ELUCIDATING THE *IN VITRO* ANTIPROLIFERATIVE PROPERTIES AND ASSOCIATED MECHANISMS OF *MOMORDICA COCHINCHINENSIS SPRENG* (GAC FRUIT) AQUEOUS EXTRACT USING COLORECTAL CANCER ORIGIN CELL LINES

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by

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A major research project like this is never the work of anyone alone. The contributions of many different people, in their different ways, have made this possible. I would like to extend my sincere appreciation.

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LIST OF SYMBOLS AND ABBREVIATIONS

µg	Micrograms
µL	Microlitre
APS	Ammonium persulphate
ATP	Adenosine triphosphate
BSA	Bovine serum albumin
CCND1	Protein-coding gene for Cyclin D1
CCNE1	Protein-coding gene for Cyclin E1
CDK2	Cyclin dependent-kinase 2
CDK4	Cyclin dependent-kinase 4
CDK6	Cyclin dependent-kinase 6
CDKN1A	Protein-coding gene for p21
CDKN2A	Protein-coding gene for p16 and p14
CDKN2C	Protein-coding gene for p18
cDNA	Complementary deoxyribose nucleic acid
CM	Complete medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2, 2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
TGFβ	Transforming growth factor beta
ER	Estrogen receptor
ERK	Extracellular signal -regulated protein kinase
FBS	Fetal bovine serum
FRAP	Ferric ion reducing antioxidant power
$FeCl_3$	Ferric chloride
g	Grams
GTP	Guanosine triphosphate
H_2SO_4	Sulfuric acid

HCl	Hydrochloric acid
HER2	Human epidermal growth factor receptor 2
JNK	c-Jun NH2-terminal kinase
L	Litre
MAPK	Mitogen-activated protein kinase
min	Minute(s)
mg	Milligrams
MTT	3-[4, 5-dimethyl thiazol-2-yl] 2, 5-diphenyl tetrazolium bromide
Na_2CO_3	Sodium carbonate
NH_3	Ammonia
p38 MAPK	p38 mitogen-activating protein kinase
p53	Tumor suppressor protein p53
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PR	Progesterone receptor
qPCR	Quantitative real-time polymerase chain reaction
R_2	correlation coefficient
RNA	Ribonucleic acid
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
SEM	Standard error of mean
TAK1	Transforming growth factor-β (TGF-β)-activated kinase 1
TGF-β1	Transforming growth factor beta 1
TBS	Tris-buffered saline
TBS-T	Tris-buffered saline with 0.1% Tween-20
TGF	Transforming growth factor

UV	Ultraviolet
v/v	Volume per volume
β-actin	Beta- actin

LIST OF APPENDICES

MENELUSIDASI SIFAT ANTIPROLIFERATIF SECARA *IN VITRO* DAN MEKANISME BERKAITAN EKSTRAK AKUEUS *MOMORDICA COCHINCHINENSIS SPRENG* (BUAH GAC) MENGGUNAKAN TITISAN SEL ASAL KANSER KOLOREKTAL

ABSTRAK

Momordica cochinchinensis S., juga dikenali sebagai buah gac, adalah buah tropika yang berasal dari Asia Tenggara. Walau bagaimanapun, ia belum diterokai sepenuhnya di Malaysia. Ekstrak yang diperolehi adalah ekstrak air aril (AW), ekstrak air pulpa (PW) dan ekstrak biji (SW), serta ekstrak etanol, iaitu ekstrak aril (AE), ekstrak pulpa (PE) dan ekstrak biji (SE). Kajian ini memberi tumpuan kepada komposisi fitokimia, antioksidan, antimikroba, antiproliferatif, dan sifat penyembuhan luka mereka. .Kedua-dua ekstrak air dan etanol dari aril, pulpa, dan biji mengandungi alkaloid, flavonoid, saponin, minyak volatile, dan gula mereduksi. Walau bagaimanapun, glikosida hanya hadir dalam ekstrak air (AW, PW, SW), sementara tanin hanya dikesan dalam SW. PW menunjukkan peningkatan dalam kandungan fenolik total (TPC); 0.0215 ± 0.00060 mg GAE/g manakala, kandungan flavonoid (TFC) telah diukur pada 0.083 ± 0.022 mg QE/g FW (TFC), masing-masing. Selain itu, ekstrak PW juga menunjukkan aktiviti antibakteria yang berpotensi, dengan nilai MIC antara 5 dan 20 mg/ml dan nilai MBC antara 10 dan 20 mg/ml terhadap *E. coli, P. aeruginosa, S. flexneri*, dan *B. cereus*. Kepekatan antara 1µg/ml dan 10 µg/ml ekstrak PE dan SW menunjukkan kesan positif dalam eksperimen penyembuhan luka. Objektif kajian ini adalah untuk menyiasat potensi sifat antiproliferatif ekstrak etanol dan air buah gac terhadap garis sel kanser kolorektal HT29 dan HCT116. Di samping

itu, kesan ekstrak pada kematian sel dan perkembangan kitaran sel dalam kedua-dua garis sel disiasat. Oleh itu, mekanisme yang mungkin terlibat dalam aktiviti ekstrak yang diperhatikan juga dijelaskan. Ujian MTT telah dijalankan untuk menilai kesan ekstrak ke atas kebolehlakuan kedua-dua baris sel, manakala sitometri aliran digunakan untuk menganalisis perkembangan kitaran sel. Ekspresi gen dianalisis menggunakan qPCR, manakala ekspresi protein dinilai menggunakan Western blotting. Penemuan menunjukkan bahawa ekstrak buah gac menyebabkan penurunan kelangsungan hidup sel kepada 60% selepas 72 jam rawatan dalam garis sel AW dan PW, dan pengurangan 50% dalam garis sel HT29 dan HCT116. Hasil ini mengesahkan kerentanan HCT116 dan HT29 terhadap AW, di mana LC_{50} adalah 1.10 µg/ml, dan 0.03 µg/ml dan PW, pada 21.44 µg/ml untuk HT29. Selain itu, ekstrak menyebabkan penangguhan kitaran sel G0 / G1 di kedua-dua garis sel HT29 dan HCT116. Kajian ekspresi gen menunjukkan tahap CCND1 yang berkurangan dan kinase bergantung siklin (CDK4 dan CDK6) berkurangan di kedua-dua barisan sel. p53 hadir dan dikawal dalam HT29 bersama dengan CDKN2A dan CDKN2C manakala CDKN2A dan CDKN1A dikawal dalam HCT116. Kajian protein menunjukkan, penurunan tahap p42 / 44 MAPK dan c-Myc dikesan dalam HT29 dan HCT116. c-Jun terlalu banyak dinyatakan dalam HCT116. Ekspresi berlebihan CDKN1A, CDKN2A dan CDKN2C bertanggungjawab untuk penangguhan kitaran sel G0 / G1 dalam HT29 dan HCT116 masing-masing. Selain itu, induksi p53 dalam HT29 juga boleh menjadi faktor yang menyumbang kepada penangguhan kitaran sel yang diperhatikan. Ekspresi gen dan protein juga menunjukkan bahawa proliferasi HT29 dan HCT116 dan penangguhan kitaran sel dihalang oleh laluan isyarat MAPK dan c-Jun. Kesan melalui p42 / 44 MAPK dan c- Jun dimulakan oleh peraturan turun siklin D1 dan c-Myc di kedua-dua garis sel. Kesimpulannya, laluan yang dicetuskan oleh ekstrak buah gac ditunjukkan

bergantung pada jenis sel. Akibatnya, ekstrak buah gac berpotensi untuk dijadikan sebagai kemungkinan untuk mewujudkan ubat baru terhadap pelbagai jenis kanser kolorektal.

