

**IN VITRO SCREENING OF *Piper Sarmentosum*
ETHANOL EXTRACT FOR SELECTIVE
ANTICANCER ACTIVITY**

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ANTICANCER ACTIVITY**

by

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For any errors or inadequacies that may remain in this work, of course, the responsibility is entirely my own.

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LIST OF SYMBOLS, ABBREVIATION AND ACRONYMN

| | |
|---------------------------------|---|
| % | Percentage |
| °C | degree Celsius |
| µg/mL | Microgram/ millilitre |
| µL | Microliter |
| A549 | Human lung cancer cell lines |
| ATCC | American Tissue Culture Collection |
| cm ² | Square centimetre |
| CO ₂ | Carbon dioxide |
| DBTRG | Human brain cancer cell lines, Glioma |
| DMSO | Dimethyl sulfoxide |
| EC ₅₀ | Half maximal effective concentration |
| EDTA | Ethylenediaminetetraacetic acid |
| FBS | Fetal bovine serum |
| FeCl ₃ | Ferric chloride |
| GC-MS | Gas Chromatography and Mass Spectroscopy |
| H ₂ O ₂ | Hydrogen peroxide |
| H ₂ SO ₄ | Sulfuric Acid |
| H ₂ SO ₄ | Sulfuric acid |
| HCL | Hydrochloric acid |
| HeLa | Human cervical cancer cell lines |
| HPLC | High Performance Liquid Chromatography |
| HPV | Human Papillomavirus |
| HT-29 | Human colorectal cancer cell lines |
| IARC | The International Agency for Research on Cancer |
| IC ₅₀ | Half-maximal Inhibitory concentration |
| MCF 7 | Human breast cancer cell lines |
| mg/mL | milligram/ millilitre |
| mL | Millilitre |
| MTT | 3-(4,5-dimethylthylthiazol-2-yl)-2,5- diphenyltetrazolium bromide |
| Na ₂ CO ₃ | Sodium Carbonate |
| NaOH | Sodium Hydroxide |

| | |
|-----------------------|---|
| NCI | Dictionary of Cancer terms |
| nm | Nanometre |
| OD | Optical density |
| <i>P. sarmentosum</i> | <i>Piper sarmentosum</i> |
| PBS | Phosphate buffered saline |
| PSAE | <i>P. sarmentosum</i> aqueous extract |
| rpm | Revolutions per minute |
| RPMI | Medium 1640 (Roswell Park Memorial Institute) |
| TPC | The total phenolic content |
| Vero | Normal monkey kidney epithelial cell lines |
| WHO | The World Health Organization |
| WLR 68 | Normal human cervical cell lines |

IN VITRO SCREENING OF *Piper Sarmentosum* ETHANOL EXTRACT FOR SELECTIVE ANTICANCER ACTIVITY

ABSTRACT

Cancer is a serious global health issue. This study investigates on the specific anticancer efficacy of *Piper sarmentosum* ethanol extract, a medicinal plant used in Southeast Asia. The extract's cytotoxic effects were evaluated in vitro on three malignant cell lines: HeLa (human cervical cancer), MCF-7 (human breast cancer), and Glioma (human brain cancer). The study also examined its effects on two non-malignant cell lines, Vero (normal kidney epithelial cells) and WLR-68 (normal human liver cells). To assess cell viability, IC₅₀ values were established using the MTT test. The extract demonstrated significant anticancer action, with IC₅₀ values showing greater selectivity for malignant cells than non-malignant ones. Phytochemical research confirmed the existence of bioactive substances with medicinal potential, including flavonoids, terpenoids, and alkaloids. The ethanol was used as extraction solvent due to its many advantages such as less toxic and have high polarity. The results demonstrated that *P. sarmentosum* extract has lower IC₅₀ (2.20±1.10 µg/mL) for DBTRG cell line than Hela (44.18±15.60 µg/mL) and MCF-7 (29.95±1.20 µg/mL) cell lines. Comparing to the cytotoxicity effect of the extract towards non-malignant cell lines, the extract showed highest cytotoxic towards Vero cell line with IC₅₀ value of (0.44±1.00 µg / mL) but no IC₅₀ value detected on WLR-68 cell line. The saponins, alkaloids, flavonoids, terpenoids were presented but tannis was absent. The extract demonstrated substantial anticancer action, with IC₅₀ values showing greater selectivity for malignant cells than non-malignant ones These findings demonstrate the potential of *P. sarmentosum* ethanol extract as an alternative and complementary

cancer treatment, underlining its promising position in natural product-based therapies. Future research is recommended to better understand the mechanisms underlying its anticancer efficacy and to assess its therapeutic application.

SARINGAN DALAM VITRO *Piper Sarmentosum* EKSTRAK ETANOL UNTUK TERPILIH AKTIVITI ANTIKANSER

ABSTRAK

Kanser adalah isu kesihatan global yang serius. Kajian ini menyiasat tentang keberkesanan antikanser khusus ekstrak etanol daripada daun *P. sarmentosum*, tumbuhan ubatan yang digunakan di Asia Tenggara. Kesan sitotoksik ekstrak telah dinilai secara in vitro pada tiga jenis kanser sel: HeLa (kanser serviks manusia), MCF-7 (kanser payudara manusia), dan DBTRG (kanser otak manusia). Kajian ini juga mengkaji kesannya pada dua garisan sel bukan kanser, Vero (sel epitelium buah pinggang normal) dan WLR-68 (sel hati manusia normal). Penyelidikan fitokimia mengesahkan kehadiran bahan bioaktif dengan potensi perubatan, termasuk flavonoid, terpenoid, dan alkaloid. Etanol digunakan sebagai pelarut ekstraksi kerana kelebihan seperti kurang toksik dan mempunyai kepolaran tinggi. Hasil menunjukkan bahawa ekstrak *P. sarmentosum* mempunyai nilai IC_{50} yang lebih rendah ($2.20 \pm 1.10 \mu\text{g/mL}$) untuk garis sel DBTRG berbanding HeLa ($44.18 \pm 15.60 \mu\text{g/mL}$) dan MCF-7 ($29.95 \pm 1.20 \mu\text{g/mL}$). Berbanding dengan kesan sitotoksik ekstrak terhadap garis sel bukan malignan, ekstrak menunjukkan sitotoksik tertinggi terhadap garis sel Vero dengan nilai IC_{50} ($0.44 \pm 1.00 \mu\text{g/mL}$) tetapi tiada nilai IC_{50} dikesan pada garis sel WLR 68. Saponin, alkaloid, flavonoid, dan terpenoid hadir tetapi tanin tidak ada. Untuk menilai daya maju sel, nilai IC_{50} telah dibaca dan dikaji menggunakan ujian MTT. Ekstrak menunjukkan nilai IC_{50} lebih rendah pada sel DBTRG dan sel Vero tetapi nilai IC_{50} tinggi pada sel WLR 68. Penyelidikan fitokimia mengesahkan kewujudan bahan bioaktif dengan potensi perubatan, termasuk flavonoid, terpenoid, dan alkaloid. Penemuan ini menunjukkan potensi ekstrak etanol *P. sarmentosum*

