kanojia, D., Liu, X., Singh, U. P., Berger, F. G., Wang, Q., & Chen, H. (2012). CD49f and CD61 identify Her2/neu-induced mammary tumor-initiating cells that are potentially derived from luminal progenitors and maintained by the integrin–TGFβ signaling. *Oncogene, 31*(21), 2614-2626. doi:10.1038/onc.2011.439

Mai, C. W., Yap, K. S. I., Kho, M. T., Ismail, N. H., Yusoff, K., Shaari, K., . . . Lim, E. S. H. (2016). Mechanisms Underlying the Anti-Inflammatory Effects of Clinacanthus nutans Lindau Extracts: Inhibition of Cytokine Production and Toll-Like Receptor-4 Activation. *Frontiers in Pharmacology, 7*(7). doi:10.3389/fphar.2016.00007

Mayer, E. L., & Burstein, H. J. (2016). Chemotherapy for Triple-Negative Breast Cancer: Is More Better? *Journal of Clinical Oncology, 34*(28), 3369-3371. doi:10.1200/jco.2016.68.4068

McDonald, E. S., Clark, A. S., Tchou, J., Zhang, P., & Freedman, G. M. (2016). Clinical Diagnosis and Management of Breast Cancer. *Journal of Nuclear Medicine, 57*(Supplement 1), 9S-16S. doi:10.2967/jnumed.115.157834

Momenimovahed, Z., & Salehiniya, H. (2019). Epidemiological characteristics of and risk factors for breast cancer in the world. *Breast cancer (Dove Medical Press), 11*, 151-164. doi:10.2147/BCTT.S176070

Mostert, B., Kraan, J., Sieuwerts, A. M., van der Spoel, P., Bolt-de Vries, J., Prager-van der Smissen, W. J. C., . . . Sleijfer, S. (2012). CD49f-based selection of

circulating tumor cells (CTCs) improves detection across breast cancer subtypes. *Cancer Letters, 319*(1), 49-55. doi:https://doi.org/10.1016/j.canlet.2011.12.031

Mutazah, R., Hamid, H. A., Mazila Ramli, A. N., Fasihi Mohd Aluwi, M. F., & Yusoff, M. M. (2020). In vitro cytotoxicity of Clinacanthus nutans fractions on breast cancer cells and molecular docking study of sulphur containing compounds against caspase-3. *Food and Chemical Toxicology, 135*, 110869. doi:https://doi.org/10.1016/j.fct.2019.110869

Mutebi, M., Anderson, B. O., Duggan, C., Adebamowo, C., Agarwal, G., Ali, Z., . . . Eniu, A. (2020). Breast cancer treatment: A phased approach to implementation. *Cancer, 126*(S10), 2365-2378. doi:https://doi.org/10.1002/cncr.32910

Nik Abd Rahman, N. M. A., Nurliyana, M. Y., Afiqah, M. N. F. N. N., Osman, M. A., Hamid, M., & Lila, M. A. M. (2019). Antitumor and antioxidant effects of Clinacanthus nutans Lindau in 4 T1 tumor-bearing mice. *BMC Complement Altern Med, 19*(1), 340. doi:10.1186/s12906-019-2757-4

Niu, P., Shi, D., Zhang, S., Zhu, Y., & Zhou, J. (2018). Cardamonin enhances the anti-proliferative effect of cisplatin on ovarian cancer. *Oncol Lett, 15*(3), 3991-3997. doi:10.3892/ol.2018.7743

Nordin, F. J., Pearanpan, L., Chan, K. M., Kumolosasi, E., Yong, Y. K., Shaari, K., & Rajab, N. F. (2021). Immunomodulatory potential of Clinacanthus nutans extracts in the co-culture of triple-negative breast cancer cells, MDA-MB-231, and THP-1 macrophages. *PLoS One, 16*(8), e0256012. doi:10.1371/journal.pone.0256012

O'Conor, C. J., Chen, T., González, I., Cao, D., & Peng, Y. (2018). Cancer stem cells in triple-negative breast cancer: a potential target and prognostic marker. *Biomarkers in Medicine, 12*(7), 813-820. doi:10.2217/bmm-2017-0398