ELUCIDATING THE *IN VITRO* ANTIPROLIFERATIVE PROPERTIES AND ASSOCIATED MECHANISMS OF *MOMORDICA COCHINCHINENSIS SPRENG* (GAC FRUIT) AQUEOUS EXTRACT USING COLORECTAL CANCER ORIGIN CELL LINES

ABSTRACT

Momordica cochinchinensis S., also known as gac fruit, is a tropical fruit native to Southeast Asia. However, it has not yet been fully explored in Malaysia. The extracts obtained were aril water extract (AW), pulp water extract (PW) and seed extracts (SW), and its ethanolic counterpart, namely aril extract (AE), pulp extract (PE) and seed extract (SE). The focus of this study in terms of their phytochemical composition, antioxidant, antimicrobial, antiproliferative, and wound healing properties. Both water and ethanolic extracts of the aril, pulp and seed contain alkaloids, flavonoids, saponins, volatile oil and reducing sugars. However, glycosides were only present in water extracts (AW, PW, SW), meanwhile tannins were detected only in SW. The PW exhibited an increased level of total phenolic content (TPC); 0.0215 ± 0.00060 mg GAE/g whereas, total flavonoid content (TFC) was quantitated at 0.083 ± 0.022 mg QE/g FW (TFC), respectively. Apart from that, the PW extract also exhibited potent antibacterial activity, with MIC values between 5 and 20 mg/ml and MBC values between 10 and 20 mg/ml against *E. coli*, *P. aeruginosa*, *S. flexneri*, and *B. cereus*. The concentrations ranged between 1µg/ml and 10 µg/ml of PE and SW extracts showed positive effects in the wound healing experiment. The aril of the fruit is known for its high concentration of carotenoids, such as beta-carotene and lycopene. These compounds are powerful antioxidants and have been shown to have an anti-proliferative effect on various types of cancer-causing cells. The aim of this study was

to investigate the potential antiproliferative properties of ethanolic and aqueous extracts of gac fruit against the colon cancer cell lines HT29 and HCT116. In addition, the influence of the extracts on cell death and cell cycle progression in both cell lines was investigated. In this way, the possible mechanisms involved in the observed activity of the extract were also elucidated. The MTT assay was performed to evaluate the effect of the extract on the viability of both cell lines, while flow cytometry was used to analyse cell cycle progression. Gene expression was analysed by quantitative real-time polymerase chain reaction while protein expression was examined by Western blotting. The results showed that the gac fruit extracts reduced the cell viability of the AW and Pulp Water PW by 60% and that of the HT29 and HCT116 cell lines by 50% after 72 hours of treatment. The results confirmed the susceptibility of HCT116, and HT29 towards AW, for which the LC_{50} were 1.10 µg/ml, and 0.03 µg/ml and PW, at 21.44 µg/ml for HT29. In addition, the extracts caused G0/G1 cell cycle arrest in the HT29 and HCT116 cell lines. Examination of gene expression showed that the levels of CCND1 and the cyclin-dependent kinases (CDK4 and CDK6) were reduced in both cell lines. p53 was present and upregulated in HT29 along with CDKN2A and CDKN2C, while CDKN2A and CDKN1A were upregulated in HCT116. The proteins expression revealed that p42/44 MAPK and c-Myc were decreased in HT29 and HCT116. c-Jun was overexpressed in HCT116. Overexpression of CDKN1A, CDKN2A and CDKN2C were responsible for G0/G1 cell cycle arrest in HT29 and HCT116, respectively. Apart from this, the induction of p53 in HT29 could also be a factor for the observed cell cycle arrest. Gene and protein expression also showed that proliferation of HT29 and HCT116 and cell cycle were inhibited by MAPK and c-Jun signalling pathways. The effect by p42/44 MAPK and c-Jun was triggered by downregulation of cyclin D1 and c-Myc in both cell lines. In

conclusion, the signalling pathways triggered by gac fruit extracts are cell type dependent. Consequently, gac fruit extract has the potential in the development of new drugs against various forms of colorectal cancer.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Cancer occurs when abnormal cells divide and multiply uncontrollably, leading to the massive growth of benign (non-cancerous) or malignant (cancerous) tumors. Benign tumors are non-invasive and can be easily removed by surgery. Malignant tumors, however, can invade the surrounding tissue and cause severe damage to vital organs or pose a life-threatening risk. The risk of developing this disease is often associated with lifestyle factors such as smoking, alcohol consumption, exposure to carcinogenic substances, radiation, stress and lack of exercise, especially in developing countries (Ng et al., 2020).

The World Health Organisation (WHO) has predicted that the global cancer burden will rise to around 30 million new cancer cases by 2040, with the largest increase occurring in low- and middle-income countries (WHO, 2023). According to the National Registration Department Malaysia, a total of 82,601 medically confirmed and non-medically confirmed cancer deaths were reported between 2012 and 2016 (National Cancer Institute, 2016). Colorectal cancer is the second most common cancer in Malaysia, with about 7,000 new cases per year, according to GLOBOCAN 2020 (GLOBOCAN, 2020). Colorectal cancer is the second most common cancer in Malaysian men and women and its prevalence is increasing with the growing population of more than 32 million.

Conventional cancer treatments include surgery at an early stage, followed by chemotherapy and radiotherapy. However, all these therapies are often associated with negative side effects such as fatigue, vomiting, skin irritation and pain. These therapies not only damage the tumor cells, but also normal cells. Furthermore, the high mortality

rate of cancer patients in developing countries, which is as high as 60 %, is due to the harmful effects of chemotherapeutic drugs on healthy tissue and organs (Jemal et al., 2010). Against this background, scientists have repeatedly tried to find novel cancer drugs or preparations that can serve as a more efficient alternative with fewer negative side effects.

Herbal medicinal products (HMs) have gained popularity in various parts of the world as an alternative to conventional cancer treatments (Bidram et al., 2019). The use of herbal medicines as a form of treatment shows a remarkable increase from 5.3% before cancer diagnosis to 13.9 % after diagnosis, making it the most used treatment category (Olaku and White, 2011). HMs are extracted from a variety of plant parts, including leaves, fruits, flowers, tubers, seeds and even roots (Nontokozo and Mthokozisi, 2018). These herbal compounds such as campthotecin, taxol, resveratrol and curcumin are known for their anti-cancer effects (Olaku and White, 2011, Wang et al., 2015). HMs improve the side effects of conventional therapies, prevent recurrence, and increase general well-being (Poonthananiwatkul et al., 2015).