sebagai rawatan kanser alternatif dan pelengkap, menggariskan kedudukannya yang menjanjikan dalam terapi berasaskan produk semula jadi. Penyelidikan masa depan disyorkan untuk lebih memahami mekanisme yang mendasari keberkesanan antikansernya dan untuk menilai aplikasi terapeutiknya.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Uncontrolled proliferation and spread of aberrant cells within the body is a hallmark of the condition known as cancer. These cells may develop into tumours, which may then infiltrate surrounding organs and tissues. There are numerous varieties of cancer, each called after the body part in which it first appears.

Cancer is a serious global health issue and one of the main causes of death worldwide (Liu *et al.*, 2016). The World Health Organization (WHO) reported in 2019, 112 out of 183 nations, cancer is both the primary and secondary cause of death before the age of 70. In 2020, there were 48,639 new cases of cancer reported in Malaysia and expected to double by 2040 (Ministry of Health Malaysia, 2020). The International Agency for Research on Cancer (IARC) reported the number of new cases of cancer in 2022 was around 20 million (Sung *et al.*, 2021). Cancer is fourth most common cause death in Malaysia and breast cancer is top 5 among others cancer such as lung cancer, liver cancer, colon cancer, nasopharyngeal cancer (Malaysian Study on Cancer Survival My Scan, 2018).

Current cancer treatments, including as surgery, chemotherapy, radiotherapy, and targeted medicines, have greatly increased patient survival rates and outcomes. However, these treatments have some disadvantages. While chemotherapy and radiotherapy are efficient in targeting cancer cells, they frequently cause severe side effects such as nausea, exhaustion, hair loss, decreased immunity, and harm to healthy organs. Targeted therapies, while more precise, can occasionally result in resistance as cancer cells adapt, making treatments less effective over time. Furthermore, these medicines are frequently expensive and may not be available to all patients,

particularly those in low-income or resource-constrained settings. Because of these obstacles, many people are turning to natural resources as supplemental or alternative medicines. Natural compounds produced from plants, herbs, and marine organisms are increasingly being studied for their possible anticancer capabilities, as they are thought to be less toxic, and less expensive than traditional treatments. Furthermore, natural goods frequently contain bioactive substances with antioxidant, anti-inflammatory, and immune-boosting qualities, which might benefit overall health and possibly improve the efficacy of existing treatments. So, most people turn to alternative cancer treatment because natural products like plant-based products are less harmful to human beings (Sana *et al.*,2024).

Piper sarmentosum also known as Thai betel leaf or in Malay Daun Kaduk. The class of this leaf is Magnoliopsida, in order of Piperales and family of Piperaceae (Biotropics Malaysia Berhad.,2007). *Piper sarmentosum* Roxb. (Piperaceae) is a traditional medicinal plant found throughout Southeast China, including Fujian, Guangdong, and Guizhou, as well as India, Malaysia, and Thailand. Moreover, the species in the genus *Piper* are distributed across the tropical regions. They are mostly found in the understory of lowland tropical rainforests, although they can also be found in clearings and higher-altitude places like cloud forests. For millennia, people have used it to cure a variety of conditions, including wind-cold cough, fever, arthralgia, rheumatism, diarrhoea, dysentery, postpartum foot oedema, diabetes, toothache, and traumatic injuries (Sun *et al.*,2020). *P. sarmentosum* ethanol extract is morphologically similar to *P. longum* and *P. hapnium* but can easily be distinguished by its procumbent fruit-bearing branches with large, stout, sweet fruits on maturity (Mathew *et al.*, 2004).

However, *P. sarmentosum* ethanol extract anticancer properties are poorly defined. In this study, we examined the ethanol extract of *P. sarmentosum* for the presence of anticancer activity against selected cancer cell lines. Human cervical cancer cell lines (HeLa), human breast cancer cell lines (MCF7) and human brain cancer cell lines (Glioma)(DBTRG). The cytotoxic activity of the plant extract on selected normal cell lines, liver cell (WLR- 68) and human kidney epithelial cell (Vero). All cells are obtained from American Type of Culture Cell (ATCC).

Half maximal inhibitory concentration (IC₅₀) was measured by MTT assay. Qualitative phytochemical analysis of *P. sarmentosum* ethanol extract was flavonoid, saponins, tannis, terpenoids and alkaloids presence in this plant extract. These types of compounds have been suggested in previous research study at lapuyan zamboanga del sur, Philippines (Sakilan *et al.*, 2019). IC₅₀ values derived from cell-based assays help drive the medical chemistry efforts toward improved drug design. So that *P. sarmentosum* ethanol extract can be commercialized used as an anticancer drug in future. The IC₅₀ value is crucial in drug design because it measures a compound's ability to inhibit a target, such as an enzyme or a cancer cell, by 50%. A lower IC₅₀ value indicates that the compound is more powerful. This allows researchers to compare different chemicals, increase their efficacy by altering their structure, and assure their safety by testing their effects on healthy cells. It also aids in determining the appropriate dosage and assessing the compound's selectivity for its intended target, lowering the likelihood of adverse effects. Overall, IC₅₀ values aid in the development of safer and more effective medications.

1.2 Rationale of the study

This study is conducted to evaluate the anticancer property of *P. sarmentosum* ethanol extract on several cancer cell lines for potential alternative treatment. It is because nature products like plant-based products less harm to human being (Sana *et al.*,2024). Moreover, modern cancer therapy can have serious adverse effect. Fatigue, nausea, hair loss, reduced immune function, and long-term problems are among the potential adverse effects. This has motivated many individuals to pursue complementary or alternative treatments, including natural products, in order to potentially improve outcomes, eliminate side effects, or promote general well-being.

1.3 Research objectives

1.4 General objective

- To screen the anticancer activity of *P. sarmentosum* ethanol extract on several cancer cell lines.