Oshima, R. G., Baribault, H., & Caulín, C. (1996). Oncogenic regulation and function of keratins 8 and 18. *Cancer Metastasis Rev, 15*(4), 445-471. doi:10.1007/bf00054012

Palma, G., Frasci, G., Chirico, A., Esposito, E., Siani, C., Saturnino, C., . . . D'Aiuto, M. (2015). Triple negative breast cancer: looking for the missing link between biology and treatments. *Oncotarget, 6*(29), 26560-26574. doi:10.18632/oncotarget.5306

Panicker, N. G., Balhamar, S. O. M. S., Akhlaq, S., Qureshi, M. M., Rizvi, T. S., Al-Harrasi, A., . . . Mustafa, F. (2019). Identification and Characterization of the Caspase-Mediated Apoptotic Activity of Teucrium mascatense and an Isolated Compound in Human Cancer Cells. *Molecules, 24*(5), 977. doi:10.3390/molecules24050977

Pareja, F., Geyer, F. C., Marchiò, C., Burke, K. A., Weigelt, B., & Reis-Filho, J. S. (2016). Triple-negative breast cancer: the importance of molecular and histologic subtyping, and recognition of low-grade variants. *npj Breast Cancer, 2*(1), 16036. doi:10.1038/npjbcancer.2016.36

Park, S.-Y., Choi, J.-H., & Nam, J.-S. (2019). Targeting Cancer Stem Cells in Triple-Negative Breast Cancer. *Cancers, 11*(7), 965.

Pascua, S. M., McGahey, G. E., Ma, N., Wang, J. J., & Digman, M. A. (2020). Caffeine and Cisplatin Effectively Targets the Metabolism of a Triple-Negative Breast Cancer Cell Line Assessed via Phasor-FLIM. *International Journal of Molecular Sciences, 21*(7), 2443. doi:10.3390/ijms21072443

Pauzi, A. Z. M., Yeap, S. K., Abu, N., Lim, K. L., Omar, A. R., Aziz, S. A., . . . Alitheen, N. B. (2016). Combination of cisplatin and bromelain exerts synergistic cytotoxic effects against breast cancer cell line MDA-MB-231 in vitro. *Chinese medicine, 11*, 46-46. doi:10.1186/s13020-016-0118-5

Petrelli, F., Coinu, A., Borgonovo, K., Cabiddu, M., Ghilardi, M., Lonati, V., & Barni, S. (2014). The value of platinum agents as neoadjuvant chemotherapy in triple-negative breast cancers: a systematic review and meta-analysis. *Breast Cancer Res Treat, 144*(2), 223-232. doi:10.1007/s10549-014-2876-z

Pines, A., Kelstrup, C. D., Vrouwe, M. G., Puigvert, J. C., Typas, D., Misovic, B., . . . Olsen, J. V. (2011). Global phosphoproteome profiling reveals unanticipated networks responsive to cisplatin treatment of embryonic stem cells. *Mol Cell Biol, 31*(24), 4964-4977. doi:10.1128/mcb.05258-11

Plasilova, M. L., Hayse, B., Killelea, B. K., Horowitz, N. R., Chagpar, A. B., & Lannin, D. R. (2016). Features of triple-negative breast cancer: Analysis of 38,813 cases from the national cancer database. *Medicine, 95*(35), e4614-e4614. doi:10.1097/MD.0000000000004614

Pongmuangmul, S., Phumiamorn, S., Sanguansermsri, P., Wongkattiya, N., Fraser, I. H., & Sanguansermsri, D. (2016). Anti-herpes simplex virus activities of monogalactosyl diglyceride and digalactosyl diglyceride from Clinacanthus

nutans, a traditional Thai herbal medicine. *Asian Pacific Journal of Tropical Biomedicine, 6*(3), 192-197. doi:https://doi.org/10.1016/j.apjtb.2015.12.014

Prabhakaran, P., Hassiotou, F., Blancafort, P., & Filgueira, L. (2013). Cisplatin Induces Differentiation of Breast Cancer Cells. *Frontiers in Oncology, 3*, 134. doi:10.3389/fonc.2013.00134

