

**THE SCREENING OF *Alpinia purpurata* ETHANOL EXTRACT FOR  
SELECTIVE ANTICANCER ACTIVITY IN VITRO**

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**by**

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**Dissertation submitted in partial fulfillment of the requirements for the  
degree of Bachelor of Health Science (Honours) (Biomedicine)**

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## DECLARATION

I hereby declare that this dissertation is the result of my own investigations, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at Universiti Sains Malaysia or other institutions. I grant Universiti Sains Malaysia the right to use the dissertation for teaching, research and promotional purposes.

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Nuraniessa Juliana binti Saidin

Date: .....

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## LIST OF SYMBOLS AND ABBREVIATIONS

ATCC	American Tissue Culture Collection
WHO	The World Health Organization
IARC	The International Agency for Research on Cancer
<i>A. purpurata</i>	<i>Alpinia purpurata</i>
$IC_{50}$	Half-maximal Inhibitory concentration
MTT	3-(4,5-dimethylthylthiazol-2-yl)-2,5- diphenyltetrazolium bromide
mg/mL	Milligram/ milliliter
HPV	Human Papillomavirus
HeLa	Human cervical cancer cell lines
MCF-7	Human breast cancer cell lines
DBTRG	Human brain cancer cell lines
Vero	Normal monkey kidney cell lines
WLR-68	Normal human liver cell lines
DMSO	Dimethyl sulfoxide
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum

PBS	Phosphate buffered saline
%	Percentage
°C	Degree Celsius
µg/mL	Microgram/ milliliter
g	Gravitational force
RPMI	Medium 1640 (Roswell Park Memorial Institute)
H <sub>2</sub> SO <sub>4</sub>	Sulfuric Acid
Na <sub>2</sub> CO <sub>3</sub>	Sodium Carbonate
NaOH	Sodium Hydroxide
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HCL	Hydrochloric acid
CO <sub>2</sub>	Carbon dioxide
FeCL <sub>3</sub>	Ferric chloride
µL	Microliter
nm	Nanometer
cm <sup>2</sup>	Square centimeter
mL	Milliliter
OD	Optical density

## ABSTRAK

Kanser, termasuk kanser payudara, serviks, dan otak, kekal sebagai masalah kesihatan utama di seluruh dunia memerlukan rawatan yang berkesan. Kajian ini menyiasat kesan sitotoksik ekstrak etanol *Alpinia purpurata* dan tamoksifen terhadap sel HeLa, MCF-7, DTBRG, WLR-68 dan Vero menggunakan ujian MTT dan ujian fitokimia untuk menilai potensi mereka sebagai agen antikanser. Keputusan menunjukkan bahawa ekstrak etanol *A. purpurata* menunjukkan sitotoksiti yang ketara, dengan nilai IC<sub>50</sub> sebanyak 18.19±1.5 µg/mL untuk HeLa, 158.70±1.1 µg/mL untuk MCF-7, 68.47±1.7 µg/mL untuk DTBRG, 3.31±2.5 µg/mL untuk sel Vero, dan nilai IC<sub>50</sub> yang tinggi terhadap sel WLR-68±2.2 µg/mL. Tamoksifen, sebuah ubat antikanser yang telah terbukti berkesan, menunjukkan kesan sitotoksik yang lebih kuat, dengan nilai IC<sub>50</sub> sebanyak 0.13±0.5 µg/mL untuk sel HeLa dan 1.61±0.7 µg/mL untuk sel MCF-7. Namun, tamoksifen menunjukkan toksisiti yang lebih tinggi kepada sel-sel normal. Kesimpulannya, ekstrak etanol *Alpinia purpurata* menunjukkan aktiviti antikanser yang menjanjikan ketoksikan selektif terhadap sel kanser, menawarkan potensinya untuk digunakan sebagai alternatif yang lebih selamat daripada tamoksifen.