Specific objectives

- To determine the cytotoxic activity of *P. sarmentosum* ethanol extract on selected cancer cell lines human breast cancer (MCF - 7), human cervical cancer (HeLa) and human brain cancer glioma (DBTRG) by using MTT assay.
- To elucidate the cytotoxic activity of *P. sarmentosum* ethanol extract on selected normal cell lines normal human cervical cell lines (WLR- 68) and normal monkey kidney epithelial cell lines (Vero) by using MTT assay.
- To evaluate phytochemical constituents *P. sarmentosum* ethanol extract which suggested to responsible for anticancer activity by using phytochemical test.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer, a complex and multidimensional disease, is still one of the top causes of morbidity and mortality globally. It is characterized by unregulated cell development and affects practically every organ and tissue in the human body. The global burden of cancer is significant, with the World Health Organization (WHO) estimating over 19.3 million new cases and approximately 10 million deaths in 2020 alone (Sung *et al.*, 2021). The prevalence and incidence vary widely by geographic region, influenced by factors such as lifestyle, genetic predisposition, and healthcare infrastructure. Notably, lifestyle factors such as tobacco use, unhealthy diets, physical inactivity, and alcohol consumption account for nearly one-third of all cancer deaths (Bray *et al.*, 2018).

2.2 Breast Cancer

In 2016, women over 40 accounted for the majority of breast cancer deaths in the United States, with a significant number among those over 60. The chance of developing breast cancer can be increased by a number of factors, including sex, aging, oestrogen, family history, gene mutations, and an unhealthy lifestyle. The majority of incidences of breast cancer occur in women, who also account for 100 times more cases than males do (Sun *et al.*, 2017). A woman's risk is increased if there is a family history of breast cancer, such as a mother or sister who has the disease. The risk of breast cancer is increased by 1.75 times if one close family has the disease, according to a UK study involving over 113,000 women. In addition to contemporary habits like drinking alcohol and overindulging in fat foods, factors such as early menstruation, a

late menopause, having children later in life, or fewer children altogether can raise the risk of breast cancer. The most frequent cancer among Malaysians is breast cancer (Azizah *et al.*,2019). A total of 21,634 cases of female breast cancer were diagnosed for the period of 2012-2016 compared with 18,206 cases in 2007-2011 report (Figure 2.1.0). In Malaysia, 1 in 9 women are at risk of developing breast cancer (Lee *et al.*, 2019).

2.3 Cervical Cancer

Worldwide, cervical cancer is still a serious problem, particularly in developing and disadvantaged nations. It seriously affects the sexual, physical, and social wellbeing of women. Cervical cancer is the fourth most frequent cancer worldwide and the fourth major cause of cancer-related deaths among women. In 2020, there were predicted to be 604,127 new cases and 314,831 deaths due to cervical cancer. Ninety percent of cervical cancer deaths occur in low- and middle-income countries (LMICs). With an age-adjusted prevalence of 6.2 per 100,000 women, cervical cancer was the third most frequent cancer among women in Malaysia between 2012 and 2016, accounting for 3,981 new cases. In Malaysia, cervical cancer is the third most common cancer among females and ranked ninth in Malaysia among other cancers (Azizah *et al.*, 2019). The number of new cases and deaths is estimated to increase by 64 per cent and 87 per cent in 2040, respectively, if no immediate action is taken (Zhao *et al.*, 2022).

The narrow, lower end of the uterus that joins the vagina to the uterus is called the cervix. The internal OS, which is the opening between the cervix and the upper part of the uterus, the external OS, which is the opening between the cervix and vagina, the ectocervix, which is the outer part of the cervix that opens into the vagina, and the

endocervix, which is the inner part of the cervix that forms the endocervical canal, make up this structure. The region that unites the endocervix and ectocervix is known as the squamocolumnar junction. It is comprised of squamous cells from the ectocervix and glandular cells (column-shaped mucus-producing cells) from the endocervix. The squamocolumnar junction is sometimes referred to as the transformation zone and this part is the most cervical cancer start to build (Figure 2.1.1) (NCI Dictionary of Cancer terms, 2021). In addition, HPV is a common virus that passed from one to another during sex. At least half of sexually active people will have HPV at some point in their lives, but few women will get cervical cancer. Over time, an HPV infection with oncogenic HPV types can persist and transform normal cells into abnormal cells, ultimately leading to cancer, if the immune system is unable to eradicate the virus. So, persistent high-risk genital HPV infection accounts for approximately 99.7% of cases of cervical cancer. With 528,000 new instances of cervical cancer recorded in 2012, it is one of the most common cancers in women worldwide (Okunade, 2020).

2.4 Glioma/Brain Cancer

According to World Health Organization's GLOBOCAN 2012 database, brain tumours account for roughly 2% of all malignancies in Malaysia. It was listed as the eleventh and thirteenth most prevalent cancers in men and women, respectively (Heng *et al.*, 2023). Meningiomas, gliomas (especially glioblastoma), and intracranial metastases from systemic malignancies are the most common types of brain tumours. The most prevalent and deadly primary malignant brain tumour is glioblastoma (McFaline-Figueroa *et al.*, 2018). The frontal lobe and multiple lobes (overlapping tumors) have the highest incidence of glioblastoma, which is most frequently found in the supratentorial region (frontal, temporal, parietal, and occipital lobes). The temporal

and parietal lobes are the next most prevalent locations for glioblastomas (Tamimi, 2017) (Figure 2.4) ((Glioblastoma multiforme (GBM)). Gliomas have an annual incidence rate of 6.6 per 100,000 people worldwide, with a higher prevalence among adults aged 45 to 65 years (Ostrom *et al.*, 2021). Glioblastoma, the most malignant kind, accounts for almost 60% of all gliomas. Glioma risk factors include advanced age, male sex, ionizing radiation exposure, and certain genetic predispositions, such as mutations in isocitrate dehydrogenase (IDH) genes (Ohgaki & Kleihues, 2005).

