ANTICANCER-IMMUNE RESPONSE TOWARDS BREAST CANCER CELL LINES MDA-MB-231 AND TERATOGENIC ASSESSMENT OF *Pereskia bleo* LEAVES

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TINDAK BALAS ANTIKANSER-IMUN TERHADAP SEL KANSER PAYUDARA MDA-MB-231 DAN PENILAIAN TERATOGENIK DAUN *Pereskia bleo*

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LIST OF SYMBOLS

°C	Degree Celsius
min	Minutes
mL	millilitre
cm	centimeter
%	Percentage
α	Alpha
γ	Gamma
g	Gram
mg	Milligrams
µg	Micrograms
/	Per, and, or
mg/mL	Milligrams/ millilitre
µg/mL	Micrograms/ millilitre
μl	Microliter
mm	Millimeter
nm	Nanometer
pg/mL	Picograms/ millilitre
no	Number
n	Number
Kg	Kilogram
mg/kg	Milligrams/Kilogram
+	Positive
-	Negative
vs	versus
Q	Quarter
kD	Kilo Dalton
hrs	Hours

LIST OF ABBREVIATIONS

ADCC	Antibody-Dependent Cell Cytotoxicity
AIF	Apoptosis-inducing factor
Apaf-1	Apoptotic protease activation factor-1
APCs	Antigen presentation cells
API	Apoptotic protein inhibitors
Bcl-2	B-cell lymphoma 2
BSA	Bovine serum albumin
BW	Body weight
CO_2	Carbon dioxide
CRL	Crown–rump length
Cyto C	Cytochrome C
DISC	Death inducing signalling complex protein
DMEM	Dulbecco's Modified Eagles Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DRs	Death receptors
DW	Distill water
ELISA	Eenzyme-linked immunosorbent assay
End G	Endonuclease G
ER	Estrogen receptor
FADD	Fas-associated death domain
FasL	Fas Ligand
FBS	Foetal bovine serum
FDA	Food and Drug Adminstration
G1 phase	Gap phase 1
G2 phase	Gap phase 2
GD	Gestation day
GM-CSF	Granulocyte-macrophage colony-stimulating factor
HtrA2	High-temperture requirement protein A2
IAPs	Inhibitor of apoptosis (IAP) proteins
IC_{50}	Half maximal inhibitory concentration
ICAD	Caspase-activated DNas
IFN-γ	Interferon gamma
ILs	Interlukines
IP	Intraperitoneal
ITIMs	Immunoreceptors tyrosine based inhibitory motifs
KIR	Killer cell immunoglobulin-like receptors
LIRs	Leukocyte inhibitory receptors
$\log_{10}$	*Logarithm* with base 10
LSM	Lymphocytes Separation Media
MDM2	Mouse double minute protein 2
MEPB	Methanol extract of *Pereskia bleo*
MHC	Major histocompatibility complex
MOMP	Mitochondrial Outer Membrane Permeabilization
NK cells	Natural killer cells
NKT	Natural Killer T

OD	Optical density
OECD	Organisation for Economic Cooperation and Development
P. bleo	*Pereskia bleo*
P53	Tumour suppressor protein
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffer saline
PD-1	Programmed cell death 1
PD-L1	Programmed cell death ligand 1
PHA	Phythaemagglutinin
PI	Propidium iodide
PS	Phosphatidylserine
RNA	Ribonucleic acid
ROI	Reactive oxygen intermediates
ROS	Reactive oxygen species
S phase	Synthesis Phase
SEM	Stander error mean
Smac	Second mitochondrial-dervied activator of caspases
tBID	Turncated BID
TME	Tumour microenviroment
TRADD	TNF receptor-associated death domain
TRAIL	TNF-related apoptosis-inducing ligand
vs	Versus
WHO	World Health Organization

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TINDAK BALAS ANTIKANSER-IMUN TERHADAP SEL KANSER PAYUDARA MDA-MB-231 DAN PENILAIAN TERATOGENIK DAUN *Pereskia bleo*

ABSTRAK

Rawatan konvensional bagi kanser payudara, terutamanya terapi radiasi dan kemoterapi, memberi kesan sampingan kepada pesakit. Oleh itu, fokus di peringkat global telah meningkat dalam mencari kaedah rawatan yang tidak toksik dan bersifat sitoselektif, termasuk kajian ke atas herba. Pelbagai jenis herba menunjukkan bioaktiviti yang sangat baik walau bagaimanapun terdapat juga herba yang bersifat toksik dan teratogenik pada dos-dos tertentu. Kajian ini dijalankan untuk menilai sifat anti-kanser daun *P. bleo* dari segi keupayaannya untuk mengaruh apoptosis dan untuk menilai kebolehannya mengaruh tindakbalas imun anti-kanser dengan meningkatkan ketoksikan sel Pembunuh Semulajadi (sel NK) terhadap sel kanser payudara MDA-MB-231, serta menilai ketoksikan dan keteratogenannya dalam model haiwan. Ekstrak heksana, etil asetat dan metanol daun *P. bleo* telah diuji ketoksikannya ke atas sel normal MCF-10A dan MDA-MB-231 melalui ujian MTT. Esktrak yang paling aktif ialah metanol, maka metanol digunakan untuk eksperimen yang seterusnya. Annexin V/PI dan analisis sitometri aliran digunakan untuk meneroka keupayaan ekstrak metanol daun *P. bleo* (MEPB) dalam mengaruh apoptosis, penyekatan kitaran sel dan pengekspresan protein apoptotik. Ujian imunosorben berkait enzim (ELISA) digunakan untuk mengukur aras interferon-gamma (IFN-γ), interleukin (IL)-8, IL-10, IL-12, IL-18, perforin, dan granzyme B dalam darah penderma sihat bagi menentukan kepekatan MEPB terbaik dalam mengaktifkan sel NK. Ketulenan dan kiraan sel NK dinilai menggunakan analisis sitometri aliran dan tripan biru. Analisis sitometri aliran