Prat, A., Parker, J. S., Karginova, O., Fan, C., Livasy, C., Herschkowitz, J. I., . . . Perou, C. M. (2010). Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. *Breast Cancer Research, 12*(5), R68. doi:10.1186/bcr2635

Prat, A., Pineda, E., Adamo, B., Galván, P., Fernández, A., Gaba, L., . . . Muñoz, M. (2015). Clinical implications of the intrinsic molecular subtypes of breast cancer. *The Breast, 24*, S26-S35. doi:10.1016/j.breast.2015.07.008

Roberts, M. S., Anstine, L. J., Finke, V. S., Bryson, B. L., Webb, B. M., Weber-Bonk, K. L., . . . Keri, R. A. (2020). KLF4 defines the efficacy of the epidermal growth factor receptor inhibitor, erlotinib, in triple-negative breast cancer cells by repressing the EGFR gene. *Breast Cancer Research, 22*(1), 66. doi:10.1186/s13058-020-01305-7

Rodriguez, E., Pei, G., Zhao, Z., Kim, S. T., German, A., & Robinson, P. (2021). Substance P Antagonism as a Novel Therapeutic Option to Enhance Efficacy of Cisplatin in Triple Negative Breast Cancer and Protect PC12 Cells against Cisplatin-Induced Oxidative Stress and Apoptosis. *Cancers, 13*(15), 3871.

Rosenberg, B., Vancamp, L., & Krigas, T. (1965). INHIBITION OF CELL DIVISION IN ESCHERICHIA COLI BY ELECTROLYSIS PRODUCTS FROM A PLATINUM ELECTRODE. *Nature, 205*, 698-699.

Rosenberg, B., VanCamp, L., Trosko, J. E., & Mansour, V. H. (1969). Platinum compounds: a new class of potent antitumour agents. *Nature, 222*(5191), 385-386.

Schliemann, D., Ismail, R., Donnelly, M., Cardwell, C. R., & Su, T. T. (2020). Cancer symptom and risk factor awareness in Malaysia: findings from a nationwide cross-sectional study. *BMC Public Health, 20*(1), 464. doi:10.1186/s12889-020-08581-0

Shi, R., Wang, C., Fu, N., Liu, L., Zhu, D., Wei, Z., . . . Wang, Y. (2019). Downregulation of cytokeratin 18 enhances BCRP-mediated multidrug resistance through induction of epithelial-mesenchymal transition and predicts

poor prognosis in breast cancer. *Oncol Rep, 41*(5), 3015-3026. doi:10.3892/or.2019.7069

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics, 2016. *CA Cancer J Clin, 66*(1), 7-30. doi:10.3322/caac.21332

Silver, D. P., Richardson, A. L., Eklund, A. C., Wang, Z. C., Szallasi, Z., Li, Q., . . . Garber, J. E. (2010). Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. *J Clin Oncol, 28*(7), 1145-1153. doi:10.1200/JCO.2009.22.4725

Smith, S. E., Mellor, P., Ward, A. K., Kendall, S., McDonald, M., Vizeacoumar, F. S., . . . Anderson, D. H. (2017). Molecular characterization of breast cancer cell lines through multiple omic approaches. *Breast Cancer Research, 19*(1), 65. doi:10.1186/s13058-017-0855-0

Sun, C.-Y., Zhang, Q.-Y., Zheng, G.-J., & Feng, B. (2019). Phytochemicals: Current strategy to sensitize cancer cells to cisplatin. *Biomedicine & Pharmacotherapy, 110*, 518-527. doi:https://doi.org/10.1016/j.biopha.2018.12.010

Telli, M. L., Jensen, K. C., Vinayak, S., Kurian, A. W., Lipson, J. A., Flaherty, P. J., . . . Ford, J. M. (2015). Phase II Study of Gemcitabine, Carboplatin, and Iniparib As Neoadjuvant Therapy for Triple-Negative and BRCA1/2 Mutation–Associated Breast Cancer With Assessment of a Tumor-Based Measure of Genomic Instability: PrECOG 0105. *Journal of Clinical Oncology, 33*(17), 1895-1901. doi:10.1200/jco.2014.57.0085

Teoh, P. L. (2021). A minireview on phytochemical and medicinal properties of Clinacanthus nutans. *Journal of Applied Pharmaceutical Science, 11*(06), 015-021.