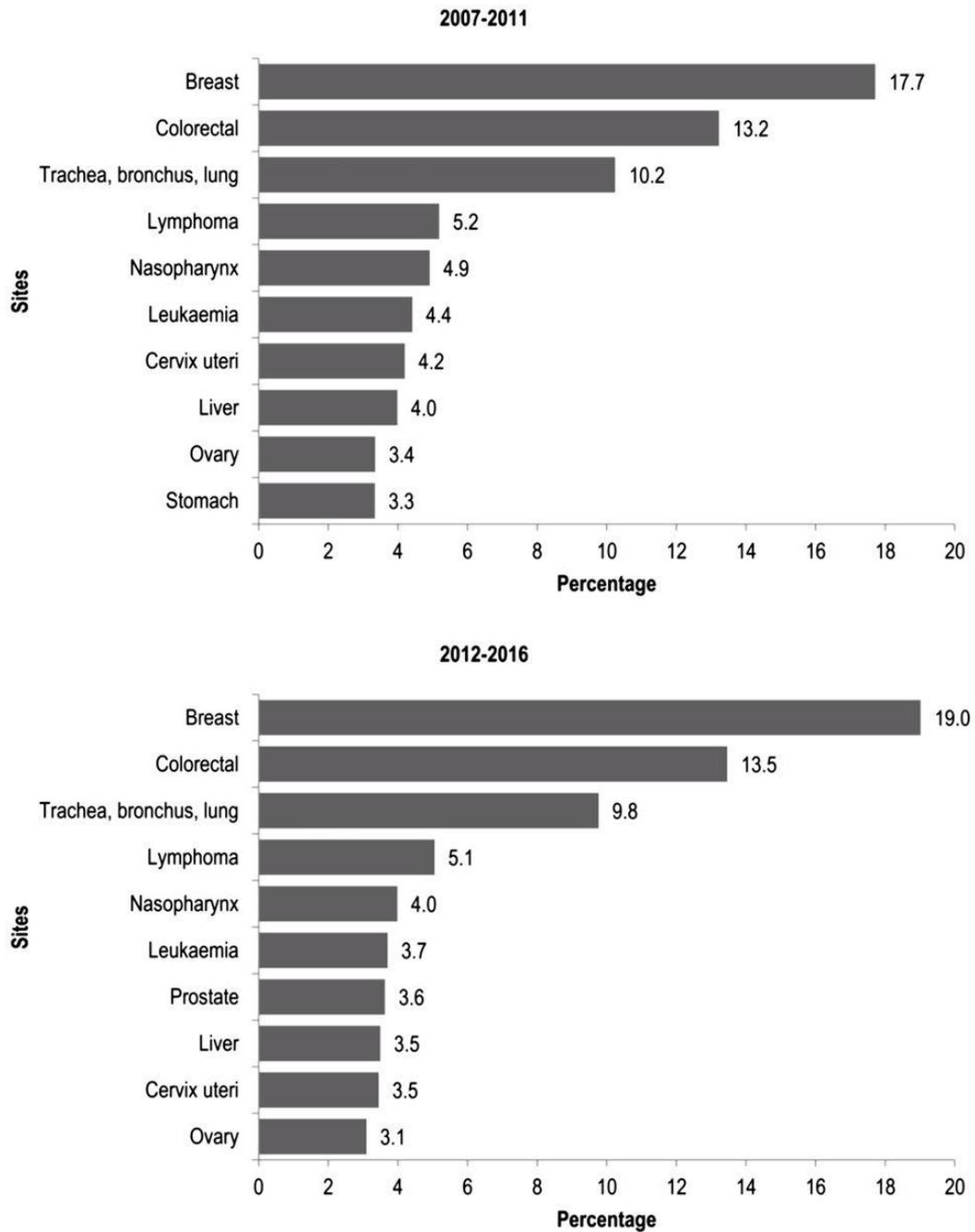


Figure 2.1 The comparison of ten most common cancers (Azizah *et al.*,2019)

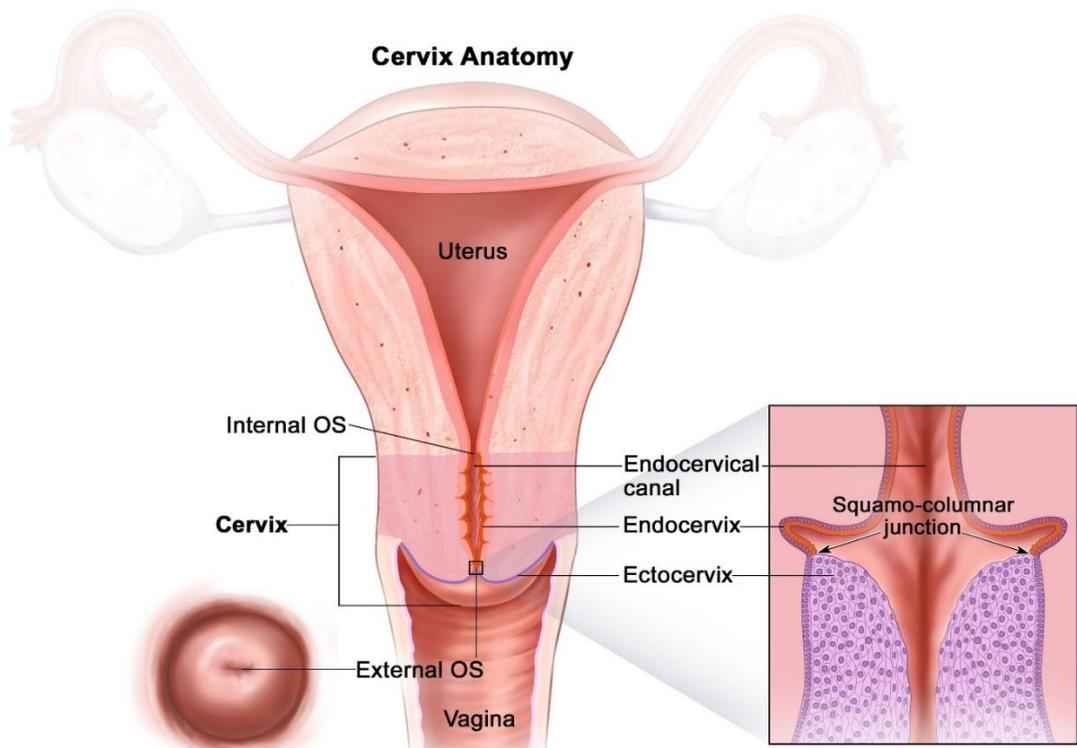


Figure 2.2 The female reproduction system (NCI Dictionary of Cancer terms, 2021)