The gac fruit "*Momordica cochinchinensis*" is an annual, dioecious fruit from the Cucurbitaceae family (also commonly referred as cucumber, gourd, melon or pumpkin (Gunasekaran et al., 2014, Chuyen et al., 2015). Originally, the gac fruit was cultivated in Thailand, Vietnam, Laos, Cambodia, and Myanmar. It is now also popular in several Southeast Asian countries such as India, China, Indonesia and Malaysia. The exceptional therapeutic and nutritional properties of the gac fruit have led to it being referred to as a "superfruit" or "heavenly fruit". This fruit, with its bright red colour, is easy to enjoy and contains high amounts of lycopene and β-carotene (Parks et al., 2013). In addition, it has significant amounts of phytonutrients such as α-tocopherol, fatty acids, polyphenolic compounds and flavonoids that contribute to human well-

being, including anti-inflammatory, antimicrobial and anti-cancer compounds (Yu et al., 2017, Innun, 2013, Kang et al., 2010).

According to estimates by the World Health Organisation (WHO, 2022), the global spending on health continually rose between 2000 and 2018 and reached US$ 8.3 trillion. In the year 2020, a year dominated by emergence of covid-19, a sharp increase in government spending on health at all country income levels underpinned the rise in health spending to a new high of US $9 trillion. It has been estimated that the funding for global healthcare has increased over the last two decades and this trend is expected to continue in the future. This increase in the trend would put a strain on both the economies of developing countries and healthcare systems. The return to folk practices and the use of herbal medicine, which is both cost-effective and side-effect free, could therefore help to alleviate this burden. Therefore, further studies on gac should be conducted to scientifically prove its benefits as a cancer drug.

The hypothesis of the study postulates that the diverse bioactive compounds in the gac fruit (*Momordica cochinchinensis*), particularly lycopene, β-carotene, and polyphenols, may contribute to its anti-proliferation properties by affecting multiple signaling pathways, including the MAPK pathway. These compounds may provide a synergistic effect with conventional cancer therapies, reducing drug resistance and enhancing the overall effectiveness of treatment. This study proposes that integrating gac fruit extract into standard cancer treatment regimens will lead to improved outcomes in terms of reduced proliferation of cancer cells and increased rates of apoptosis, compared to treatment with conventional cancer therapies alone.

1.2 Research Rationale

The gac fruit, *Momordica cochinchinensis*, also known as *terupang, makkao, mu bie zi, torobok* and *khaawz* in Southeast Asian countries (SA) (Chuyen et al., 2015). Gac, a high value-added fruit, contains five times as much lycopene as tomatoes, eight times as much carotene as carrots and an exceptional amount (60 times) of vitamin C compared to oranges (Thavamany et al., 2020). Its high content of lycopene and β-carotene contributes to the maintenance and improvement of vision (Abdulqader et al., 2018). In addition, it also contains fatty acids, vitamin E, polyphenols such as phenolic acids, flavonoids, and trypsin inhibitors (Lee et al., 2017, Bruno et al., 2018) These compounds are associated with a variety of bioactivities, including antioxidant, antimicrobial, anti-ulcer and anti-cancer properties, making them a source of potentially useful leads for the development of a novel anti-cancer drug (Thavamany et al., 2020). The current limitation in cancer treatment is drug resistance, side effects and limited effectiveness. This study was conducted to investigate Gac fruit as a natural source of these compounds providing an alternative to synthetic drugs, which often comes with side effects. Gac fruit may have the potential to act as complementary therapy along with conventional treatments to boost the efficacy of existing therapies. The study also focuses on MAPK pathways which are involved in regulating cell growth, proliferation, and apoptosis. The elucidation on the anti-proliferation properties of Gac and the possible mechanism(s) of action via MAPK signaling pathways helps to identify specific molecular targets that may be manipulated in cancer therapy. Many cancer drugs target single pathway or single molecule, making it easier for cancer cells to become resistant. Gac fruit which comes with diverse bioactive compounds may affect multiple signaling pathways which may contribute to more comprehensive and less resistant treatment.

1.3 Problem statement

Cancer continues to be a major global health challenge, with the number of cases projected to increase sharply, particularly in low- and middle-income countries. Current conventional treatments, such as chemotherapy and radiotherapy, often cause severe side effects and can damage healthy cells, contributing to high mortality rates. The increasing prevalence and mortality associated with cancers, such as colorectal cancer in Malaysia, highlight the urgent need for alternative therapeutic strategies that are both effective and have fewer negative side effects. Herbal medicinal products (HMs) have emerged as promising alternatives due to their potential to reduce side effects and enhance well-being. Among these, the gac fruit *(Momordica cochinchinensis*) is noted for its rich composition of anti-cancer compounds like lycopene, β-carotene, and polyphenols. However, the efficacy and mechanisms of gac fruit extracts in cancer treatment remain unexplored. This research aims to investigate the therapeutic potential of gac fruit extract in enhancing cancer treatment efficacy and reducing side effects, thereby providing a scientifically validated, cost-effective, and less harmful cancer treatment alternative.