dan ELISA digunakan untuk menilai keupayaan MEPB untuk meningkatkan ketoksikan sel NK terhadap sel MDA-MB-231. Ketoksikan dan keteratogenan daun MEPB dinilai dengan meneliti kitaran estrus, berat badan, tingkah laku umum dan tanda klinikal, analisis histopatologi, berat badan mutlak organ viseral janin, dan hasil kehamilan termasuk bilangan corpora lutea dan tapak implantasi, kematian sebelum dan selepas implantasi (%), berat rahim gravida, bilangan janin hidup dan mati, berat badan janin, nisbah jantina, dan pemeriksaan keseluruhan janin. Kajian ini menggunakan 40 ekor tikus betina yang termasuk 10 kumpulan tikus sebagai kawalan (air suling) dan 30 kumpulan tikus yang dirawat MEPB (250, 500, dan 1000 mg/kg/hari). Ujian MTT menunjukkan ketoksikan sederhana MEPB terhadap sel kanser payudara MDA-MB-231 dengan nilai IC_{50} 64.57µg/ml. Data sitometri aliran menunjukkan bahawa MEPB boleh menyekat sel dalam fasa G0/G1 dan merangsang apoptosis dalam sel MDA-MB-231, dengan meningkatkan ekspresi Bax, p53, dan caspase-3 sambil menurunkan ekspresi Bcl-2. Hasil kajian menunjukkan bahawa daun MEPB mempunyai keupayaan untuk meningkatkan aras IFN-γ, IL-12, IL-18, perforin, dan granzyme B dan menurunkan aras IL-8 dan IL-10 dalam darah penderma sihat. Pesakit kanser payudara didapati mempunyai kurang sel NK berbanding penderma yang sihat, dan kira-kira 87.09% sel NK telah ditulenkan dengan berkesan. MEPB mengaruh sel NK untuk membunuh sel MDA-MB-231 secara apoptosis, melalui peningkatan perforin, granzyme B dan IFN-γ, dalam darah pesakit kanser payudara dan penderma sihat. Keputusan menunjukkan bahawa kumpulan tikus yang dirawat dengan pelbagai dos daun MEPB tidak menjejaskan parameter ketoksikan, hasil kehamilan, dan parameter ketoksikan ke atas fetus. Penemuan kami menyimpulkan bahawa daun MEPB mengaruh apoptosis dalam sel MDA-MB-231, mempunyai keupayaan yang signifikan untuk mengatur sitokin, meningkatkan ketoksikan sel NK

terhadap sel kanser, tanpa sebarang bukti ketoksikan dan keteratogenan dalam kumpulan tikus yang dirawat dengan MEPB.

ANTICANCER-IMMUNE RESPONSE TOWARDS BREAST CANCER CELL LINES MDA-MB-231 AND TERATOGENIC ASSESSMENT OF *Pereskia bleo* LEAVES

ABSTRACT

Conventional treatment for breast cancer, especially radiation and chemotherapy, have significant adverse effects on patients. Thus, an increased global focus on finding nontoxic and cytoselective treatments has emerged, which includes the study on herbs. Various herbs showed excellent bioactivities; however, there are herbs that can be toxic and teratogenic at certain dosage. The current study was conducted to assess the anti-cancer properties of *P. bleo* leaves in terms of their ability to induce apoptosis and to determine its anti-cancer-immune response by stimulating Natural Killer (NK) cells' cytotoxicity against MDA-MB-231 breast cancer cells, as well as to evaluate its toxicity and teratogenicity in the animal model. Hexane, ethyl acetate and methanolic extracts of *P. bleo* leaves were tested for their cytotoxicity against normal cells MCF-10A and MDA-MB-231 cell lines by MTT assay. Methanolic extract showed the best activities and was used for subsequent experiments. Annexin V/PI assay and flow cytometric analysis were used to measure the induction of apoptosis, cell cycle arrest, and apoptotic protein expression by methanolic extract of *P. bleo* leaves (MEPB). Enzyme-linked immunosorbent assay (ELISA) was utilised to measure the level of interferon-gamma (IFN-γ), interleukins (IL)-8, IL-10, IL-12, IL-18, perforin, and granzyme B in healthy blood donors to determine the best concentration of MEPB leaves for activating NK cells. Flow cytometry and trypan Blue were used to measure NK cell counts and purity for subsequent experiments. Flow cytometric analysis and ELISA were used to determine

the ability of MEPB to enhance NK cell cytotoxicity against MDA-MB-231 cells. The toxicity and teratogenicity of MEPB leaves were evaluated by observing the oestrous cycle, body weight, general behaviour and clinical signs, histopathological analyses, absolute body weights of dam's visceral organs, and pregnancy outcomes, including the numbers of corpora lutea and implantation sites, pre- and post-implantation death (%), gravid uterine weight, number of live and dead foetuses, foetal body weight, sex ratio, and gross examination of the foetuses. The study used 40 female rats and was divided into 10 rat-control groups (distilled water) and 30 rat-MEPB groups (250, 500, and 1000 mg/kg/day). The MTT assay showed moderate cytotoxicity of MEPB leaves towards MDA-MB-231 breast cancer cells with an IC_{50} value of 64.57µg/mL. The flow cytometry data indicated that MEPB can arrest the cell cycle at the G0/G1 phase and stimulate apoptosis in MDA-MB-231 cells, increasing the Bax, p53, and caspase-3 while decreasing Bcl-2 expression. The results indicated that MEPB leaves could upregulate IFN-γ, IL-12, IL-18, perforin, and granzyme B levels and downregulate IL-8 and IL-10 levels in healthy blood. Breast cancer patients were found to have fewer NK cells than healthy donors, and approximately 87.09% of NK cells were effectively isolated. MEPB enhanced NK cells to kill MDA-MB-231 cells via apoptosis by upregulating perforin, granzyme B, and IFN-γ in healthy and breast cancer patient donors. The study also showed that the rat groups treated with various doses of MEPB leaves did not affect toxicity parameters, pregnancy outcomes, and foetotoxicity parameters. Our findings concluded that MEPB leaves induced apoptosis in MDA-MB-231 cells, with a significant capacity to regulate cytokines and increase NK cell cytotoxicity towards cancer cells without any evidence of toxicity and teratogenicity in rat-MEPB groups.

CHAPTER 1

INTRODUCTION

1.1 Background of the study

Cancer is a debilitating disease and one of the biggest threats to life, often leading to a slow and gradual decline (de Martel *et al*., 2020). Hippocrates, a Greek physician, invented the term "cancer" between 460 and 370 B.C. It originates from the word "karkinos," which means "carcinoma" (Sudhakar, 2009). Globally, the mortality rate of cancer is rising yearly. Statistically, cancer is reported to be the 4th most common cause of death (National Cancer Registry, 2018). Effective treatments for breast cancer commonly include surgery, chemotherapy, targeted therapy, and endocrine therapy, such as tamoxifen (Dhanasekaran, 2020). These therapies cause side effects, such as headaches, fatigue, weakness, hair loss, nausea, vomiting, diarrhoea, mouth sores, dry mouth, damage to the immune system, potential organ failure, and digestion of both normal and cancer cells (Ko *et al*., 2018).

Owing to the significant side effects of most therapies, researchers have been prompted to look at natural substances, particularly medicinal plants, for their possible anticancer benefits (Panyajai *et al*., 2022). In the Holy Qur'an, about 19 medicinal plants are listed (Urbi *et al*., 2014). The phytochemical compounds confer therapeutic qualities to plant-based medicines (Cui *et al*., 2018). Due to their abundance of bioactive chemicals, the medicinal plants recognised by the World Health Organization (WHO) showed that 5–15% of them contain anticancer medicines (Shabani, 2016).