## ABSTRACT

Cancer, including breast, cervical, and brain cancers, remains a major health challenge globally, requiring effective treatments. This study investigates the cytotoxic effects of *Alpinia purpurata* ethanol extract and tamoxifen on HeLa, MCF-7, DTBRG, and Vero cells using the MTT assay and qualitative phytochemical tests to evaluate their potential as anticancer agents. The results revealed that the *A. purpurata* ethanol extract exhibited significant cytotoxicity, with IC<sub>50</sub> values of 18.19±1.5 µg/mL for HeLa, 158.70±1.1 µg/mL for MCF-7, 68.47±1.7 µg/mL for DTBRG, and 3.31±2.5 µg/mL for Vero cells. Tamoxifen, a well-established anticancer drug, demonstrated stronger cytotoxic effects, with IC<sub>50</sub> values of 0.13±0.5 µg/mL for HeLa and 1.61±0.7 µg/mL for MCF-7 cells. However, tamoxifen exhibited higher toxicity to normal cells. In conclusion, *Alpinia purpurata* ethanol extract shows promising anticancer activity with selective toxicity towards cancerous cells, suggesting its potential for development as a safer alternative to tamoxifen.

# CHAPTER 1

## 1.0 INTRODUCTION

### 1.1 Background of study

In the mid-20th century, it had become important to understand that cancer cells developed from normal cells that undergone a sequence of driver mutations, or "hits," leading to their successful invasion of normal tissue, uncontrolled proliferation, and failure to differentiate. Cancer was described as "a disease of the genes" (Brown *et al.*, 2023). Moreover, cancer as a disease kills 10 million people worldwide each year, demonstrating how prevalent and lethal it remains. Additionally, cancer has consumed over a trillion dollars per year for treatment (Chen *et al.*, 2023). In 2022, Malaysia's cancer incidence rate was 46.1% per 100,000 persons (Dee *et al.*, 2023).

Current treatment for cancer includes surgery. Cancer surgery's principles and objectives include the complete removal of the tumour, an assessment of the tumour stage, and, in some situations, surgical removal of adjacent lymph nodes (Sen *et al.*, 2024). However, infection is the most common complication in surgical treatments, affecting 10% of patients and up to 20% with multiple infections (Wan *et al.*, 2021). Moreover, this treatment may lead to excessive intraoperative blood loss, requiring perioperative allogeneic blood transfusion (Nakanishi *et al.*, 2019). Radiation therapy, also referred to as radiotherapy, is also an important component of cancer treatment. It inhibits the growth and division of cancer cells by destroying or damaging them with high-energy radiation (Sen *et al.*, 2024). Common side effects of radiotherapy may include difficulty swallowing, sore throat, cough, weakness, and loss of appetite, as well as skin reactions (Temiz *et al.*, 2024). Patients who undergo radiotherapy for nasopharyngeal cancer are more likely to

develop chronic sinusitis (Brook, 2020). Another current cancer treatment is chemotherapy, which uses drugs to kill or inhibit the growth of cancer cells. It can be used as a primary treatment or in combination with other treatments, such as radiation therapy and surgery. Despite its efficacy, chemotherapy causes several adverse effects, including drug resistance, besides common side effects such as nausea, vomiting, hair loss, fatigue, anemia, and immunosuppression, as well as infertility, cardiotoxicity, and cognitive impairment (Sen *et al.*, 2024). To overcome the limitations, this study focuses on exploring alternative treatment for cancer by using plant extract.

Natural products, particularly those derived from medicinal plants, are gaining popularity for their therapeutic potential. Additionally, their low toxicity to normal cells and enhanced tolerability have gained attention among scientists and clinicians in the modern drug discovery area (Muhammad *et al.*, 2022). Flavonoids, alkaloids, terpenoids, saponins, and other secondary metabolites derived from plants have been reported as significant sources of potent anticancer agents (Talib *et al.*, 2020). A study has reported that grapes, soybean, green tea, garlic, olive, and pomegranate are the most effective plants against colon cancer (Aiello *et al.*, 2019).

This study used *Alpinia purpurata* ethanol extract on several cell lines; breast cancer cell line, MCF-7, cervical cancer cell line, HeLa, brain cancer cell line, DBTRG, normal liver cell line, WLR-68 and normal kidney cell line, Vero. Moreover, qualitative phytochemical test also utilized to investigate the presence of secondary metabolite in the plant extract, which is responsible for therapeutic effects. The study intends to investigate the extract's cytotoxic effects on selective cancer cell lines and normal cell lines, as well as analyses its phytochemical constituents for anticancer activity. Thus, understanding how these

substances interact with cancer cells may lead to innovative treatment strategies that enhance therapeutic efficacy while minimizing negative effects.