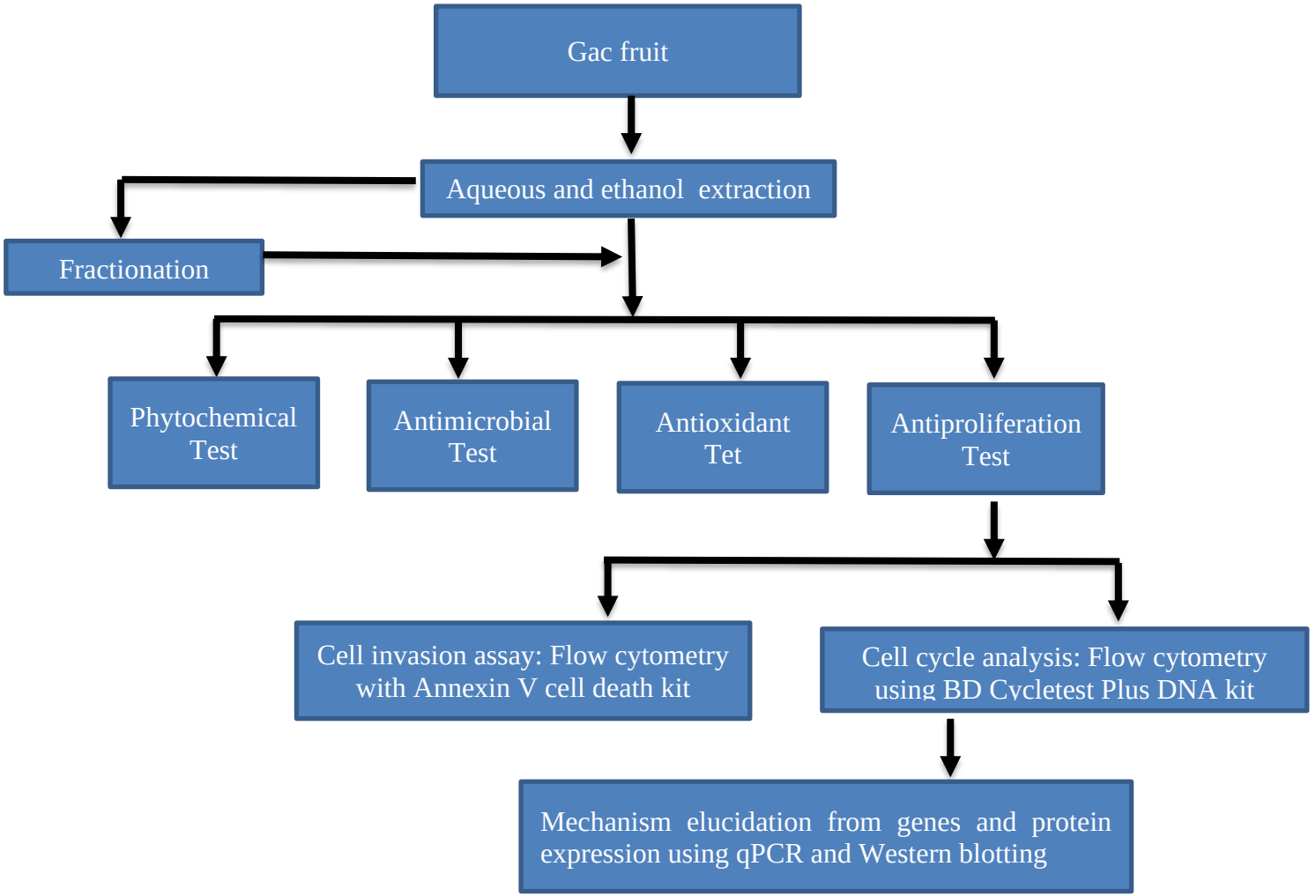
1.4 Research Objectives

The general objective of this thesis is to investigate the antiproliferative activities of Gac fruit and to determine the mechanisms by which these activities might be achieved.

The specific aims are as follows:

1. To examine the phytochemical composition, antimicrobial and antiproliferative activities of Gac fruit extracts.
2. To identify active subfractions of Gac fruit extracts through cytotoxicity testing against HT29 and HCT116 cancer cells lines.
3. To determine the effect of the active Gac fruit extracts on MAPK signaling pathway.

1.5 Flow chart of the research



1.6 Experimental design

The diagram outlines the various parts of scientific study on the Gac fruit. The objectives have been used as a guide to depict the experimental design of this study in Figure 1.1. Objective 1 involves converting the fruit into extracts and then subdividing these into subfractions. Subsequently, *in vitro* analyses such as phytochemical screenings, antioxidant tests, antimicrobial assessments, and wound healing experiments are carried out. In Objective 2, MTT assays are utilized to examine the extract's ability to inhibit cell growth, along with studies on cell death mechanisms and cell cycle impacts. Finally, Objective 3 focusses on determining the changes in gene and protein activities to elucidate the underlying biochemical pathways affected by the fruit.

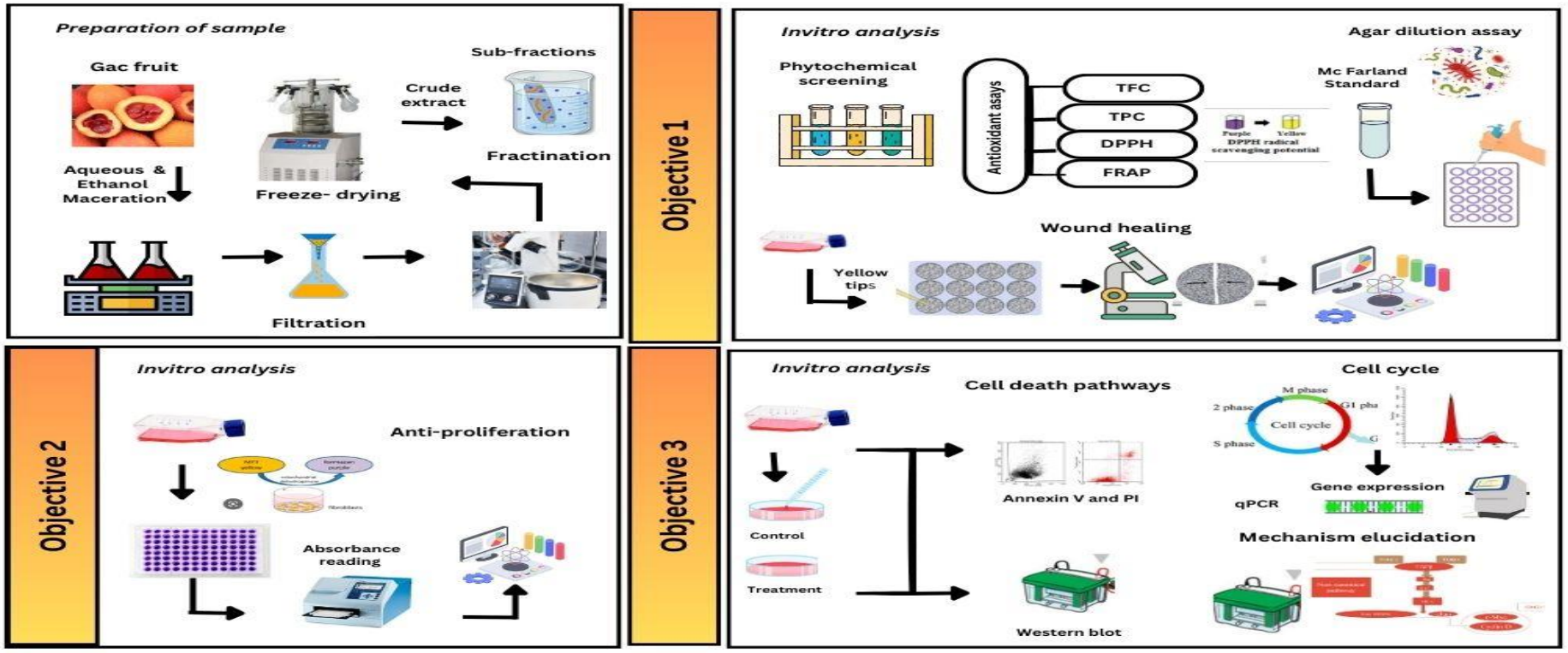


Figure 1.1 Schematic drawing of the experimental design. The image was created using Canva.com.