Malaysia ranks 12th in biodiversity, especially for *Pereskia bleo* (*P. bleo*) (Paramanick & Sharma, 2017). *P. bleo* is described as a traditional medicine and a member of the Cactaceae family. Previous studies acknowledged the activity of this plant towards various diseases, such as headaches, diabetes, cardiovascular diseases,

neurological disorders, ulcers, gastric pain, cancer, blood pressure, rheumatism oedema, and obesity (Vijayablan *et al*., 2021). Malaysians consume *P. bleo* leaves as a vegetable in soups and salads (Garcia *et al*., 2019). *P. bleo* leaves are approved for their antibacterial, antioxidant, anti-inflammatory, and anti-cancer properties (Fattepur *et al*., 2020). However, according to previous data, the methanolic extract of *P. bleo* leaves had cytotoxic effects and induced apoptosis against breast cancer cells (MCF-7) (Malek *et al*., 2007).

Several studies have reported that the bioactive compounds of medicinal plants can enhance immune cells, such as NK cells, to fight cancer (Grudzien & Rapak, 2018). Natural killer (NK) cells are employed in cancer immunotherapy (Morvan & Lanier, 2016). NK cells, one of the most common innate immune systems, play a role in the natural defences of the body (Choucair *et al*., 2019). On the other hand, NK cells are designed to digest target cells, such as infection or cancer, while avoiding harm to normal cells (Voss & Bryceson, 2017). Many studies demonstrated that activated NK cells act as an anti-tumour agent (Gauthier *et al*., 2019). Both perforin and granzyme B, found on the surface of NK cells, play an essential role in digesting target cells, eventually resulting in the death of cells (Liesche *et al*., 2018). However, NK cells can release cytokines like interferon-gamma (IFN-γ), which help to destroy the target cells (Chiossone *et al*., 2018).

Most of the therapeutic drugs used to cure related and unrelated pregnancy issues are harmful to pregnancy and exhibit teratogenic effects on the foetus (de Faria *et al*., 2004). Due to concerns about the health of the foetus, many pregnant women prefer to treat their symptoms with medicinal plants instead of pharmaceutical medicines (Holst *et al*., 2009). The foetus is most vulnerable to teratogenicity during the first trimester of pregnancy; therefore, pregnant women must exercise caution

during this time (Calvasina *et al*., 2007). There is currently a lack of data on the risk or safety of medicinal plants during pregnancy. Previous studies showed that the adverse side effects of anticancer-derived plants on normal cells can lead to teratogenic, mutagenic, structural malformations, damage, growth retardation, as well as carcinogenic and sometimes leading to death or congenital disability (Bentil, 2015). For instance, an experiment was conducted on a pregnant woman who utilised anticancer-derived plants such as cyclophosphamide, which resulted in the newborn foetuses having abnormalities in structure and function (Nama & Shehata, 2012). According to a previous study, Methotrexate, which is used to treat leukaemia, lymphoma, and breast cancer, led to anomalies, miscarriages, and skeletal abnormalities with ambiguous genitalia during the first trimester (Addar, 2004). A study revealed that the drug Bleomycin, used to treat lymphoma, ovarian cancer, and teratoma can cause plagioccephaly syndactyly (fourth and fifth fingers) during the second and third trimesters (Cardonick *et al*., 2010). Furthermore, the side effects of the *Carthamus tinctorius* plant turned out to be eyelid defects, brain defects, renal toxicity, and hepatic toxicity (Baradaran *et al*., 2014). According to the findings of an experiment conducted on rats, large dosages of ginger administered to pregnant rats resulted in increased foetal weight, foetal loss, and bone maturation (Hepner *et al*., 2002).

1.2 Problem statement and rationale of the study

Radiation and chemotherapeutic medicines are used to treat cancer today, and both have significant side effects on patients. Thus, an increased global focus on finding nontoxic treatments for healthy cells that are toxic to cancer cells has emerged. *P. bleo* has been proven in earlier investigations to have anti-cancer properties. To

provide useful basic pharmacological information on this medicinal plant, further research is needed to understand the potential of *P. bleo* extract to produce cytotoxicity and increase immunological activation. This study might help researchers better understand the role of *P. bleo* leaves in apoptosis induction and cytotoxicity of NK cells against cancer cells. However, the findings of this study may potentially aid alternative medicine, as improved *P. bleo* leaves have been shown to have beneficial effects on cancer cytotoxicity. However, information on the interaction with toxicity is still lacking. Previously, the acute oral toxicity of *P. bleo* on mortality or adverse effects of the plant at a maximum dose of 2500 mg/kg was tested by Sim and colleagues, but it is still unclear. Therefore, in this study, the teratogenicity assessment of *P. bleo* was done similar to Sim *et al*. (2010a). Medicinal herbs may help relieve related and unrelated pregnancy issues (Westfall, 2004). On the other hand, they play a vital role in abortion induction and teratogenic effects (Tang *et al*., 2012). During the first trimester, the foetus is most sensitive to teratogenicity; thus, pregnant mothers must be cautious (Calvasina *et al*., 2007).

1.3 Objective of study

1.3.1 General objective

To assess the anti-cancer immune response towards MDA-MB-231 breast cancer cell lines, toxicity and teratogenicity of *P. bleo* leaves extract.

1.3.2 Main objective of the study

1. To determine the antiproliferative activities of *P. bleo* leaves extract on breast cancer cell lines (MDA-MB 231) and normal breast cell lines (MCF-10A).

2. To assess the mode of cancer cell death induced by the *P. bleo* leaves extract on the selected cancer cells via a cell cycle arrest assay, Annexin V staining, and apoptotic protein expression, including Bax, Caspase-3, p53, and Bcl-2.
3. To analyse the killing effect of NK cells on breast cancer cells induced by *P. bleo* leaves extract by evaluating the expression of interferon-gamma (IFN-γ), perforin, and granzyme B.
4. To evaluate the female toxicity and teratogenic effects of *P. bleo* leaves extract.

CHAPTER 2

LITERATURE REVIEW

2.1 Medicinal plants

Plants have always been a source of medicine. Many modern medications have been derived from plants formerly used in traditional treatments. They play an essential role in preserving health and developing novel therapies in various regions of the world (Dutta *et al*., 2020).

In basic terms, "herb" refers to medicinal plants. Medicinal plants are a broad category of plants utilised in medicine to treat disease and have health-promoting properties. Worldwide, more than 94 plant species are used medicinally and in traditional healthcare systems. Previous studies have reported around 122 medicinal compounds isolated from these plants (Yuan *et al*., 2016). Statistics show that 65–85% of the world's population uses herbal medicine as their major source of healthcare (Kifle *et al*., 2021). The use of herbal medicine is estimated to be between 5.9% and 48.3% in Europe, 17.9% in the United States, and 12% in Canada (Eardley *et al*., 2012). Over 80% of people in Asia and Africa rely on medicinal plants (Chemburkar *et al*., 2014). Traditional medicine is commonly practised in many Asian nations despite the availability of allopathic treatment (Gunjan *et al*., 2012). Medicinal plants are described in Malaysia as crude plant concoctions used in alternative medicine techniques, such as Malay medicine, traditional Malay medicine, Ayurvedic medicine, naturopathy, homoeopathy, and some are also sold as nutritious foods or dietary supplements (Jantan, 2006). According to a previous study, most patients supplement their conventional medical prescriptions with herbal medicine because most individuals believe herbal medication is natural, harmless, and has fewer adverse effects than synthetic medicines or the notion that natural treatments are more

dependable and effective than pharmaceuticals (Vidya & Lohit, 2019). Phytochemicals are natural compounds found in various parts of medicinal plants (e.g., roots, leaves, and flowers) that act together with nutrients and fibres to protect against disease (Hussein & El-Anssary, 2019). Therefore, medicinal plants that are rich in phytochemicals have been utilised as anti-cancer, anti-inflammatory, anti-microbial, and antioxidant agents (Ashaari *et al*., 2020) (Table 2.1).