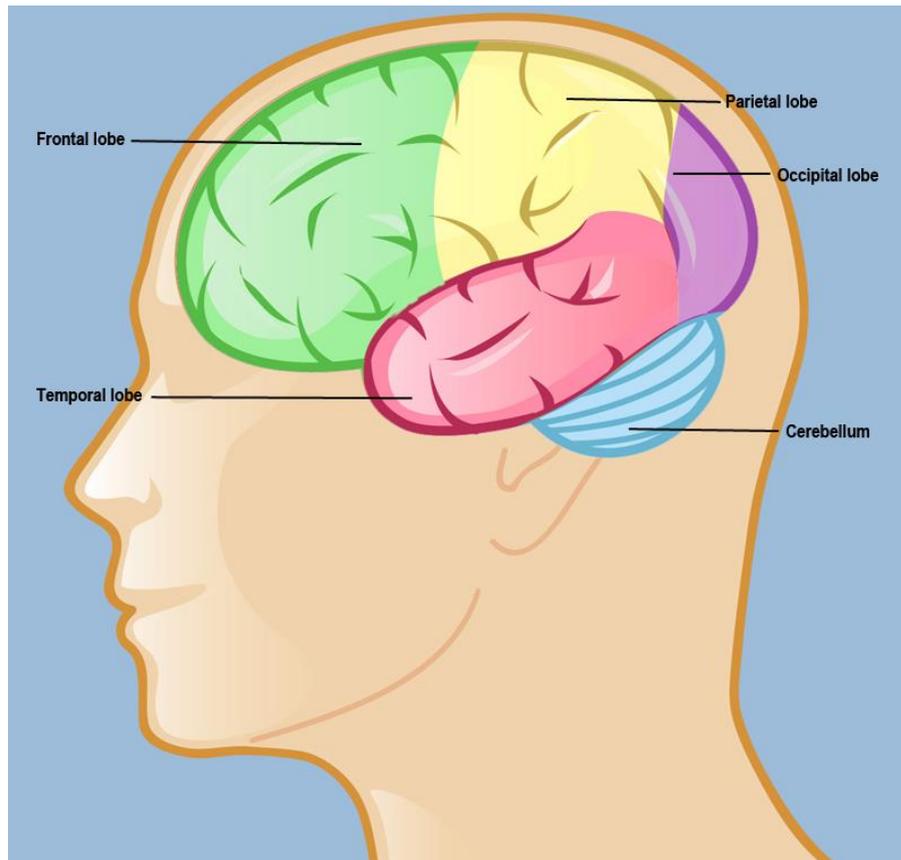


Figure 2.3 The location of glioblastoma ((Glioblastoma multiforme (GBM)) (McFaline-Figueroa *et al.*, 2018)

2.5 Cancer Treatment

The word "chemotherapy" was coined by German chemist Paul Ehrlich, who studied the use of medicines to cure infectious disorders. He was also the first scientist to use animal models to test the efficacy of various drugs against diseases (Amjad *et al.*, 2020). Chemotherapy is the primary treatment for many malignancies (Tilsed *et al.*, 2022). Chemotherapy used drugs to kill cancer cell and slow their growth in human body. Hair loss, nausea, and weariness were the most commonly reported side effects. Tiredness might impede daily tasks on a consistent basis (Love *et al.*, 1989). The term "mucositis" was first used in late 1980 to characterize chemotherapy-induced inflammation of the oral mucosa. Oral mucositis is regarded as the most severe non-haematological consequence of cancer therapy (Chaveli-López *et al.*, 2016).

Gliomas are brain tumours that develop from glial cells, with an annual frequency of 4 to 11 per 100,000 people. Low-grade gliomas are often treated with surgery and subsequent radiotherapy. However, there are concerns regarding the potential long-term effects of radiation, particularly on neurocognitive functioning (Lawrie *et al.*, 2019). Radiotherapy can have long-term consequences on the brain, including as memory issues, slower thinking, and difficulty focusing and making decisions. These problems occur because radiation can harm brain tissue, particularly white matter, which allows different areas of the brain to communicate. Younger patients, such as children, are more vulnerable since their brains are still developing, posing obstacles in learning and daily life. Radiation can also cause emotional changes, such as depression or anxiety (Lavergne *et al.*, 2015), as well as disrupt hormone levels and raise the risk of stroke. However, modern radiation techniques and treatments are helping to alleviate these side effects.

Cancer surgery is the procedure in which a surgeon removes cancer cells from the body. This treatment can give high risk of infection, have surgery leaves scars and can cause painful to patients. Persistent pain in the surgical area following breast cancer surgical treatment affects roughly 25% to 50% of individuals (Gärtner *et al.*, 2009). Due to current treatment which have negative impact on patients' quality of life (Lewandowska *et al.*, 2020). For example, current treatment can cause hair loss, fatigue and nausea and it cost also expensive. So, most people turn to natural product because of low cost, easy to get the sources and less toxic. Natural products have long been seen as a promising source of treatment for cancer, which is expected to become one of the leading causes of mortality this century. However, there is still a need for the creation of new anticancer medications, drug combinations, and chemotherapy

techniques through methodical and scientific research of a vast pool of synthetic, biological, and natural products (Mukherjee *et al.*, 2001).

2.6 Medicinal plants

Throughout ancient times, people have utilized medicinal plants as healers and cures. We have a rich legacy of undocumented materials because knowledge of their medical benefits was mostly transmitted orally from generation to generation. Over 35,000 different plant species are utilized medicinally in different human societies worldwide (Lewington, 1993). Furthermore, Malaysia is the fourth most biodiversity-rich country in the world, behind China, India, and Indonesia, out of the 12 countries in the globe. Additionally, the Malaysian rain forest contains possible "Viagra" substitutes, such as "Tongkat Ali" (*Eurycoma longifolia*) and an extract that treats impotence by combining "nadas pedas" (*Foeniculum vulgare*), "mempelas hari" (*Alyxia reinwardtii*), black pepper seeds, and turmeric. Table 2.2 shows a list plant including vegetables as a medicinal remedy for many common diseases.

Many trees and plants are related to species that are already known for their medicinal properties, and with research and testing, we may discover their health benefits too. Using modern techniques to identify bio-active compounds will speed up this process and help uncover their potential uses in medicine. One of the main sources of pharmaceuticals for treating human ailments is obtained from plant compounds, along with their semi-synthetic and synthetic analogs. Considering this, the World Health Organization (WHO) has calculated that roughly 80% of people on the planet receive traditional medical care. One of these therapies is phototherapy, sometimes referred to as phytomedicine, which is the application of plant extracts or a combination of plant extracts to the treatment of illness. Utilizing medicinal plants can

help the body regain its capacity to defend, regulate, and heal itself, thereby enhancing mental, emotional, and physical health (Aiello *et al.*, 2019).