CHAPTER 2

LITERATURE REVIEW

2.1 Momordica cochinchinensis

2.1.1 Background of the plant

Momordica cochinchinensis Spreng is commonly found in Southeast Asian (SA) countries, particularly in Vietnam, China, Thailand, India, Indonesia and Malaysia. The fruit is popularly known as gac, Chinese bitter gherkin and cochinchin gourd (Thavamany et al., 2020). This fruit belongs to the melon family (Cucurbitaceae), which also includes cucumbers, pumpkins and bitter melons (Kubola et al., 2011). The fruit is classified botanically as follows: *Plantae* (kingdom); *Cucurbitales* (order); *Cucurbitaceae* (family); *Cucurbitoideae* (subfamily); *Momordica* (genus); *Momordica cochinchinensis* (species).

2.1.2 Local names

This fruit is called Tepurang in Malaysia and Indonesia. However, the name of the fruit varies by country Table 2.1.

2.1.3 Description of plants

The vines of the gac plant can grow up to 6 metres long over a fence. Gac is a perennial dioecious plant with separate male and female plants (Parks et al., 2013). The plant has 3-5 palmately lobed leaves and white to ivory-yellow flowers as Figure 2.1 (Bharathi and John, 2013). Gac plants typically bloom two months after planting. The female flowers have an unformed fruit that forms a protrusion at the base, while the male flowers have pale coloured petals leading to an open flower (Parks et al., 2013). Hand pollination by dusting the receptive stigmas increases fruit setting and yield, especially in the absence of native pollinators (Parks et al., 2013). On average,

a gac plant can bear up to 60 fruits in a season (Burke et al., 2005). The fruits ripen about 9 to 10 weeks after pollination. The ripe fruit can be harvested when the fruit skin changes colour from green to yellow, dark orange and finally red, as shown in (Tran et al., 2016). The fruit is hard at harvest but softens quickly after harvest, which causes problems in transporting the fruit and in its shelf life (Win et al., 2015). The fruit is mainly grown from the seeds, twigs and roots (Chuyen et al., 2015).

The gac fruit is valued for its health benefits as it contains an exceptional source of lycopene and beta-carotene. The content of lycopene in the fruit has been reported to be five times higher than that of tomatoes (Thavamany et al., 2020) and that of beta-carotene eight times higher than that of carrots (Thavamany et al., 2020) has also been reported that the fruit contains an astonishing amount (60 times) of vitamin C compared to oranges and 40 times more zeaxanthin than in yellow corn (Thavamany et al., 2020).

2.1.4 Gac fruit anatomy

The fruits of the gac are normally round or egg-shaped. However, a gac cultivar grown in India has been reported to have an elongated shape (Thavamany et al., 2020). The fruit consists of two main parts, namely the mesocarp and the endocarp. The mesocarp comprises orange/yellow spines covering the skin and a thick, spongy, orange-coloured layer called the pulp, as shown in Figure 2.2. The distribution of fruit spines ranges from smooth and dense to hard and widely spaced. The endocarp contains red, soft and sticky seeds that cover the black seeds (Vuong, 2000). Each fruit contains an average of 15 to 20 seeds. The seeds are mostly round, compressed and have a sculptured seed coat covered by a red aril, as shown in Figure 2.2. The yellow pulp accounts for 50 % of the total weight, while the aril, which contains the highest proportion of carotenoids, accounts for 10 – 25 % of the fruit weight. The skin and

seeds make up 17 % and 16 % of the total weight of the fruit respectively (Vuong, 2000; Kha et al., 2013; Chuyen et al., 2015). Parks et al. (2013) have shown a correlation between the size of the fruit, the weight and the proportion of seeds. The increase in fruit weight and size would lead to higher aril production. However, lower fruit weight may result from water loss during storage (Nhung et al., 2010; Win et al., 2015).

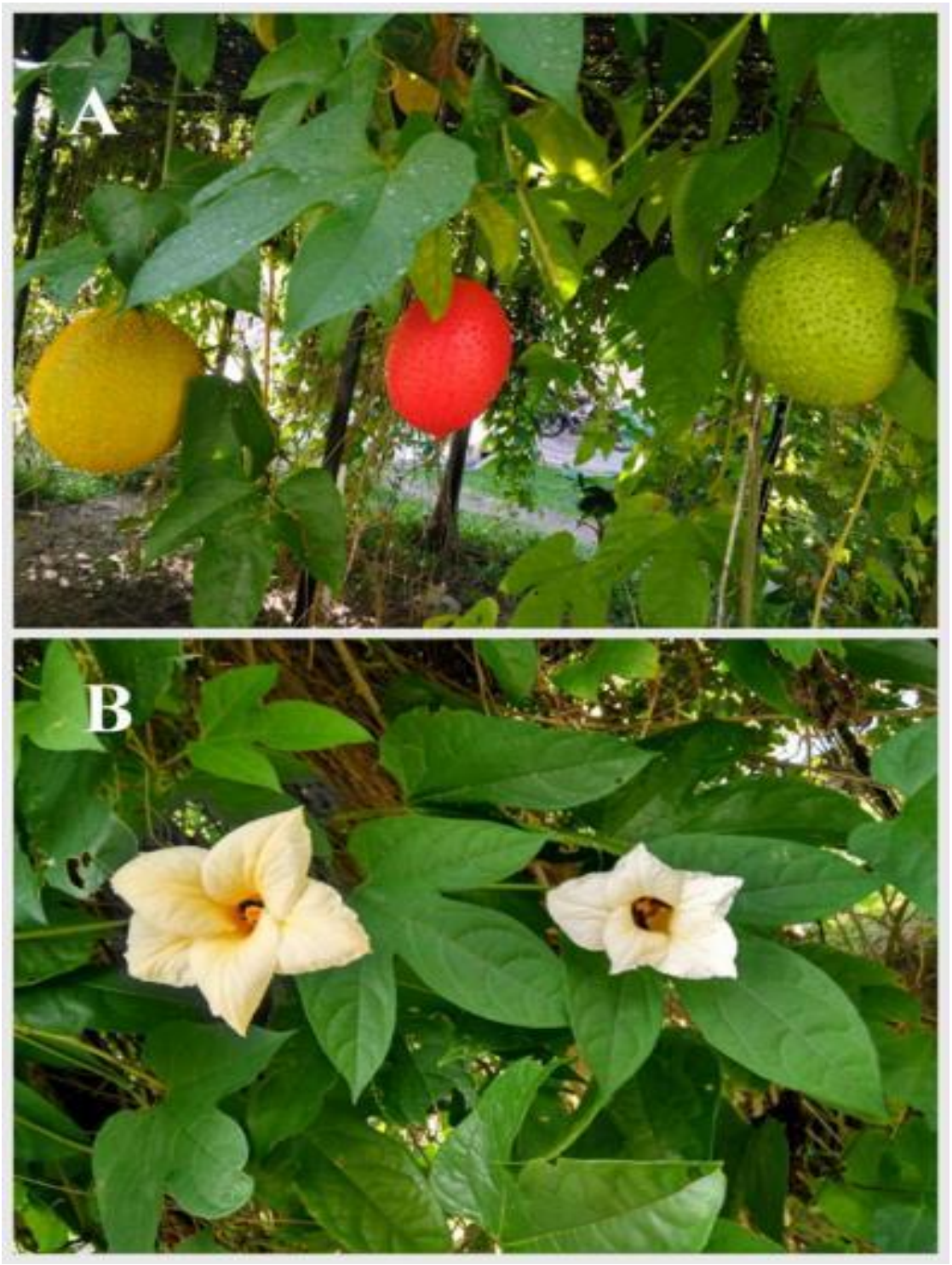


Figure 2.1 A) Leaves and fruits of Gac plant and B) Female (right) and male (left) flowers of Gac plant.