Table 2.1: List of bioactive compounds of plants that have anti-cancer activities.

Source of plant	Name of Phytochemical compound	Treatment for Anti-cancer effects
Podophyllum peltatum and *Podophyllum emodii*	Podophyllotoxin	Skin cancers, warts lymphomas, bronchial, and testicular cancer (Tan *et al*., 2011)
Podophyllum hexeandrum	Podophyllotoxin	Testicular, lung cancer, leukemias, ulcers, wounds, constipation, and tuberculosis therapy (Imbert, 1998)
Cephalotaxus harringtonia	Cephalotaxus alkaloids	Chronic and acute myelogenous leukemia (Feldman *et al*., 1996)
Colchicum autumnale (Colchicaceae)	Colchicine	Crystal arthritis, cirrhosis, and gout (Negi *et al*., 2015)
Bleekeria vitensis and *Ochrosia elliptica*	Ellipticine	Cure ependymoblastoma, leukemia, myeloma, melanoma, breast, and colon cancer (Isah, 2016)
Tinospora cordifolia, Berberis vulgaris, Berberis aquifolium, and *Rhizoma coptidis*	Berberine	Breast, prostate, and colorectal cancer (Barzegar *et al*., 2015)
Combretum caffrum (Combretaeae)	Combretastatins	Leukemia, lung, and colon cancer (Lauritano *et al*., 2016)
Terminalia bellerica (Combretaceae)	Combretastatins	Cure medullary thyroid and anaplastic thyroid cancer (Garon *et al*., 2016)
	Triterpenoid acids	Both *in vitro* and *in vivo* against leukemia, pancreatic, and breast cancer (Cragg & Newman, 2005)

Table 2.1: Continued

Ziziphus mauritiana, Ziziphusrugosa, Ziziphus oenoplia, and *Betula Sp.* (Betulaceae)	Betulinic acid	Melanoma (Prakash *et al*., 2013)
Red pepper	Capsaicin	Anti-cancer, antimutagenic, antimetastatic, anti-angiogenic, and chemopreventive functions in pancreatic, prostatic, liver, skin, leukemia, lung, bladder, colon, and endothelial cells (Clark & Lee, 2016)
Zingiber officinale	Gingerol	Colon, pancreas, ovarian, and breast cancer (Park *et al*., 2006; Oyagbemi *et al*., 2010)
Crocus sativus L.	Saffron (Crocetin)	Lung, liver, skin, pancreatic, colorectal, and breast cancer therapy (Hoshyar & Mollaei, 2017)
Mushroom	Vitamin D	Colon cancer (Gorham *et al*., 2007), breast, pancreatic, and ovarian cancer (Buyru *et al*., 2003)

2.1.1 Anti-cancer activities of medicinal plants

The immune system comprises special cells, tissues, and organs that substantially defend the body from external invasion of infectious or harmful microorganisms caused by immune system malfunction (Pandey *et al*., 2023). Neutrophils, macrophages, basophils, monocytes, dendritic cells, natural killer cells (NK cells), and lymphocytes (T and B) play a vital role in the effector functions of innate and adaptive immunity and serve as the first lines of defence against various pathogens, such as viruses, bacteria, and cancers in the human body (Miyake *et al*., 2017).

Cancer is triggered by DNA damage in cells or can be caused by changes in DNA (mutations), which results in abnormal growth and division (Alhmoud *et al*., 2020). Currently, the most common types of cancer therapy are surgery,

chemotherapy, and radiotherapy. These methods negatively impact healthy cells, leading to more studies aimed at discovering new and safe cancer treatments (Knight *et al*., 2021). The plant kingdom has significant impacts on human health. The major role of herbal medicines is to restore the capacity of the body to defend, control, and cure diseases. They are usually taken in the form of powders, pills, and extracts (Lichota & Gwozdzinski, 2018). Approximately 60% of anti-cancer drugs are derived from plant products (Kooti *et al*., 2017) (Table 2.2).

Table 2.2: Plants derived anti-cancer drugs.

Source of plant	Drug	Use of treatment
Catharanthus roseus (Apocynaceae)	Vinca Alkaloids, vinblastine (VLB), and vincristine (VCR)	Lymphomas, leukemias, breast cancer, testicular cancer, lung cancer, and Kaposi's sarcoma (Singh *et al*., 2013)
	vincristine (VCR)	Against leukemia, acute lymphocytic leukemia in childhood (Zaid *et al*., 2012)
Taxus baccata, *Tsuga canadensis*, and *Corylus avellana*	Taxanes (Paclitaxel and taxol), milataxel, ortataxel, and tesetaxel)	Metastatic breast cancer, AIDS-related Kaposi sarcoma, non-small cell lung carcinoma, and bladder cancer (Christensen, 2022) Ovarian, lung, pancreas, and prostate cancer (Xie & Zhou, 2017)
	Larotaxel	Urethral bladder, pancreatic, lung, and breast cancer (Ojima *et al*., 2016)
Podophyllum peltatum	Etoposide	Hodgkin`s and non- Hodgkin`s lymphoma, lung, gastric, breast, and testicular cancer (Montecucco *et al*., 2015)
Camptotheca acuminata	Campothecin derivatives, such as topotecan (hycamtin) and irinotecan	To cure colorectal, ovarian, and small-cell lung cancer (Rahier *et al*., 2005)

Table 2.2: Continued

Cephalotaxus harringtonia	Cephalotaxus (harringtonine and homoharringtonine cephalotaxus alkaloids) (Feldman *et al*., 1996)	Homoharringtonine: To cure chronic and acute myelogenous leukemia (Feldman *et al*., 1996) Harringtonine plus with Homoharringtonine: To treat chronic myelogenous leukemias, acute myelogenous leukemia (Cragg *et al*., 2006)
Bleekeria vitensis and *Ochrosia elliptica* (stem, bark, leaf and root) (Stiborová *et al*., 2014)	Ellipticine derivatives (N-2-(diethylaminoethyl)–9–hydroxyellipticinium chloride, 2-N-methyl 9-hydroxyellipticine)	To cure ependymoblastoma, leukemia, myeloma, melanoma, breast and colon cancer (Kizek *et al*., 2012; Isah, 2016) Ellipticine (elliptinium): To cure breast cancer (Ohashi *et al*., 1995)
Tinospora cordifolia, *Berberis vulgaris*, *Berberis aquifolium*, and *Rhizoma coptidis* (root and rhizome) (Mantena et al., 2006)	Berberine	To treat breast, prostate and colorectal cancer (Barzegar *et al*., 2015)
Combretum caffrum	Combretastatins	To cure leukemia, lung cancer and colon cancers (Lauritano *et al*., 2016)