## **1.2 Rationale of study**

Many cancer patients seek alternative treatments (Regassa *et al.*, 2022). It is due to side effects such as nausea, vomiting, hair loss, fatigue, anemia, and immunosuppression caused by current cancer treatment (Sen *et al.*, 2024). *Alpinia purpurata* phytochemicals, such as flavonoids and alkaloids compounds, have been revealed to exhibit various biological actions, including anti-inflammatory, antioxidant, and anti-cancer properties (Lintao *et al.*, 2021). Hence, *Alpinia purpurata* ethanol extract could be a potential alternative treatment for cancer. Thus, this study is conducted to evaluate the anticancer property of *Alpinia purpurata* ethanol extract on several types of cancer cell lines for potential alternative treatment for cancer.

### **1.3 Research objectives**

#### **1.3.1 General objective**

1. To screen the selective anticancer activity of *Alpinia purpurata* ethanol extract on several types of human cell lines.

#### **1.3.2 Specific objectives**

1. To evaluate the cytotoxic activity of *Alpinia purpurata* ethanol extract on several types of cancer cell lines, breast cancer cell line, MCF-7, cervical cancer cell line, HeLa, and brain cancer cell line, DBTRG.
2. To elucidate the cytotoxic activity of *Alpinia purpurata* ethanol extract towards non-malignant cell lines; normal liver cell line, WLR-68 and normal kidney cell line, Vero.
3. To determine the phytochemical constituents of the *Alpinia purpurata* ethanol extract responsible for anticancer activity.

## CHAPTER 2

### 2.0 LITERATURE REVIEW

#### 2.1 Cancer

Cancer is a major societal, public health, and economic issue in the 21st century, responsible for about one in six (16.8%) and one in four (22.8%) deaths from noncommunicable diseases (NCDs) worldwide. In 2022, lung cancer was the most frequently diagnosed cancer, responsible for about 2.5 million new cases, or one in eight cancers worldwide (12.4% of all cancers), followed by female breast cancer (11.6%), colorectum cancer (9.6%), prostate cancer (7.3%), and stomach cancer (4.9%). Lung cancer was the main cause of cancer death, responsible for an estimated 1.8 million deaths (18.7%), followed by colorectal (9.3%), liver (7.8%), female breast (6.9%), and stomach (6.8%) (Bray *et al.*, 2024).

Breast cancer has now surpassed lung cancer as the most commonly diagnosed cancer worldwide (Figure 2.0), which responsible for one in all six cancer deaths among women, according to statistics released by the International Agency for Research on Cancer (IARC) in December 2020 (Centers for Disease Control and Prevention, 2024). According to the Malaysian National Cancer Registry (MNCR), the most prevalent five cancers among men and women are breast, colorectal, trachea, bronchus, lung, lymphoma, and nasopharynx cancer (Azizah *et al.*, 2019).