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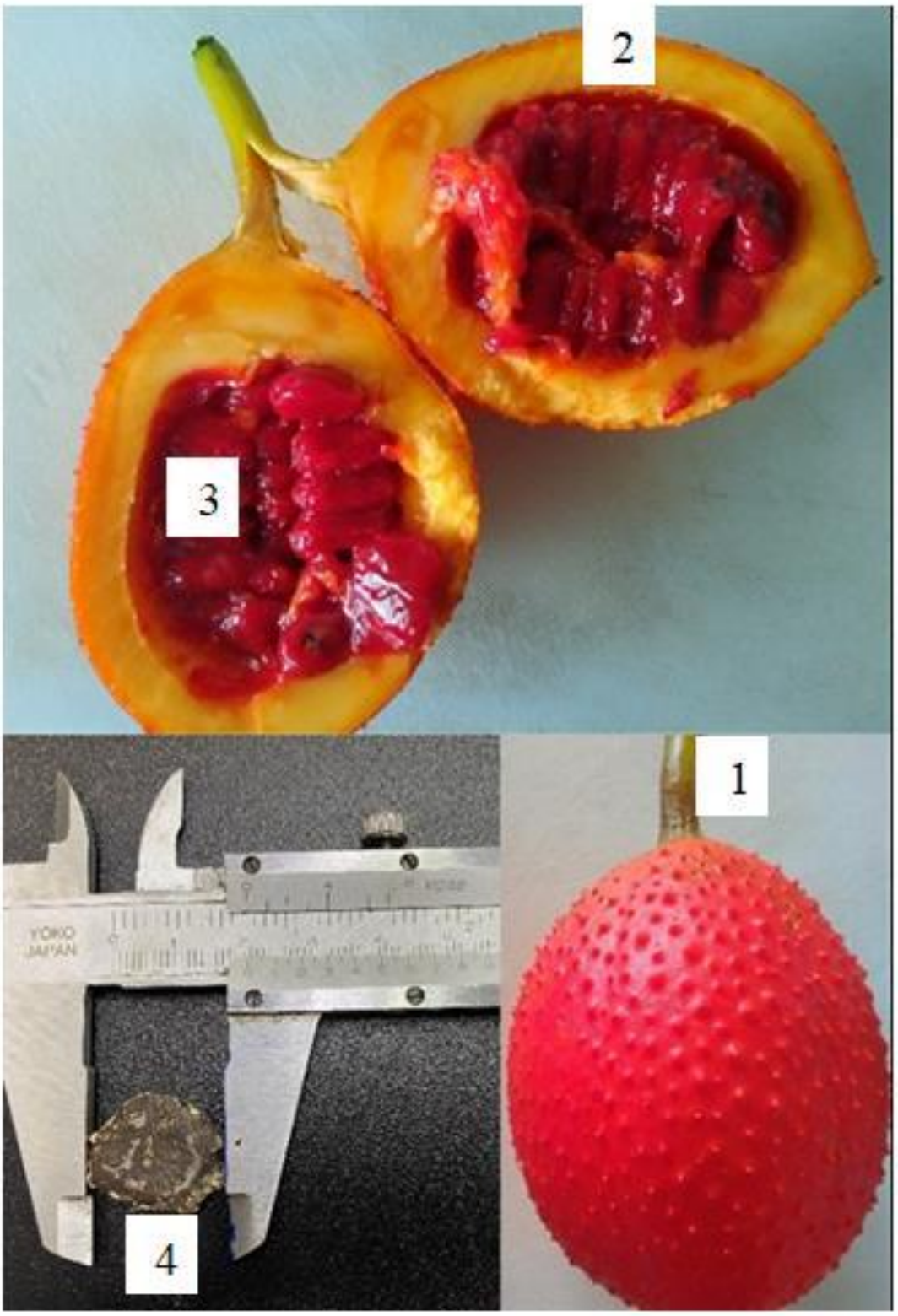

Figure 2.2 Anatomical parts of Gac fruit (1. Peel with spines, 2. Pulp,3. Aril and 4. Seed).

Table 2.1 Vernacular names of *Momordica cochinchinensis* based on the different Asian countries. Adopted from Lim, (2012).

Country	**Vernacular names**
Bangladesh	Kakrol
China	Da Ye Mu Bie Zi, Mu Bie, Mù-Biē Guǒ , Mu Bie Zi, Teng Tong, Tu Mu Bie
India	Bhat Kerala, Golkara, Kakrol, Gangerua, Gulkakra, Kakur, Kakrol, Kantola, Kathaamla
Indonesia	Pupia, Torobok, Toropu
Laos	Khaawz
Malaysia	Teruah, Terupang
Philippines	Tabog-Ok, Tabog- Uak
Thailand	Bai- Khai- Du, Fak-khao, Phak-Khao
	Khika-Khrua Yawd-Fak-Kao
Vietnam	Red Gac, Mộc Miết Tử
Cambodia	Makkao

2.1.5 Traditional uses

The consumption of gac fruit has been seen in diverse cultures worldwide (Tuyen et al., 2013). The delightful flavour of the ripe gac seed is commonly used as a colouring agent in the preparation of red sticky rice or xoi gac (Tuyen et al., 2013). This dish is usually served at New Year celebrations and weddings in Vietnam (Zheng et al., 2015). On the other hand, the young green fruits can be cooked with chilli paste or prepared as a meal. In Thailand, it is also used to prepare curries (Kubola and Siriamornpun, 2011). It is reported that fried dishes and soups become flavourful with the addition of arillus, resulting in a glossy appearance and distinct taste of the dish (Chuyen et al., 2015). Xerophthalmia, also known as dry eyes and night blindness, is mainly caused by provitamin A deficiency. According to a study by Chuyen et al. (2015), the consumption of aril oil from Gac fruit as a supplement can help prevent

xerophthalmia (Chuyen et al., 2015). The seeds of the fruit, called *mubiezhi* (in Chinese: 木鳖子), play an important role in the treatment of breast cancer in traditional Chinese medicine (Zheng et al., 2015). It has been shown that the ethyl acetate seed extract effectively suppressed the proliferation of MDA-MB231 cells and induced triggered cell cycle arrest and apoptosis (Zheng et al., 2015). Traditionally, the seeds have been utilized for the treatment of many skin problems such as boils, pyodermatitis, ringworm, freckles, sebaceous glands and conditions such as mastitis, tuberculous cervical lymphadenitis, haemorrhoids and haemangiomas (Zheng et al., 2015). The ground gac seeds are used both orally and externally to treat inflammatory scrofula, swellings and tinea. Additionally, it can also be used to treat diarrhoea and purulent skin infections such as wounds, carbuncles, boils and furuncles in humans and animals (Thavamany et al., 2020). In Vietnam, the roots of the plant can promote blood circulation and urination by eliminating damp heat (Tran et al., 2016). The roots are applied for the treatment of rheumatism, inflammation, leg swelling and oedema (Tuyen et al., 2013, Tran et al., 2016).