Terpenoids, flavonoids, alkaloids, and steroids are secondary metabolites found in medicinal plants with anti-cancer properties (Sumner, 2000). These secondary metabolites inhibit cancer-stimulating enzymes, repair DNA, stimulate anti-cancer enzyme synthesis in cells (caspase-3, caspase-7, caspase-8, caspase-9, caspase-10, and caspase-12), and induce antioxidant effects (Sakarkar & Deshmukh, 2011). Plant metabolites stimulate apoptosis in cancer cells (Sohi *et al*., 2003). *In vitro* experiments show that several medicinal plants have anti-cancer properties due to their abundant bioactive compounds, which are essential in inhibiting cancer growth and can enhance the ability of the immune system to target cancer cells. For instance, the

root extract of the *Dicoma anomala Sond* plant exhibited anti-cancer agents on MCF-7 breast cancer cells (Shafiq *et al*., 2020), the *Fagaropsis angolensis* plant on prostate cancer cells known as DU-145 and breast cancer cells known as HCC1395 (Misonge *et al*., 2019), whereas the *Prunus avium* plant on breast cancer cells known as MDA-MB-453 (Layosa *et al*, 2021). Ji and co-workers showed that polysaccharides isolated from the *Cynanchum paniculatum* plant had a powerful anti-cancer effect on both mice hepatic cancer cells (H22), human liver cancer cells (HepG2), mice sarcoma cells (S180), and lung cancer cells (A549), which they attributed to the capacity to activate immune cells, splenic NK cells and peritoneal macrophages (Ji *et al*., 2022). The list of medicinal plants with anti-cancer properties is in Table 2.3.

Table 2.3: Type of medicinal plants that have anti-cancer activities towards various cancers.

Name plant	Extracts	Mechanism of action
Achillea wilhelmsii	Methanol extract of leaves	Induce apoptosis in colon, stomach, breast, and melanoma cells (Uddin *et al*., 2011)
Allium sativum	Methanolic extract	Anti-cancer activity against MCF-7 breast cancer and bladder cancer cells (Abdullaev, 2001; Karmakar *et al*., 2011)
Vernonia amygdalina	Extract	Reverses the cancer in MCF-7 breast cancer cells and increased the basal apoptotic but decreased the angiogenic activity in mice (Sigstedt *et al*., 2008) Anti-cancer activity in breast MCF-7 and MDA-MB-231breast cancer cells and inhibits the proliferation of cells (Gresham *et al*., 2008)
Morus alba	Methanolic extracts	Anti-proliferative effects on pulmonary carcinoma (Calu-6), colon carcinoma (HCT-116), and MCF-7 breast cancer cells (Chon *et al*., 2009)

Table 2.3: Continued

	Albanol root extract	Apoptosis-inducing, cytotoxic activity in HL-60 cells Decrease the level of pro-caspases 3, 8, and 9 by induced topoisomerase II. Bax/Bcl-2 ratio increased. Induced HL-60 apoptotic cell death through stimulation of the death receptor (Kikuchi *et al*., 2010)
	Methanolic leaf extract	Inhibition of HepG2 cells (Naowaratwattana *et al*., 2010)
	Aqueous and ethanol extracts	Anti-cancer activity against HepG2 and SMMC-7721liver cancer cells, BGC-823 gastric cancer cells, LoVo and SW-116 colon cancer cells, and CaEs-17 esophagus cancer cells (Li *et al*., 2012)
Paris polyphylla	Extract	Anti-cancer effects in ECA109 oesophageal cancer cells by: Increasing the connexin26 mRNA and protein expression Increased Bad genes expression Decreased the expression of Bcl-2 genes Inhibiting the growth of ECA109 cells by apoptosis (Li *et al*., 2012)
Fennel (Aerial part)	Alcoholic extract	Enhance lymphocytes to reduce expression of IFN-γ and IL-4 (Pacifico *et al*., 2018)
Laurus nobilis Linn. (Lauraceae)	Ethanol extract	Enhance an acute lung injury mouse model to reduce IL-1β, IL-6, and TNF-α expression (Lee *et al*., 2019)
Peppermint (*Mentha piperita*)	Maceration (Water)	Reduce IL-1, IL-6, and TNF-α in Male Wistar rats (Osman *et al*., 2020)

An alkali-soluble polysaccharide extracted from the *Angelica sinensis* plant can boost the functions of immune cells, including NK cells, macrophages, and lymphocytes. It could also raise the levels of immune cytokines, including IFN-γ, interleukin (IL)-2, and tumour necrosis factor-α (TNF-α), which ultimately enhance apoptosis in mice hepatic cancer cells (H22) by blocking the G0/G1 phase (Yu *et al*.,

2021). Almutairi and co-workers documented that the extracted seed of *Annona muricata* had anti-cancer properties and could induce apoptosis by expressing pro-apoptotic proteins, such as p53 and Bax protein (Almutairi *et al*., 2023).

2.1.2 Immunomodulatory effects by medicinal plants

Immunostimulators and immunosuppressants are common immunomodulators (Gruppen *et al*., 2018). Several medicinal plants have biological properties, including immunomodulatory effects (Lin *et al*., 2023). Previous research indicates that the most effective method to preventing and treating diseases is through immunostimulation with natural ingredients. Based on prior research, medicinal plants, such as *Cyrtomium macrophyllum* (Ren *et al*., 2014), *Phyllanthus urinaria* (Ilangkovan *et al*., 2013), and *Asparagus racemosus* (Gautam *et al*., 2004), have been reported to have immunomodulatory properties by suppressing or stimulating immune cells (Krensky *et al*., 2011). In a human model experiment, the researchers reported that *Astragalus membranaceus* root can decrease IL-6 levels (Denzler *et al*., 2010). Duansak and colleagues demonstrated in Wistar Furth rat models that the *Aloe vera* plant can lower TNF-α and IL-6 (Duansak *et al*., 2003). A prior study showed that the rhizome extracts of the *Acorus calamus* inhibit nitric oxide, IL-2, and TNF-α production (Mehrotra *et al*., 2003). Hussain and co-workers documented that ethanolic and aqueous extracts of the *Picrorhiza kurroa* plant can stimulate humoral responses by antibody production, the release of mediators of hypersensitivity reactions, and tissue responses to these mediators in the target organs (Hussain *et al*., 2013).