Teoh, P. L., Cheng, A. Y. F., Liau, M., Lem, F. F., Kaling, G. P., Chua, F. N., & Cheong, B. E. (2017). Chemical composition and cytotoxic properties of Clinacanthus nutans root extracts. *Pharmaceutical biology, 55*(1), 394-401. doi:10.1080/13880209.2016.1242145

Tian, J., Raffa, F. A., Dai, M., Moamer, A., Khadang, B., Hachim, I. Y., . . . Lebrun, J.-J. (2018). Dasatinib sensitises triple negative breast cancer cells to chemotherapy by targeting breast cancer stem cells. *Br J Cancer, 119*(12), 1495-1507. doi:10.1038/s41416-018-0287-3

Tiwary, R., Yu, W., Sanders, B. G., & Kline, K. (2011). alpha-TEA cooperates with chemotherapeutic agents to induce apoptosis of p53 mutant, triple-negative

human breast cancer cells via activating p73. *Breast Cancer Res, 13*(1), R1. doi:10.1186/bcr2801

Tu, S.-F., Liu, R. H., Cheng, Y.-B., Hsu, Y.-M., Du, Y.-C., El-Shazly, M., . . . Chang, F.-R. (2014). Chemical Constituents and Bioactivities of Clinacanthus nutans Aerial Parts. *Molecules, 19*(12), 20382-20390.

Tu, S. F., Liu, R. H., Cheng, Y. B., Hsu, Y. M., Du, Y. C., El-Shazly, M., . . . Chang, F. R. (2014). Chemical constituents and bioactivities of Clinacanthus nutans aerial parts. *Molecules, 19*(12), 20382-20390. doi:10.3390/molecules191220382

Vassilopoulos, A., Chisholm, C., Lahusen, T., Zheng, H., & Deng, C. X. (2014). A critical role of CD29 and CD49f in mediating metastasis for cancer-initiating cells isolated from a Brca1-associated mouse model of breast cancer. *Oncogene, 33*(47), 5477-5482. doi:10.1038/onc.2013.516

Velázquez-Quesada, I., Ruiz-Moreno, A. J., Casique-Aguirre, D., Aguirre-Alvarado, C., Cortés-Mendoza, F., de la Fuente-Granada, M., . . . Velasco-Velázquez, M. A. (2020). Pranlukast Antagonizes CD49f and Reduces Stemness in Triple-Negative Breast Cancer Cells. *Drug design, development and therapy, 14*, 1799-1811. doi:10.2147/DDDT.S247730

Vermeulen, L., Sprick, M. R., Kemper, K., Stassi, G., & Medema, J. P. (2008). Cancer stem cells--old concepts, new insights. *Cell Death Differ, 15*(6), 947-958. doi:10.1038/cdd.2008.20

Wahba, H. A., & El-Hadaad, H. A. (2015). Current approaches in treatment of triple-negative breast cancer. *Cancer biology & medicine, 12*(2), 106-116. doi:10.7497/j.issn.2095-3941.2015.0030

Wang, H., Guo, S., Kim, S.-J., Shao, F., Ho, J. W. K., Wong, K. U., . . . Deng, C.-X. (2021). Cisplatin prevents breast cancer metastasis through blocking early EMT and retards cancer growth together with paclitaxel. *Theranostics, 11*(5), 2442-2459. doi:10.7150/thno.46460

Wang, T., Fahrmann, J. F., Lee, H., Li, Y.-J., Tripathi, S. C., Yue, C., . . . Yu, H. (2018). JAK/STAT3-Regulated Fatty Acid β-Oxidation Is Critical for Breast Cancer Stem Cell Self-Renewal and Chemoresistance. *Cell metabolism, 27*(1), 136-150.e135. doi:10.1016/j.cmet.2017.11.001