2.1.6 Bioactive compounds of Gac fruit

The gac fruit is renowned not only as a prime source of carotenoids, such as beta-carotene and lycopene but it also boasts essential fatty acids and other compounds such as α- tocopherol (vitamin E), phenolic compounds and flavonoids. As a reservoir of bioactive compounds, it contributes to health benefits through its provitamin A activity, antioxidant, antimicrobial and anticancer properties (Tinrat et al., 2014, Chuyen et al., 2015, Zheng et al., 2015).

2.1.6(a) Carotenoids

Carotenoids were first identified in gac in the early 1990s by Guichard and Bui (1941). Compared to other lycopene-rich vegetables, gac has a high content of beta-carotene and lycopene (Burke et al., 2005). Numerous studies have reported that a lycopene-rich diet is associated with a lower risk of cardiovascular disease and cancers such as lung, breast, stomach and prostate cancer (Lu et al., 2011, Kha et al., 2013,). Gac aril was found to have the highest concentration of β-carotene of all fruits and vegetables. The β-carotene content in gac aril was found to be eightfold more than that amounts observed in carrots (Thavamany et al ., 2020).In addition, the consumption of gac-enriched xoi gac (rice dishes) containing 3.5mg of β-carotene also showed a substantial increase in β-carotene and retinol concentrations in human plasma than the rice dishes enriched with synthetic β-carotene (Vuong, 2000). Since β-carotene is a precursor of vitamin A, gac fruit is therefore a potentially valuable source of this vitamin and could be extremely useful in combating vitamin A deficiency, which is prevalent in developing nations (Yu et al., 2017).

The aril membrane of the gac fruit is a potentially valuable source of carotenoids. In a study on the total concentration of lycopene in the ripest sample of Gac, a value of 305.3 mg/ 100g fresh weight (FW) was determined, compared to 4-5

mg/ 100g FW in commercially available tomatoes (Thavamany et al., 2020). Other studies on Gac fruit also observed Gac fruit, but the results of the studies varied, such as 48.1mg/100g fresh weight (FW) (Thavamany et al., 2020), 49.7mg/100g FW 2mg/100g FW (West and Poortvliet, 1993) and 410.7mg/ 100g FW (Nhung et al., 2010). Factors that may be responsible include variety, genotype, season, geographical location, maturity stage, growing conditions and storage conditions (Kha et al., 2013). The factors affecting carotenoid content in gac fruit need to be further investigated to improve carotenoid yield.

In addition to these carotenoids, other carotenoids such as lutein, zeaxanthin and β-cryptoxanthin have also been detected in the gac fruit. Lutein is also frequently used in the treatment of eye disease. The concentration of lutein is higher in the peel than in the fruit skin or flesh (Kubola and Siriamornpun, 2011). Lutein was found to be present in all parts of the fruit, with the peel and pulp having higher concentrations, 12 480 and 1448 µg g -1 FW, respectively. According to Aoki et al., (2002), 9µg zeaxanthin g -1 FW and 2µg β-cryptoxanthin g-1 FW were found in gac aril, while 1.6µg zeaxanthin g -1 FW and 3.5µg β-cryptoxanthin g -1 FW were found in gac pulp. In addition to the aril, the yellow pulp and peel are also good sources of carotenoids and should not be overlooked as sources of carotenoids.

2.1.6(b) α- Tocopherol

Vitamin E (α-tocopherol) was found in gac oil at a concentration of 357μg/ mL-1 and 76 μg/ mL-1 FW, which is higher compared to other fruits (Le Khac Lam Dien and Minh, 2013). Thus, the consumption of vitamin E in gac oil or aril could contribute to the daily intake of vitamin E. In addition, vitamins A, C and E are used in cosmetic components due to their antioxidant effects. Gac aril oil is a valuable source of vitamin E and omega-3 fatty acids (Le Khac Lam Dien and Minh, 2013). The antioxidant content in gac aril is 5 to 10 times higher than in other sources such as grapefruit, guava and tomato (Kubola et al., 2013). In a clinical study conducted by Leevutinun et al., (2015), gac cream was a non-irritating product that brightened the skin, significantly increased its smoothness and moisturization and reduced skin wrinkles. Gac extract can therefore be considered an effective component of an anti-wrinkle cream.

2.1.6(c) Polyphenolics and flavonoids

The gac fruit contains phenolic compounds and flavonoids (Kubola and Siriamornpun, 2011). The aril had the highest concentrations of total phenolic acid 4.3 mg gallic acid equivalents and 2.1 mg rutin equivalents of total flavonoids. Gallic acid, vanillic acid, ferulic acid, caffeic acid and proto-catechuic acid were also present in the fruit. Among the flavonoids, apigenin and rutin were the most important compounds found in the fruit peel and pulp, compared to other flavonoids such as myricetin, luteolin, quercetin and kaempferol. Phenolic compounds and flavonoids have attracted much attention due to their antioxidant activity and free radical scavenging ability, which may have beneficial effects on human health, e.g. in preventing coronary heart disease and cancer (Kubola and Siriamornpun, 2011).

2.1.6(d) Oils and fatty acids

Gac fruit is rich in fatty acids, especially monounsaturated and polyunsaturated fatty acids. 70% of the total fatty acids of 102 mg/g fresh weight are unsaturated and 50% are polyunsaturated. Soxhlet extraction with petroleum ether found the total oil content of gac aril to be between 18 and 34% on a dry basis (Tuyen et al., 2013). The fatty acid composition studies by (Thavamany et al., 2020) analyzing the extracted oil showed oleic acid, palmitic acid and linoleic acid as the main fatty acids in the aril as shown in Table 2, while stearic acid, linoleic acid, oleic acid and palmitic acid were the main fatty acids in the seeds (Thavamany et al., 2020) The fatty acids in the peel are important for the absorption of fat-soluble nutrients, including carotenoids, in a typically low-fat diet (Thavamany et al., 2020, Yuan et al., 2023). The peels and the oil extracted from the gac fruit are also used as a dietary supplement for the treatment of xerophthalmia and night blindness caused by a deficiency of provitamin A carotenoids (Abdulqader et al., 2018).