2.1.3 Phytochemicals in medicinal plants

Plants produce a wide range of phytochemicals, which are classified into two categories based on their role in plant metabolism: primary metabolites, which comprise proteins, amino acids, sugars, and chlorophyll (Hussein & El-Anssary,

2019), and secondary metabolites, including alkaloids, flavonoids, terpenoids, phenolics, and steroids, sesquiterpenes, diterpenes, triterpene saponins, and triterpene aglycones. These chemical compounds are naturally synthesised in the various parts of plants, such as, leaves, stems, roots, seeds, and flowers (Jan *et al*., 2021). Flavonoids, tannins, alkaloids, and phenolic chemicals have the most significant biological properties and exert substantial physical effects on the human body. For example, phenolic compounds play a role in increasing bile secretion and reducing blood cholesterol (Ghasemzadeh *et al*., 2010), and flavonoids have potential as a treatment for various malignancies (Kleemann *et al*., 2011). Alkaloids, including vindesine, vinorelbine, vinblastine, and vincristine, have been proven in previous studies to have potent anti-cancer properties. For instance, vincristine and vinblastine against breast cancer, lymphoblastic leukaemia, and skin cancer (Mishra & Verma, 2017), vinorelbine against liver and colon cancer (Liu *et al*., 2020), whereas vindesine against lung cancer (Arora *et al*., 2010).

Previous studies demonstrated and confirmed that secondary metabolites play an essential role in human therapy against various ailments, including chronic or infectious diseases and cancer (Sharifi-Rad *et al*., 2016). Previous studies documented that bioactive compounds have anti-bacterial, antioxidant, anti-fungal, anti-diabetic, anti-cancer, anti-viral, and anti-inflammatory properties (Umaru *et al*., 2018). A variety of phytochemicals, including tannins, alkaloids, phenols, naphthoquinones, flavonoids, saponins, steroids, carbs, mucilage, gum, and resin, were extracted from the methanolic extract of *Carissa macrocarpa* leaves. According to the findings, these secondary metabolites boost the anti-bacterial effects of *Carissa macrocarpa* on *Escherichia coli* and *Staphylococcus aureus* (Ramasar *et al*., 2022). Four secondary chemicals were extracted from *Senna petersiana* leaves, including Gamma-

Linolenate, columnidin, L-lysine citrate, and hercynine. These compounds exhibited their anti-bacterial properties towards *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Mycobacterium smegmatis* (Matotoka *et al*., 2023). According to the phytochemical profile, the stem bark of *Beilschmiedia roxburghiana* is abundant with flavonoids, polyphenols, alkaloids, and tannins. These phytochemicals enhance antioxidant and anti-bacterial agents of various bacteria, such as *Salmonella typhi*, *Staphylococcus aureus*, *Shigella sonnei*, *Acinetobacter baumannii*, and *Klebsiella pneumonia* (Khanal *et al*., 2022).

Apart from that, *Abutilon indicum*, comprising leaf, root, flower, and seed has been used as an antioxidant due to its rich bioactive compounds, such as mucilage, tannins, organic acids, traces of asparagine, magnesium phosphates, calcium carbonate, alkaline sulphates, sterols, alkaloids, glycosides, titerpenoids, amino acids, oils, terpenoids, steroids, terpenes, and flavonoids, which might contribute to its wide range of uses in the human body, including treatment of fever, toothache, blood dysentery, allergies, piles, leprosy, cystitis, bronchitis, and gonorrhoea. Additionally, it is used as an aphrodisiac and a diuretic (Panda, 1999).

2.2 *Pereskia bleo* (Cactaceae)

Cacti are well-known desert plants distinguished by their distinctive stem and leaf development forms. There are roughly 2000 species in the Cactaceae family, which has 100 genera (Zareisedehizadeh *et al*., 2014). Maihuenioideae, Pereskioideae, Opuntioideae, and Cactoideae are the four subfamilies. Over 90% of the species belong to the Opuntioideae and Cactoideae subfamilies (Ortega-Baes *et al*., 2010). The ecological characteristics are connected to morphological and physiological changes that enable them to retain more water and survive in arid environments (Edwards & Diaz, 2006; Edwards & Donoghue, 2006).

2.2.1 General description of *Pereskia bleo*

P. bleo belongs to the plant botanical family Cactaceae, commonly known as "Pokok Jarum Tujuh Bilah" in Malaysia (Abdul-Wahab *et al*., 2012), while in Chinese, it is called "Cak Sing Cam" or "Qi Xing Zhen" (Wahab *et al*., 2009). It is recognised by many English names, including rose cactus, wax rose, and leaf cactus (Yen *et al*., 2013). The genus Pereskia belongs to the Pereskioideae (Hunt, 2016). Except for *P. bleo*, which lives in areas with higher annual rainfall, most *Pereskia* species are found in dry forests or thorny scrubs in tropical regions with a dry season and very arid forest life (Edwards & Donoghue, 2006). *P. bleo* is used as a medicinal plant that originated from South America, Brazil, Mexico, and Central America. It is widely distributed and cultivated in many tropical and subtropical regions, including Malaysia, China, and India (Zareisedehizadeh *et al*., 2014). *P. bleo* can reach a height of 0.8–8 m. It has bright green and large leaves, orangish-red blooms (depending on the species; flowers might be white, yellow, or red) that can occur singly or in groups, and long spiky stems (5 to 7 black spines of 1 cm in length). However, there are only 1 to 4 young shoots. The formation of areoles may be seen in these spines, which have 1 to 5 cm diameter and resemble roses (Figure 2.1). Fruits are commonly waxy, round, and green with dark shading, which changes to yellow when ripe (Yen *et al*., 2013). *P. bleo* has been shown to have anti-cancer, anti-rheumatic, anti-ulcer, anti-inflammatory, antioxidant, and anti-microbial properties in previous research (Zareisedehizadeh *et al*., 2014).

Figure 2.1: A: The stem of *P. bleo* (Zareisedehizadeh *et al*., 2014), B. leaves and the orangish-red flowers of *P. bleo* (Abdul-Wahab *et al*., 2012).

2.2.2 Classification of *P. bleo*

Table 2.4: *P. bleo* categorization (Butterworth & Wallace, 2005).

Division	Class
Kingdom	*Plantae*
Phylum	*Tracheophyta*
Class	*Magnoliopsida*
Order	*Caryophyllales*
Family	*Cactaceae*
Subfamily	*pereskioideae*
Genus	*Pereskia*
Species	*Pereskia bleo*

2.2.3 Medicinal uses of *P. bleo*

The leaves of *P. bleo* contain carbon (50.6%), magnesium (0.4%), sulphur (1.5%), oxygen (35.4%), chloride (1.2%), potassium (10.16%), phosphorus (0.4%), and calcium (0.3%) (Abbdewahab *et al*., 2009); hence, it is used either in healthy soups or eaten raw as salad (Garcia *et al*., 2019). *P. bleo* leaves are commonly used in traditional medicine for cancer, high blood pressure, diabetes, rheumatism and inflammation, atopic dermatitis, ulcers, gastric pain, headaches, and haemorrhoids (Vijayablan *et al*., 2021). Potassium is abundant in *P. bleo* leaves (10.16%), and a high-potassium diet has been demonstrated to impact blood pressure reduction significantly (Geleijnse *et al*., 1994). *P. bleo* is also used to cure gastrointestinal

disorders in Panama (Gupta *et al*., 1996). The plant has been reportedly prepared in various ways, commonly consumed raw or as a decoction made from the leaves. The traditional uses and preparation methods of *P. bleo* are shown in Table 2.5.