Wang, X., Lao, Y., Xu, N., Xi, Z., Wu, M., Wang, H., . . . Xu, H. (2015). Oblongifolin C inhibits metastasis by up-regulating keratin 18 and tubulins. *Scientific*

Reports, 5, 10293. Retrieved from http://europepmc.org/abstract/MED/25973684 doi:10.1038/srep10293

Weaver, B. A. (2014). How Taxol/paclitaxel kills cancer cells. *Molecular Biology of the Cell, 25*(18), 2677-2681. doi:10.1091/mbc.e14-04-0916

Wöckel, A., Albert, U.-S., Janni, W., Scharl, A., Kreienberg, R., & Stüber, T. (2018). The Screening, Diagnosis, Treatment, and Follow-Up of Breast Cancer. *Deutsches Arzteblatt international, 115*(18), 316-323. doi:10.3238/arztebl.2018.0316

Wong, F.-C., Yong, A.-L., Ting, E. P.-S., Khoo, S.-C., Ong, H.-C., & Chai, T.-T. (2014). Antioxidant, Metal Chelating, Anti-glucosidase Activities and Phytochemical Analysis of Selected Tropical Medicinal Plants. *Iran J Pharm Res, 13*(4), 1409-1415.

World Health Organization. (2020a). Global Cancer Observatory. Retrieved from https://gco.iarc.fr/

World Health Organization. (2020b). Global Health Estimates 2019. Retrieved from https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates

Xu, X.-M., Zhang, Y., Qu, D., Liu, H.-B., Gu, X., Jiao, G.-Y., & Zhao, L. (2013). Combined anticancer activity of osthole and cisplatin in NCI-H460 lung cancer cells in vitro. *Experimental and therapeutic medicine, 5*(3), 707-710. doi:10.3892/etm.2013.889

Xu, Z., Mei, J., & Tan, Y. (2017). Baicalin attenuates DDP (cisplatin) resistance in lung cancer by downregulating MARK2 and p-Akt. *Int J Oncol, 50*(1), 93-100. doi:10.3892/ijo.2016.3768

Yagata, H., Kajiura, Y., & Yamauchi, H. (2011). Current strategy for triple-negative breast cancer: appropriate combination of surgery, radiation, and chemotherapy. *Breast Cancer, 18*(3), 165-173. doi:10.1007/s12282-011-0254-9

Yang, J., Gao, S., Xu, J., & Zhu, J. (2018). Prognostic value and clinicopathological significance of serum- and tissue-based cytokeratin 18 express level in breast cancer: a meta-analysis. *Bioscience reports, 38*(2), BSR20171145. doi:10.1042/BSR20171145

Ye, F., Qiu, Y., Li, L., Yang, L., Cheng, F., Zhang, H., . . . Bu, H. (2015). The Presence of EpCAM(-)/CD49f(+) Cells in Breast Cancer Is Associated with a Poor

Clinical Outcome. *Journal of breast cancer, 18*(3), 242-248. doi:10.4048/jbc.2015.18.3.242

Ye, F., Zhong, X., Qiu, Y., Yang, L., Wei, B., Zhang, Z., & Bu, H. (2017). CD49f Can Act as a Biomarker for Local or Distant Recurrence in Breast Cancer. *Journal of breast cancer, 20*(2), 142-149. doi:10.4048/jbc.2017.20.2.142

Yin, L., Duan, J.-J., Bian, X.-W., & Yu, S.-c. (2020). Triple-negative breast cancer molecular subtyping and treatment progress. *Breast Cancer Research, 22*(1), 61. doi:10.1186/s13058-020-01296-5

Yin, L. L., Wen, X. M., Lai, Q. H., Li, J., & Wang, X. W. (2018). Lenalidomide improvement of cisplatin antitumor efficacy on triple-negative breast cancer cells in vitro. *Oncol Lett, 15*(5), 6469-6474. doi:10.3892/ol.2018.8120