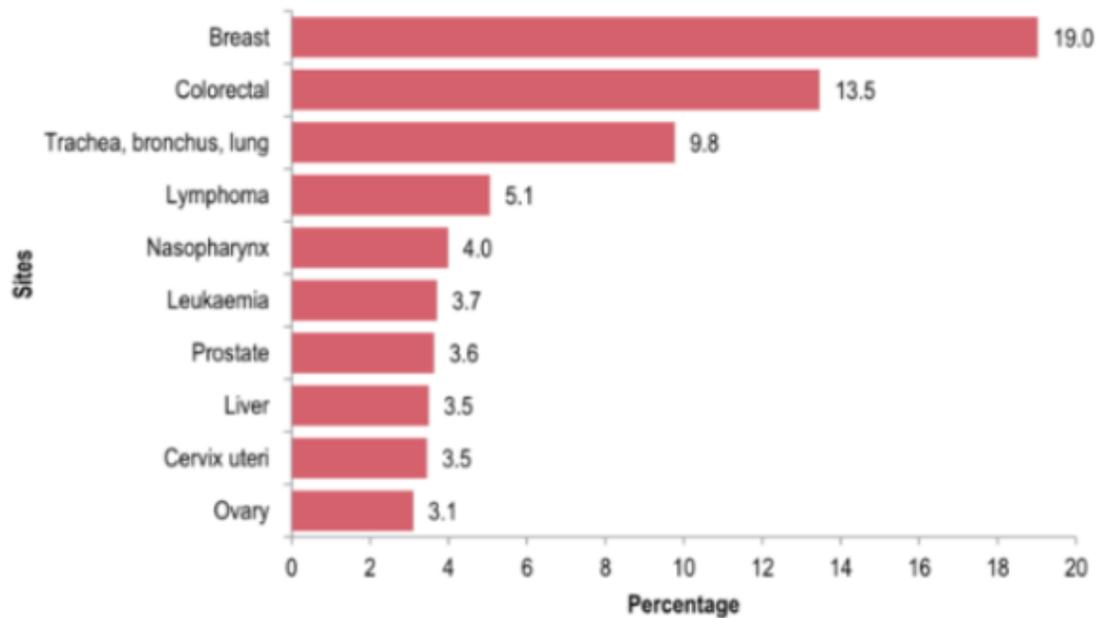


Figure 2.1: Top 10 cancers in worldwide (IARC, 2020)

## 2.2 Breast cancer

According to the GLOBOCAN 2020 data, breast cancer currently ranks as one of the most prevalent cancers diagnosed and the 5th major cause of cancer-related deaths, with an estimated 2.3 million new cases worldwide (Sung *et al.*, 2021). Besides being the most prevalent, breast cancer is also the leading cause of cancer death in women worldwide. Breast cancer caused 684,996 deaths worldwide, with an age-adjusted rate of 13.6/100,000. Although developed countries have the highest incidence rates, Asia and Africa were responsible for 63% of the total death rate in 2020 (Ferlay *et al.*, 2020). Moreover, women in high-income countries are more likely to survive breast cancer compared to those in low- and middle-income countries (Ginsburg *et al.*, 2016).

In Figure 2.2, it is reported that Malaysia has 66.8% of 5-years relative survival rate of breast cancer, which located Malaysia at 11th place compared to other countries worldwide (Beaumont, 2022). In 2018, Malaysia SCAN reported 5-year relative survival rates of 87.5%, 80.7%, 59.7%, and 23.3% for Stages I, II, III, and IV breast cancer, respectively. The variances are most likely due to inconsistencies caused by time differences in treatment types and staging, as not all patients in government hospitals have access to CT scans. Furthermore, relative survival is not equivalent to total survival (The Malaysian Reserve, 2022). Another study indicated a five-year survival rate was 60.5%, which is notably lower than many developed countries (Kadir *et al.*, 2022).