The WHO has recognized the use of medicinal plants as a complementary treatment for cancer since 1978. For example, the active component of the violet plant, known scientifically as *Viola tricolor*, is ethyl acetate, which has a potent inhibitory impact on the growth of cervical cancer when incorporated into an aqueous extract of the plant. Numerous chemicals in this plant have strong cell-cytotoxic properties. Studies have revealed that flavonoids can have anticancer effects (Kooti *et al.*, 2017)

Table 2.1 The list of some Malaysian plants yielding clinically useful drugs (Latiff.A,1991)

| Species | Local name | Drug | Clinical use |
|-----------------------------|----------------|--------------|-------------------|
| <i>Ananas comosus</i> | nenas | bromelain | proteolytic agent |
| <i>Areca catechu</i> | pinang | arecoline | anthelmintic |
| <i>Azadirachta indica</i> | mambu | azadiracthin | insecticide |
| <i>Carica papaya</i> | betik | chymopapain | proteolytic |
| <i>Catharanthus roseus</i> | kemunting cina | vinblastine | antitumor |
| <i>Centella asiatica</i> | pegaga | asiaticoside | vulnerary |
| <i>Curcuma longa</i> | kunyit | curcumin | choloretic |
| <i>Datura metel</i> | kecubung | scopolamine | sedative |
| <i>Diocorea spp.</i> | gadung | diosgenin | contraceptive |
| <i>Nicotiana tabacum</i> | tembakau | nicotine | insecticide |
| <i>Ricinus communis</i> | jarak | castor oil | laxative |
| <i>Strychnos nux-vomica</i> | - | strychnine | CNS stimulant |

2.6.1 Piper sarmentosum Roxb.

Southeast Asian cuisine frequently uses *Piper sarmentosum*, is a plant from the *Piperaceae* family (Table 2.3) (Biotropics Malaysia Berhad.,2007). Though smaller and with a different flavour, its leaves resemble those of betel. It's an herb that grows creepingly up to ten meters long and tastes less peppery. The heart-shaped leaves have a waxy surface when they are young and measure roughly 7–14 cm in length and 6–13 cm in width (Figure 2.3) (Jabatan Perhutanan Semenanjung Malaysia, 2023). Little white flowers bloom, and the fruit is small, dry, black, and has a little rounded tip.

This plant's native range includes India, Laos, Cambodia, Vietnam, Malaysia, the Philippines, Indonesia, and Southeast Asia, including Northeast India, Southern China, and the Andaman Islands. In Indonesia this plant knows as *cha plu*, in Thailand know as *bo la lot*, in Vietnam known as *phak i leut*, and *xi ye qing wei teng* or *qing ju* in China. The plant easily grows in shady areas (Ismail *et al.*, 2018). However, in

Malaysia it known as *daun kaduk*. Asian traditional medicine often uses *Kaduk* leaves. The fruit's amides have been demonstrated to have anti-plasmodial and anti-tuberculosis properties, while chemical investigation has revealed that the leaves contain the antioxidant naringenin. Additionally, this herb functions as an expectorant to assist remove phlegm, cures fever, and facilitates digestion including treating toothaches, coughs, pleurisy, asthma, and fungal dermatitis of the foot are all possible using its roots.

Table 2.2 The taxonomy of *P. sarmentosum* (Biotropics Malaysia Berhad.,2007)

| Plant/Product name: | |
|----------------------------|--------------------------|
| Scientific Name | <i>Piper sarmentosum</i> |
| Class | Magnoliopsida |
| Order | Piperales |
| Family | Piperaceae |



Figure 2.4 The morphology of *P. sarmentosum* ethanol extract (Jabatan Perhutanan Semenanjung Malaysia, 2023)

2.6.2 Antimalarial activity

Studies on medicinal plants from Malaysia, including *P. sarmentosum* ethanol extract, *Andrographis paniculata*, and *Tinospora crispa*, showed significant antimalarial effects both *in vitro* and *in vivo* (Table 2.3.1) (Rahman *et al.*, 1999).

Table 2.3 Plant species used for antimalarial evaluation (Rahman *et al.*, 1999)

| Species | Family | Parts used | Local name |
|--------------------------------|----------------|-------------|--------------|
| <i>Piper sarmentosum</i> | Piperaceaea | Leaves | Kaduk/Kudak |
| <i>Andrographis paniculata</i> | Acanthaceae | Whole plant | Hempedu Bumi |
| <i>Tinospora crispa</i> | Menispermaceae | Stem | Patawali |

The three plant species' methanol extracts, as well as the chloroform extracts of *Andrographis paniculata* and *P. sarmentosum* ethanol extract, were examined for their ability to inhibit parasite growth. Tests were run in duplicate in 24-well culture plates (FALCON) with 2% and 35% haematocrit (1 millilitre suspension/well) for initial and experimental parasitaemia. Giemsa-stained blood films were created periodically for the purpose of microscopically assessing parasitaemia and parasite development. The three plant species exhibited strong antimalarial activity against the parasites *Plasmodium berghei* (*in vivo*) and *Plasmodium falciparum* (*in vitro*), with the chloroform extract generally outperforming the methanol extract in the results of the *in vitro* tests. Those extracts' antimalarial properties may have resulted from the active principle's potential presence (Rahman *et al.*, 1999).

The emergence of multidrug-resistant *Plasmodium falciparum* makes the development of novel antimalarial medications from medicinal plants imperative. Therefore, the antimalarial and toxicological properties of the methanol and water leaf extracts of *P. sarmentosum* ethanol extract (*kaduk*), a plant that is frequently used as

an herbal remedy to treat malarial symptoms, were investigated. Using a malarial SYBR Green I-based fluorescence (MSF) test, the inhibitory concentration (IC₅₀) of *P. sarmentosum* ethanol extract methanol and water extracts against a chloroquine-sensitive strain (3D7) of *Plasmodium falciparum* was ascertained. The brine shrimp lethality test (BSLT) was used to determine the plant extracts' lethal concentration (LC₅₀). The IC₅₀ values of the methanol and water extracts were 229.7 ± 0.125 µg/mL and 138.8 ± 0.122 µg/mL, respectively, indicating that they lacked antimalarial activity (Zin *et al.*, 2019).

2.6.3 Anti-inflammatory activity

It was also shown that the aqueous extract of *P. sarmentosum* ethanol extract (AEPS) reduced the volume of paw edema caused by carrageenan, suggesting that (AEPS) may have anti-inflammatory properties. Interestingly, *P. sarmentosum* ethanol extract has long been used to treat pain and inflammatory disorders and this is supported by the anti-inflammatory and anti-nociceptive properties of (AEPS). A flavonoid component has been discovered as one of *P. sarmentosum* ethanol extract's active constituents (Zakaria *et al.*, 2010).

2.6.4 Anti-diabetic

P. sarmentosum ethanol extract and *P. betle* aqueous extracts have potential as anti-obesity and anti-diabetic medicines, according to a comparative investigation of their anti-obesity and anti-diabetic activities conducted in vitro. *P. sarmentosum* ethanol extract aqueous extract (PSAE) and *P. betle* aqueous extract (PBAE) included a high concentration of flavonoids, phenols, and tannins. Flavonoids are an abundant group of naturally occurring plant-phenolic chemicals with numerous biological and pharmacological activities against obesity and obesity-related diabetes. Phenols,

Tannins, and Saponins are secondary chemicals with antioxidant properties, as well as anti-obesity and insulin sensitivity properties (Daud *et al.*, 2021).