Yong, M., Tani, A., Mohamed Salih, F., Pare, R., Norgainathai, R., & Fong, S. (2020). Aqueous leaf extract of Clinacanthus nutans inhibits growth and induces apoptosis via the intrinsic and extrinsic pathways in MDA-MB-231 human breast cancer cells. *Pharmacognosy Magazine, 16*(72), 689-694. doi:10.4103/pm.pm_121_20

Yong, Y. K., Tan, J. J., Teh, S. S., Mah, S. H., Ee, G. C., Chiong, H. S., & Ahmad, Z. (2013). Clinacanthus nutans Extracts Are Antioxidant with Antiproliferative Effect on Cultured Human Cancer Cell Lines. *Evid Based Complement Alternat Med, 2013*, 462751. doi:10.1155/2013/462751

Yu, K.-R., Yang, S.-R., Jung, J.-W., Kim, H., Ko, K., Han, D. W., . . . Kang, K.-S. (2012). CD49f Enhances Multipotency and Maintains Stemness Through the Direct Regulation of OCT4 and SOX2. *Stem cells (Dayton, Ohio), 30*(5), 876-887. doi:https://doi.org/10.1002/stem.1052

Yusuf, S. N. A. M., Mood, C. N. A. C., Ahmad, N. H., Sandai, D., Lee, C. K., & Lim, V. (2020). Optimization of biogenic synthesis of silver nanoparticles from flavonoid-rich Clinacanthus nutans leaf and stem aqueous extracts. *Royal Society Open Science, 7*(7), 200065. doi:doi:10.1098/rsos.200065

Zakaria, Y., Yee, L. W., & Nik Hassan, N. F. (2017). Anti-Cancer Effects of Clinacanthus nutans Extract Towards Human Cervical Cancer Cell Line, HeLa. *2017, 2*(1), 9.

Zaparoli Zucoloto, A., Swely, E., Sanches, A., Silva, L., Marques Bertozzi, M., Rangel, M., . . . Camila, P. (2015). Challenges in the treatment of triple-

negative breast cancer: chemoresistance and identification of molecular targets. *Applied Cancer Research, 35*.

Zasadil, L. M., Andersen, K. A., Yeum, D., Rocque, G. B., Wilke, L. G., Tevaarwerk, A. J., . . . Weaver, B. A. (2014). Cytotoxicity of paclitaxel in breast cancer is due to chromosome missegregation on multipolar spindles. *Sci Transl Med, 6*(229), 229ra243. doi:10.1126/scitranslmed.3007965

Zhang, Q., & Lu, Q.-B. (2021). New combination chemotherapy of cisplatin with an electron-donating compound for treatment of multiple cancers. *Scientific Reports, 11*(1), 788. doi:10.1038/s41598-020-80876-z

Zhou, J., Chen, Q., Zou, Y., Chen, H., Qi, L., & Chen, Y. (2019). Stem Cells and Cellular Origins of Breast Cancer: Updates in the Rationale, Controversies, and Therapeutic Implications. *Frontiers in Oncology, 9*, 820-820. doi:10.3389/fonc.2019.00820

Zhou, L., Jiang, Y., Yan, T., Di, G., Shen, Z., Shao, Z., & Lu, J. (2010). The prognostic role of cancer stem cells in breast cancer: a meta-analysis of published literatures. *Breast Cancer Res Treat, 122*(3), 795-801. doi:10.1007/s10549-010-0999-4

Zulkipli, A. F., Islam, T., Mohd Taib, N. A., Dahlui, M., Bhoo-Pathy, N., Al-Sadat, N., . . . My, B. C. C. S. G. (2018). Use of Complementary and Alternative Medicine Among Newly Diagnosed Breast Cancer Patients in Malaysia: An Early Report From the MyBCC Study. *Integrative cancer therapies, 17*(2), 312-321. doi:10.1177/1534735417745248

Zulkipli, I. N., Rajabalaya, R., Idris, A., Sulaiman, N. A., & David, S. R. (2017). Clinacanthus nutans: a review on ethnomedicinal uses, chemical constituents and pharmacological properties. *Pharmaceutical biology, 55*(1), 1093-1113. doi:10.1080/13880209.2017.1288749