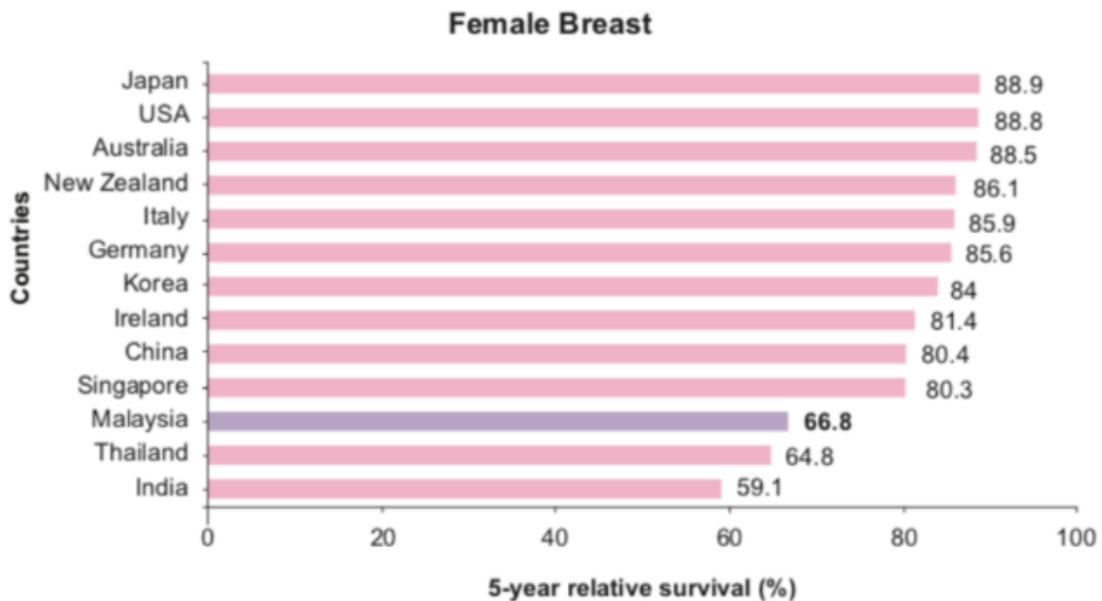


Figure 2.2: International comparison of 5-years relative survival of breast cancer (Beaumont, 2022)

### **2.3 Cervical cancer**

Cervical cancer is a major global health concern, ranking fourth among women globally. According to the Global Cancer Observatory (GCO), cervical cancer is the fourth most prevalent cancer affecting women in Malaysia, with 1,740 cases reported in 2020, 991 or 57% of fatality. The GCO estimates that more than 12 million Malaysian women aged 15 and above are at risk of acquiring cervical cancer (International Agency for Research on Cancer, 2020). According to the HPV Information Centre, it is the second most common malignancy in Malaysian women aged 15 to 44. It is also the fourth most frequent cancer in women globally, according to the World Health Organisation (WHO), with an expected 604,000 new cases identified in 2020. It is largely caused by high-risk human papillomavirus (HPV) infections that spread through sexual contact (HPV Information Centre, 2023).

According to Centers for Disease Control and Prevention (2024), the major cause of cervical cancer is chronic infection with high-risk HPV strains, notably types 16 and 18, which account for about 88.7% of all invasive cervical malignancies in the country. Despite the Malaysian government's initiatives to supply HPV vaccines, only 35.2% of women have undergone a Pap smear test in recent years, showing a large gap in preventative treatment. Moreover, women with impaired immune systems, such as those living with HIV, are more likely to acquire cervical cancer due to a lack of ability to clear out HPV infections. In addition, tobacco use has been linked to an increased risk of cervical cancer, as it can decrease the immunological response to HPV infections (Centers for Disease Control and Prevention, 2024).

## 2.4 Brain cancer

As of 2021, brain cancer is responsible for around 2% of all cancer cases globally, while it causes approximately 2.5% of cancer-related deaths. Although brain cancer can occur at any age, it is more prevalent among older individuals, with age being a significant risk factor. The survival rate for brain cancer varies significantly depending on the type of tumor, grade, and stage at diagnosis. Tumors within the brain can be difficult to treat due to their location, leading to lower survival rates compared to other types of cancer. Certain risk factors may increase the likelihood of developing a brain tumor such as exposure to ionizing radiation, certain genetic disorders, and a family history of brain cancer. Additionally, brain cancer includes various tumor types such as gliomas, meningiomas, pituitary adenomas, and medulloblastomas, each with unique characteristics and treatment strategies (Barkade *et al.*, 2023).

Brain cancer in Malaysia presents a notable health concern, with varying incidence rates and types of tumours reported across different regions. The overall incidence of brain tumours in Malaysia is estimated to be between 1.26 and 4.98 per 100,000 people per year, depending on the location analyzed. For example, research in Sibul found an incidence of 4.98 per 100,000 in Sarawak population (Heng *et al.*, 2023). While Kelantan and Terengganu had lower incidence rates of 0.44 per 100,000 of the state's population. Meningioma is the most prominent type of brain tumour, accounting for around 32% of all cases, followed by gliomas and metastatic tumours (Table 2.1). Brain tumours are more commonly diagnosed in middle-aged people, particularly those aged 51 to 60 years. There is also significant female predominance in some types of brain tumours, such as meningiomas, which have a female-to-male ratio of approximately 3:1 (Azmi, 2020).