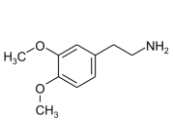
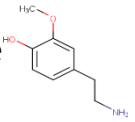
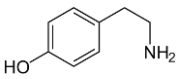
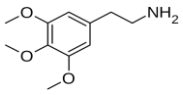
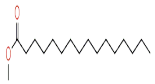
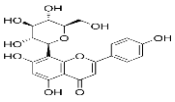
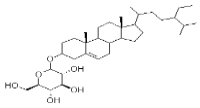
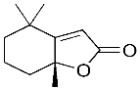
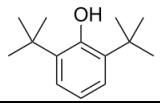
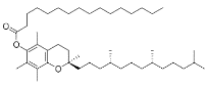
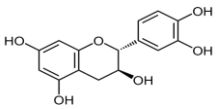
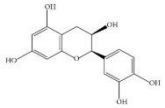
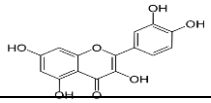
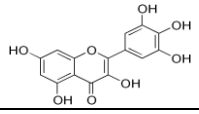
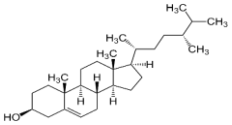
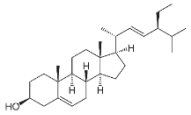
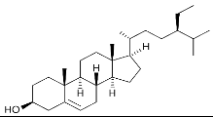
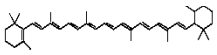
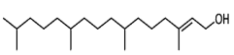
Table 2.5: Traditional usage and methods of preparation of *P. bleo.*

Purpose	Method of preparation
Detoxification and prevention of cancer	Boiling the leaves or fruit to make tea (Rahmat *et al*., 2013)
Dietary purposes and health maintenance	Eating the raw leaf, flower, and fruit (Malek *et al*., 2009)
Health maintenance and revitalising the body	Boiling the leaves in water and extracting the juice (Rahmat *et al*., 2013)
To relieve muscular pain	Making decoction from the leaves and then using as a warm bath for muscle ache (Gupta *et al*., 1993)
To treat haemorrhoids, hypertension, diabetes, infections, headaches, and inflammatory conditions (rheumatism and asthma).	No information is available in the literature (Malek *et al*., 2009; Er *et al*., 2007)

2.2.4 Chemical constituents of *P. bleo*

Alkaloids (namely 3,4-dimethoxy-β-phenethylamine, mescaline, 3-methoxytyramine, and tyramine), carotenoids, flavonoids, terpenoids, sterols (e.g., beta-carotene), fatty acids, lactone, phytosterol glycoside, phenolic compounds, and alpha-tocopherol, are phytochemical compounds of *P. bleo* leaves (Johari & Khong, 2019). On the other hand, Goh verified that stigmasterol, β-sitosterol, dihydroactinidiolide, and campesterol compounds were also isolated from *P. bleo* (Goh, 2000). Meanwhile, β-sitosterol, 2,4-ditert-butylphenol, and phytol were isolated through ethyl acetate extraction of *P. bleo* in another investigation (Malek *et al*., 2007) (Table 2.6).

Table 2.6: Components of *P. bleo* leaves (Vijayablan *et al*., 2021).

Alkaloids	3,4-Dimethoxy-β-phenethylamine		3-Methoxytyramine	
	Tyramine		Mescaline	
Fatty acids	Methyl palmitate		Methyl linoleate	
Flavonoids	Vitexin			
Phytosterol glycoside	β-Sitosterol glucoside			
Lactone	Dihydroactinidiolide			
Phenolic compounds	2,4-Ditert-butylphenol		α-Tocopherol	
	Catechin		Epicatechin	
	Quercetin		Myricetin	
Sterols	Campesterol		Stigmasterol	
	β-Sitosterol			
Terpenoids	β- Carotene		Phytol	

2.2.5 Bioactivity of *P. bleo*

The *P. bleo* plant was found to have a wide range of pharmacological activities, such as anti-nociceptive, anti-bacterial, antioxidant, anti-microbial, anti-diabetic, and anti-cancer (Azizan *et al*., 2024). The bioactive compounds discovered in the extract are linked to the powerful pharmacological properties of the plant. For instance, researchers have shown that flavonoid chrysin isolated from *P. bleo* extracts has medicinal properties for treating diabetes. It may lower triglyceride and glucose levels (Mat Darus & Mohamad, 2017), and downregulate the expression of pro-inflammatory cytokines linked to the onset of diabetes and its complications, such as atherosclerosis and other heart disorders (Ramírez-Espinosa *et al*., 2017). Additionally, chrysin and Apigenin 6-C-glucoside, discovered in *P. bleo* extract, play a major role in elevating insulin production (Mat Darus & Mohamad, 2017). Chan and co-workers reported that the extract from *P. bleo* leaves was found to exhibit antifungal activity on *Trichoderma mentagrophytes, Cryptococcus neoformans*, *Aspergillus brasiliensis, Candida albicans*, *Issatchenkia orientalis*, and *Candida parapsilosis* (Chan *et al*., 2018). Previous research findings show that the *P. bleo* leaves extract exhibited antibacterial properties on *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* (Lee HongLim *et al*., 2009). Apart from the leaves, hexane, ethyl acetate, and dichloromethane extracts of *P. bleo* exhibited notable anti-nociceptive properties (Abdul-Wahab *et al*., 2012). Research conducted *in vitro* revealed that plants abundant in phenolic compounds have antioxidant properties. *P. bleo* leaves, which are abundant in phenolic compounds, were shown to have significant antioxidant properties according to DPPH test findings (Hassanbaglou *et al*., 2012). Phytol extracted from *P. bleo* leaves also exhibited anti-cancer activity in various cancer cells (Malek *et al*., 2009). The EtOAc fraction (β-sitosterol, 2,4-di-tert-

butylphenol, α-tocopherol and phytol) exhibits specific anti-cancer effects on human carcinoma cells. Furthermore, the ethyl acetate fraction was more potent against human colon cancer (HCT116) and breast cancer cells (MCF-7) (Malek *et al*., 2007). Based on their research, Mohd-Salleh *et al*. concluded that *P. bleo* leaf extracts had significant anti-cancer activity towards HeLa cervical cancer cells and MDA-MB-231 breast cancer cells (Mohd-Salleh *et al*., 2020a).

2.3 Cancer

2.3.1 Cancer and prevalence

Cancer is derived from the Latin term carcinoma, which means crab. Previous cancer research witnessed tremendous improvement in the knowledge of cancer biology. Cancer originates from changes or mutations in genetic material (DNA). Moreover, cells in an organ proliferate uncontrollably, resulting in the formation of aberrant or abnormal cells known as cancer cells (Sitki-Copur, 2019). Tobacco, environmental contaminants, exposure to ionising radiation, infectious diseases, such as hepatitis B/C, helicobacter pylori and Human immunodeficiency virus (HIV), poor nutrition, and inherited genetic defects are among the causes of cancer (Blackadar, 2016).