Vuong and King (2003) investigated the acceptance of gac oil by typical Vietnamese housewives (Vuong and King, 2003). The authors reported that the oil was well accepted and replacing pork fat with Gac fruit peel oil (2 ml/day) increased the intake of β-carotene and essential fatty acids and decreased the intake of saturated fatty acids. In addition, the oleic and linoleic acids contained in the oil are beneficial to human health. For example, α-linoleic acid, which is one of the polyunsaturated acids (PUFAs), plays an important role in reducing cardiovascular disease (Poudyal et al., 2011, Rodriguez-Leyva et al., 2010) by lowering low-density lipoprotein cholesterol (LDL cholesterol). Oleic acid and linoleic acid have an anti-atherogenic effect (Lopez-Huertas, 2010). The composition of the fatty acids and the high proportion of

carotenoids in gac oil suggest that the gac fruit has a high nutritional value (Yuan et al., 2023).

2.2 Cancer

The global cancer burden is expected to rise from 20 million cases in 2022 (Globocan 2022). According to reports from the World Health Organisation (WHO 2022), cancer was responsible for almost one in five deaths in 2022, with a total number of around 10 million deaths worldwide. The rise in cancer incidence is a growing concern as it poses a significant threat not only to human life, but also to healthcare systems and places a significant economic burden on developing countries.

Cancer is called a genetic disorder because it can be linked to changes in certain genes (Cooper, 2000, Lahtz and Pfeifer, 2011). However, it is important to realise that cancer is not a hereditary disease. As a result of these genetic changes, cancer cells can bypass many of the restrictions that normally regulate the behaviour of normal cells (Cooper, 2000). Normal cells only divide when they are told to do so by the body's homeostatic mechanisms, and they are also able to repair damage they have sustained. Cancer cells, however, break down all these regulatory influences that protect and self-destruct the body (Lahtz and Pfeifer, 2011)

Cancer cells multiply uncontrollably and form malignant tumours that invade the surrounding healthy tissue (Sriharikrishnaa et al., 2023). A tumour that is confined to a specific area can be effectively treated and possibly cured by surgery to remove the tumour (Bisoyi, 2022). In contrast, malignant tumours tend to metastasise by secreting cells that break away from the primary tumour, travel through the lymphatic or vascular system and settle in distant sites in the body where they form deadly secondary tumours (metastases) that cannot be treated by surgical removal as it has spread throughout the body (Bisoyi, 2022). Other therapeutic approaches, such as

radiation and chemotherapy, often become less effective as the disease spreads (Roy et al.,2017). The spread of cancer to vital organs and systems results in organ failure which increases the likelihood of mortality (Roy et al.,2017). Given to its effects on human health and the possibility of finding a cure, along with the established of cancer therapies like surgery, radiation therapy, chemotherapy, immunotherapy, and targeted treatments, there is a continuous focus on discovery of novel anti-cancer compounds. The research aims to improve the effectiveness of these established treatments and provide new hope for patients facing metastasis.

2.3 Colorectal cancer

2.3.1 General introduction

Colorectal cancer is the third most common cancer in women and men and the second most common in terms of mortality rate with an estimated 1,963,000 new cases and 935,000 reported deaths worldwide (IARC, 2020). Over the past 20 years, colorectal cancer has increased throughout Asia and has become a major health concern (Center et al., 2009). According to the National Cancer Registry Report 2019 in Malaysia, CRC was identified as the second most common cancer (Azizah et al., 2019). Data from the GLOBOCAN project shows that Malaysia had the third highest incidence of colorectal cancer (CRC) among Southeast Asian countries, with a rate of 18.3 cases per 100,000 people (Globocan, 2022).

The term colorectal cancer refers to malignant growths that develop in either the colon or rectum (American Cancer Society 2020). The occurrence of colorectal cancer is due to the abnormal growth of polyps, which are abnormal outgrowths of the inner lining of the colon (Sawicki et al., 2021). Histologically, polyps can be divided into two main categories: non-neoplastic polyps, including hamartomatous, hyperplastic

and inflammatory polyps, and neoplastic polyps, especially adenomatous polyps. The adenomatous polyps are particularly important as they have the potential to develop into malignant tumours if they are not removed by colonoscopy (Sawicki et al., 2021).

2.3.2 Risk factors of CRC

The risk factors for the development of colorectal cancer can be divided into two categories: genetic factors and environmental factors. Genetic risk takes into account ethnicity, age, gender, height and family history. Carcinogenesis associated with chromosomal instability (CIN) has been linked to the accumulation of mutations in adenomatous polyposis coli (APC), Kirsten-ras (K-ras) and TP53, while MSI-associated carcinogenesis has been linked to BAT25 and BAT26 (Jeon et al., 2008). Over 60% of small, benign adenomas of the colon have mutations in both copies of the APC gene, suggesting that alterations in this gene are often the first step in the development of colon cancer (Karp et al., 2020). Mutations in one of the RAS oncogenes, called KRAS, are often found in larger adenomas and cell masses in the early stages of cancer (Karp et al., 2020). On the other hand, mutations in the TP53 gene typically occur in later stages of tumour progression, indicating a clear transition to malignancy.

Individuals with a family history of colorectal cancer (CRC) or adenomatous polyps have an increased risk of developing CRC (Haggar and Boushey, 2009). According to a comprehensive meta-analysis that included 16 studies and 8,091 cases of colorectal cancer (CRC), individuals with a family history of CRC had an average risk of developing CRC that was approximately two times higher than individuals without such a family history (Johnson et al., 2013). In addition to family history, age is also an important risk factor, as the incidence of colorectal cancer correlates strongly with increasing age (Holford et al., 2019).

Lifestyle and diet are also important risk factors associated with an increased risk of colorectal cancer. The risk of colorectal cancer (CRC) increases in proportion to the number of cigarettes smoked, making cigarette smoking a modifiable risk factor for CRC (Demb et al., 2019). Compared to non-smokers, the relative risk of colorectal cancer was 1.06 for 5 pack-years, 1.11 for 10 pack-years, 1.21 for 20 pack-years and 1.26 for 30 pack-years (Johnson et al., 2013). In addition, continuous alcohol consumption, whether weekly or daily, is strongly associated with an increased risk of colorectal cancer (CRC). A cohort study of over 3,000 men and over 1,000 women conducted in Korea found that moderate and heavy alcohol consumption was associated with an increased risk of distal colorectal cancer in men and an increased risk of rectal cancer in women (Shin et al., 2011).

There is a positive correlation between obesity and the risk of developing colorectal cancer (CRC). A comprehensive meta-analysis that investigated the relationship between colorectal cancer and body mass index (BMI) using data from 23 studies with over 66,000 colorectal cancer patients revealed a significant correlation. The study showed that the risk of colorectal cancer increased by ten per cent for every 8 kg/m^2 increase in BMI (Johnson et al., 2013). Apart from this, physical inactivity and the consumption of red meat increase the risk of colorectal cancer. Based on epidemiological studies and meta-analyses, the consumption of red meat (including beef, pork and lamb) and processed meat is associated with a 20-30% (American Cancer Society , 2017) increased risk of CRC (Bradbury et al., 2020).

2.3.3 CRC and available treatment

The treatments available for bowel cancer depend primarily on factors such as the site of tumour growth, the stage of cancer progression and the patient's general state of health. Surgical removal of tumours is a common treatment option, especially in the