Furthermore, *P. sarmentosum* ethanol extract has the potential to be utilized as an alternative therapy or in combination with other drugs to treat diabetes mellitus. *P. sarmentosum* ethanol extract leaf extract may reduce heart dysfunction and early signs of atherosclerosis in streptozotocin (STZ) diabetic rats by delaying degeneration of myocardial and aortic tissue. Only one study in this evaluation examined the effect of *P. sarmentosum* ethanol extract on the kidneys of diabetic rats. The study found that the kidneys of rats with STZ-induced diabetes mellitus had increased inflammatory cell infiltration, Bowman's capsule size, and glomerular membrane thickness. *P. sarmentosum* ethanol extract aqueous leaf extract 0.125 g/kg reduced these degenerative renal changes in rats with STZ-induced diabetes mellitus, implying that the plant extract may prevent diabetes-related renal failure (Azhar *et al.*, 2022).

2.6.5 Anti-hypertensive

According to the National Health and Morbidity Survey of 2011, 35.1% (5.7 million) of Malaysian individuals aged 18 and older had hypertension. Despite the fact that the specific cause of hypertension remains uncertain, conventional medicine continues to treat it. Studies have shown that oxidative stress and a lack of nitric oxide (NO) produce an increase in vascular wall tension and blood pressure. In a study of spontaneous hypertensive rats, administration of *P. sarmentosum* ethanol extract leaves aqueous extract (Kadukmy™) resulted in significant reductions in systolic, diastolic, and mean arterial pressure ($P < 0.05$), increased serum nitric oxide (NO) level ($P < 0.05$), reduced serum malondialdehyde (MDA) level ($P < 0.05$), and serum total cholesterol level in groups treated with So, *P. sarmentosum* ethanol extract can lower blood pressure and cholesterol levels (Mohd Zainudin *et al.*, 2015).

2.6.6 Antioxidant and Cytotoxicity

The Folin-Ciocalteu method was used to analyse a methanolic extract of *P. sarmentosum* ethanol extract leaves, as well as High Performance Liquid Chromatography. The extract was tested in vitro for antioxidant activity using the diphenyl picrylhydrazyl (DPPH) scavenging test. The total phenolic content (TPC) of the *P. sarmentosum* ethanol extract was 89.33 mg GAE/g DM, and HPLC indicated flavonoids and phenolic acids. The DPPH assay showed that *P. sarmentosum* ethanol extract has antioxidant activity, with an EC₅₀ of 60.24 µg/ml. The MTS experiment indicated that the plant extract had a cytotoxic effect on T47D cells, with an IC₅₀ value of 2.69 µg/mL. The phenolic compounds isolated from *P. sarmentosum* ethanol extract have been shown to be cytotoxic to breast cancer cells T47D (Ghazali *et al.*, 2020).

In a study evaluating the effects of *P. sarmentosum* ethanol extract on hydrogen peroxide (H₂O₂)-induced oxidative cell damage in cultured human umbilical vein endothelial cells (HUVECs), this plant extract demonstrated ferric-reducing antioxidant potential due to its high phenolic content. As a result, *P. sarmentosum* ethanol extract helps to reduce oxidative stress in HUVECs produced by H₂O₂ (Hafizah *et al.*, 2010).

2.7 Anticancer properties

It is a natural component found in plants and has complex chemical structures that can lead to the creation of new pharmaceuticals that help humanity. The most important of these bioactive substances include alkaloids, tannins, flavonoids, and phenolic compounds. The tannins and flavonoids revealed by phytochemical research, as well as steroids and alkaloids, could account for the leaf extract's antibacterial and antioxidant properties. Further examination of the bioactive compounds by GC-MS revealed the existence of phytochemicals with anticancer potential, the most prevalent

of which were asarone (73.72%), 3-(4-methoxyphenyl) propionic acid (7.58%), and Phytol (3.67%) (Shakilan *et al.*, 2019).

The ethanolic leaf extracts of the examined *Piper* species were tested for anticancer activity against three (3) human cancer cell lines: cervical carcinoma (HeLa), breast cancer (MCF-7), and colon adenocarcinoma (HT-29), using the MTT assay. The percentage of inhibition on several cell lines at various concentrations (100, 80, 60, 40, and 20) $\mu\text{g/mL}$ was used to determine the IC_{50} values of *P. sarmentosum* ethanol extract against the three cancer cell lines. IC_{50} value at $51.61 \mu\text{g/mL} \pm 23.03$ on Cervical carcinoma (HeLa) cell lines, IC_{50} value at $30.02 \mu\text{g/mL} \pm 6.84$ on Breast Cancer (MCF-7) cell lines and IC_{50} value at $24.97 \mu\text{g/mL} \pm 5.52$ on colon adenocarcinoma (HT-29) cell lines (Shakilan *et al.*, 2019).

Moreover, the MTS assay was used to determine the extract's effect on human breast cancer cells, MCF-7. The results revealed that the *P. sarmentosum ethanol extract* has a high TPC of 89.22 mg GAE/g. The extract showed significant antioxidant activity, with an EC_{50} value of $96.98 \pm 2.29 \text{ g/mL}$. In vitro cytotoxicity testing on MCF-7 cell growth yielded an IC_{50} of $24.63 \pm 0.23 \text{ g/mL}$ for the extract. Finally, the methanolic extract of *P. sarmentosum ethanol extract* had a high phenolic content and shown substantial antioxidant and anticancer activities against the tested breast cancer cell (Ibrahim *et al.*, 2023).

To test for preliminary anticancer efficacy in terms of cell viability, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl) In vitro cytotoxicity experiments using -2H-tetrazolium inner salt (MTS) are regarded as two of the most cost-effective, reliable, and convenient approaches. This is due to its ease of use, precision, and rapid detection of toxicity, as well as sensitivity and specificity.

The MTT/MTS in vitro cell proliferation assay is one of the most extensively used methods for assessing the preclinical anticancer efficacy of synthetic derivatives, natural compounds, and natural product extracts. These are the two most effective anticancer screening methods (McCauley *et al.*, 2013).

2.8 Phytochemical Constituents and Anticancer Activity of Flavonoids

Flavonoids are a promising family of phytochemicals with high anticancer activity. Flavonoids are classified into six major subclasses based on their chemical structure such as flavones, flavanols, flavanones, flavanols, anthocyanins, and isoflavones. Each subclass exhibits unique bioactivities and is distributed differently among plant species. They have a variety of modes of action and target cancer at multiple stages. Flavonoids like quercetin and kaempferol cause cancer cells to die by activating both intrinsic (mitochondrial) and extrinsic (death receptor) pathways. Quercetin, for example, was reported to upregulate pro-apoptotic proteins Bax and caspase-3 in breast cancer cells (Zhao *et al.*, 2021).