Globally, the most frequent cancer therapies are surgery, radiation therapy, chemotherapy, hormone therapy, and immunotherapy (American Cancer Society, 2015; Siegel *et al*., 2021). Radiation therapy and chemotherapy are currently prestigious medical specialties, encompassing subspecialties such as medical oncology. Chemotherapy and radiation therapy, on the other hand, can destroy DNA material while also harming normal cells, leading to a variety of side effects, such as vomiting, fatigue, nausea, hair loss, and in some cases, death (Aslam *et al*., 2014).

According to the data from the WHO in 2020, cancer is the second most significant cause of death worldwide (Ferlay *et al*., 2020). According to available data, in 2021, there were approximately 192,000 new cases of cancer and 61,000 fatalities attributed to cancer (Siegel *et al*., 2021). Globally, 2020 witnessed a significant increase in the number of cancers (Figure 2.2A), with around 19.29 million and 10 million cancer-related deaths (Figure 2.2B) (World Health Organization, 2022; Gonzalez-Valdivieso *et al*., 2021).

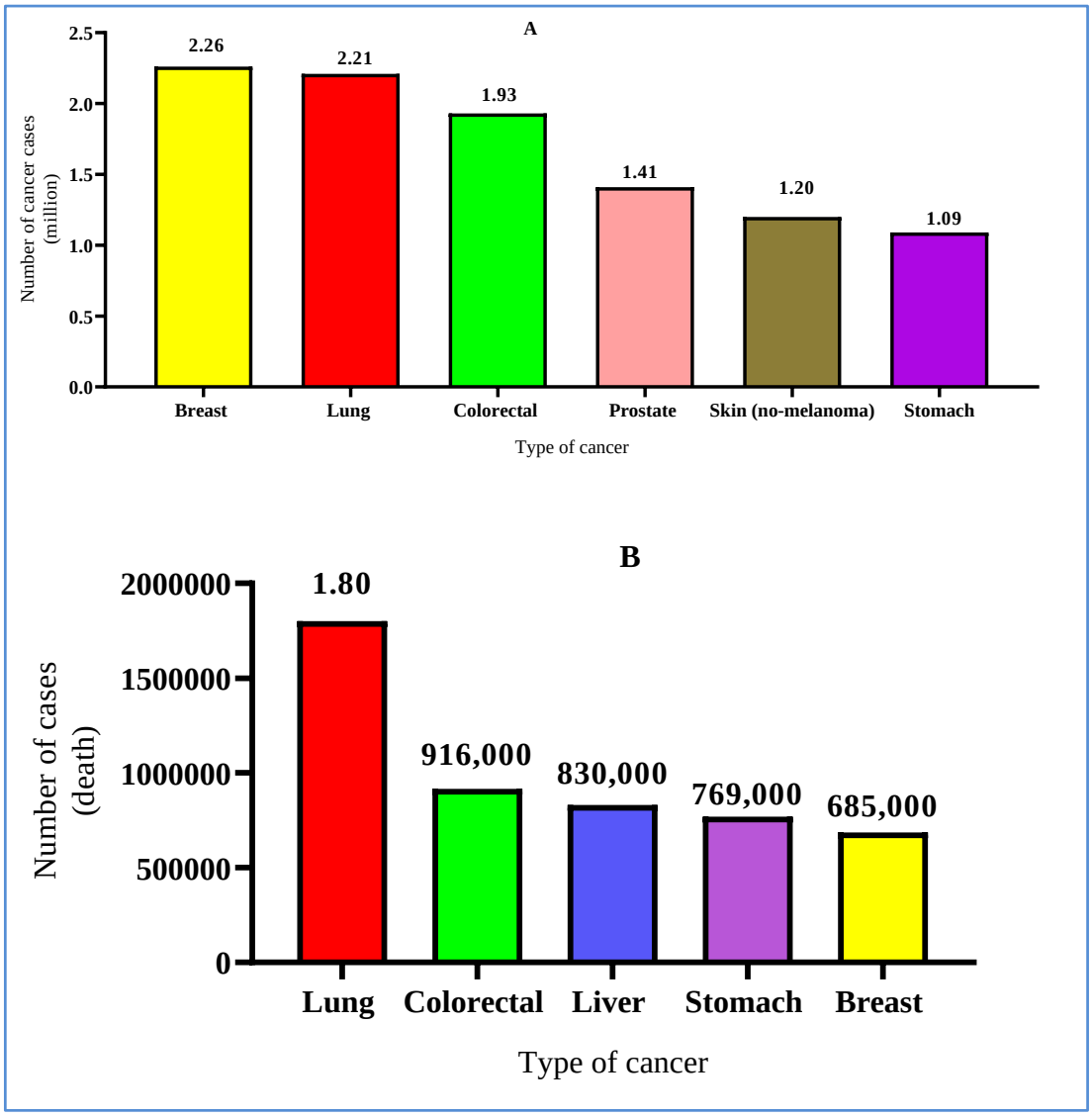


Figure 2.2: Incidence (A) and mortality rate of cancer (B) worldwide, 2020 (World Health Organization, 2022; Gonzalez-Valdivieso *et al*., 2021).

Non-communicable diseases (NCDs) are on the rise and are responsible for approximately 73% of fatalities in Malaysia. These ailments include cardiovascular diseases, diabetes, cancers, and respiratory diseases (Institute for Public Health, 2020). Nevertheless, the most prevalent ailment in Malaysia is cancer. According to the WHO, cancer incidence rate and mortality are increasing year by year in Malaysia and worldwide. For instance, around 103,507 new cases in 2007 to 2011 (MALAYSIA National Cancer Registry Report, 2007–2011), 115,238 new cases in 2012 to 2016 (MALAYSIA National Cancer Registry Report, 2012–2016), and 48,639 new cancer cases in 2020 were reported. Cancer incidence and mortality rates are expected to grow by the year 2040. The incidence rate of cancer in 2020 for both sexes of all ages is illustrated in Table 2.7 (World Health Organization, 2022).

Table 2.7: The incidence rate of cancer in both sexes for all ages (World Health Organization, 2022).

Rank	Sexes								
	Both sexes			Females			Males		
	Cancer	New cases	% of cancers	Cancer	New cases	% of cancers	Cancer	New cases	% of cancers
1	Breast	8,418	17.0%	Breast	8,418	32.9%	Lung	3,925	17%
2	Colorectal	6,597	10.6%	Colorectal	3,057	11.9%	Colorectal	3,540	15.4%
3	Lung	5,139	10.6%	Ovary	1,836	7.2%	Prostate	2,146	9.3%
4	Nasopharynx	2,222	4.6%	Cervix uteri	1,740	6.8%	Nasopharynx	1,703	7.4%
5	Liver	2,149	4.4%	Corpus uteri	1,401	5.5%	Liver	1,553	6.7%
6	Other cancers	24,114	49.6%	other cancers	9,135	35.7%	Other cancers	10,185	44.2%