2.9 Phytochemical Constituent and Anticancer Activity of Alkaloids

The anticancer properties of alkaloids stem from their ability to interfere with various cellular processes, including DNA replication, mitosis, apoptosis, and signalling pathways. Alkaloids target rapidly dividing cancer cells while sparing normal cells, making them promising candidates for chemotherapy. Many alkaloids cause programmed cell death via internal (mitochondrial) and extrinsic (death receptor) routes. For example, berberine causes apoptosis in colorectal cancer cells via activating the p53 pathway and generating reactive oxygen species (ROS). (Zhang *et al.*, 2021). Moreover, this type of alkaloids also has a strong cytotoxic effect against breast cancer (Zhang *et al.*, 2021).

2.10 Phytochemical Constituent and Anticancer Activity of Terpenoids

Terpenoids are classed by the number of isoprene units they contain, which ranges from monoterpenoids (C10) to sesquiterpenoids (C15), diterpenoids (C20), triterpenoids (C30), and polyterpenoids. They are found in essential oils, resins, and other plant exudates, contributing to the distinctive scent and biological activities of many plants. Terpenoids such as limonene and farnesol induce apoptosis in cancer cells by activating pro-apoptotic proteins like Bax and caspase-3 while inhibiting anti-apoptotic proteins such as Bcl-2 (Zhang *et al.*, 2021).

2.11 Phytochemical Constituent and Anticancer Activity of Saponins

Saponins suppress angiogenesis by downregulating vascular endothelial growth factor (VEGF). Ginsenoside Rh2 demonstrated anti-angiogenic effects in breast cancer models (Zhao *et al.*, 2023). Saponins inhibit cancer cell migration and invasion by modulating matrix metalloproteinases (MMPs). For instance, astragal side IV from *Astragalus* inhibited metastasis in hepatocellular carcinoma (Li *et al.*, 2021).

2.12 Phytochemical Constituent and Anticancer Activity of Tannins

Tannins are classified into two major types based on their chemical structure such as hydrolyzable Tannins that derived from gallic acid or ellagic acid. These tannins are esterified to a carbohydrate core (commonly glucose) and condensed Tannins (Proanthocyanins) which formed by polymerization of flavan-3-ols, such as catechin and epicatechin. Tannins inhibit tumour angiogenesis by downregulating vascular endothelial growth factor (VEGF) expression. Proanthocyanin's from grape seeds have been shown to suppress angiogenesis in breast cancer models (Zhou *et al.*, 2022).

CHAPTER 3

MATERIALS AND METHOD

3.1 Experimental design

The experimental design of this study is shown in Figure 3.1.

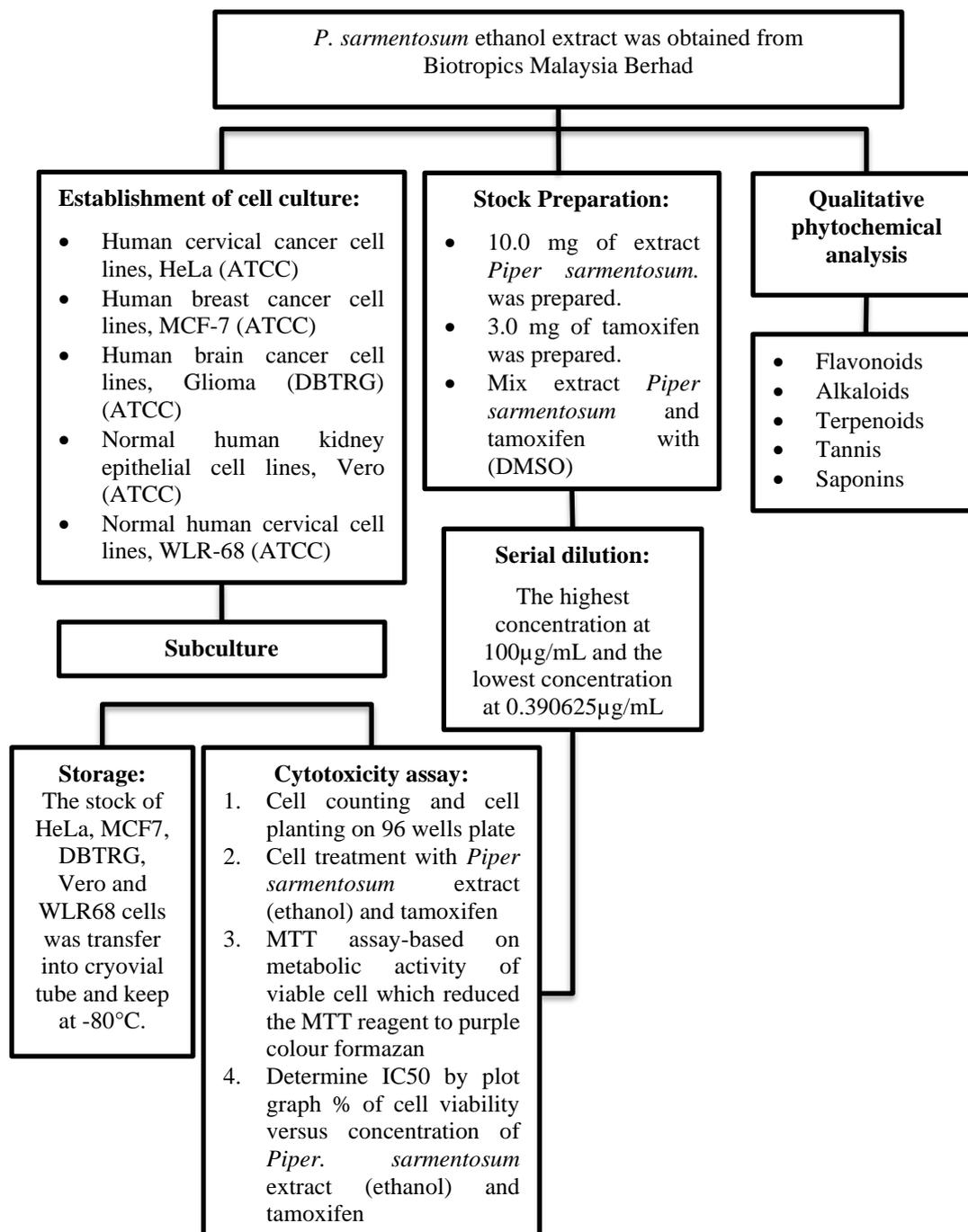


Figure 3.1 Experimental design