# EVALUATION OF *Etlingera elatior* FLOWER AQUEOUS EXTRACT (EEAE) AS TREATMENT OF COLON CANCER IN RAT MODEL (PILOT STUDY)

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**2025**

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**by**

**WEE LU SHUANG**

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

**January 2025**

# DECLARATION

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

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(WEE LU SHUANG)

**Date**: 26 January 2025

# ACKNOWLEDGEMENTS

With deepest gratitude, I would like to express my heartfelt thanks to my supervisor, Associate Professor Wan Amir Nizam Wan Ahmad, and my co-supervisor, Dr. Liza Noordin. Thank you for always being patient and helping me solve problems when I needed it. Your unwavering mentorship has been invaluable throughout my Final Year Project (FYP), and it has also inspired me to aspire to greater heights in my future endeavours.

Next, to my incredible teammates – Betty, Zeny, Hailey, Sharran, Fazalda, Liyana, and Nabillah – thank you for your kindness, cooperation, and readiness to help whenever I needed support. Your companionship made our days in the laboratory more engaging, and truly, you've made this journey unforgettable.

I am also profoundly grateful to the laboratory staff for their technical expertise and consistent assistance throughout this project. Furthermore, to those who may not have been mentioned by name but played a part in this journey, whether big or small, I extend my heartfelt thanks.

To my beloved family, thank you for your support and encouragement. Your belief in me was the driving force that kept me going through the most challenging times, and I owe this achievement to your constant love and encouragement.

Most importantly, I want to thank myself for never giving up and staying determined when facing challenges. I am proud of how far I've come, and looking forward to the road ahead, eager to go even further in the future. Also, to those I have mentioned in this text, I sincerely hope that each of you will soon achieve the success you deserve.

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| | |
|---|---|
| **β** | Beta |
| **cm** | Centimetre |
| **°C** | Degree Celcius |
| **G** | Gauge |
| **g** | Gram |
| **kg** | Kilogram |
| **<** | Less than |
| **µg** | Microgram |
| **µl** | Microlitre |
| **ml** | Mililitre |
| × | Multiple |
| **%** | Per cent |
| ± | Plus-minus |
| **:** | Ratio |

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **3D-CRT** | Three-Dimensional Conformal Radiation Therapy |
| **5-FU** | 5-Fluorouracil |
| **AC** | Adjuvant Chemotherapy |
| **ACF** | Aberrant Crypt Foci |
| **ACT** | Adoptive Cell Therapy |
| **AJCC** | American Joint Committee on Cancer |
| **AMPK** | AMP-Activated Protein Kinase |
| **Anti-CTLA** | Anti-Cytotoxic T Lymphocyte-Associated Molecule |
| **Anti-PD1** | Anti-Programmed Cell Death Receptor 1 |
| **AOM** | Azoxymethane |
| **AP-1** | Activator Protein 1 |
| **APAF1** | Apoptotic Protease Activating Factor 1 |
| **APC** | Adenomatous Polyposis Coli |
| **APCs** | Antigen-Presenting Cells |
| **ARASC** | Animal Research and Service Centre |
| **ASMR** | Age-Standardised Mortality Rate |
| **ASR** | Age-Standardised Incidence Rate |
| **ATM** | Ataxia-Telangiectasia Mutated |
| **Bcl-2** | B-Cell Lymphoma-2 |
| **BMI** | Body Mass Index |
| **BSLA** | Brine Shrimp Lethality Assay |
| **cAMP** | Cyclic Adenosine Monophosphate |
| **CAPOX** | Capecitabine + Oxaliplatin |
| **CAR** | Chimeric Antigen Receptor |
| **CCRs** | Colon Cancer Rats |
| **CDKN1A** | cyclin-dependent kinase inhibitor 1A |
| **cIAP-2** | Human Inhibitors of Apoptosis Protein (cIAP-2) |
| **CIMP** | CpG Island Methylator Phenotype |
| **CIN** | Chromosomal Instability |
| **CME** | Complete Mesocolic Excision |
| **COX-2** | Cyclooxygenase-2 |
| **CRC** | Colorectal Cancer |
| **CXCCR4** | C-X-C Chemokine Receptor Type 4 |
| **CXCL12** | C-X-C Motif Chemokine Ligand 12 |
| **DAXX** | Death Domain-Associated Protein 6 |
| **DDR** | DNA Damage Response |
| **DFS** | Disease-Free Survival |
| **DLBCL** | Diffuse Large Cell B-Cell Lymphoma |
| **DNMT** | DNA Methyltransferases |
| **DR4** | Death Receptor 4 |
| **DSS** | Dextran Sodium Sulfate |
| **EBRT** | External Beam Radiotherapy |
| **EGFR** | Epidermal Growth Factor Receptor |
| **EMT** | Epithelial-Mesenchymal Transitions |
| **ER** | Endoplasmic Reticulum |
| **FADD** | Fas-Associated Protein with Death Domain |
| **FAP** | Familial Adenomatous Polyposis |
| **Fas/FasL** | Fas-Ligand |

| | |
|---|---|
| **FDA** | Food and Drug Administration |
| **FOLFIRI** | 5-FU + Irinotecan |
| **FOLFOX** | 5-FU/LV + Oxaliplatin |
| **FRIM** | Forest Research Institute of Malaysia |
| **FYP** | Final Year Project |
| **GISTs** | Gastrointestinal Stromal Tumours |
| **GSK3** | Glycogen Synthase Kinase 3 |
| **GST** | Glutathione S-Transferase |
| **H&E** | Hematoxylin & Eosin |
| **HIF-1α** | Hypoxia-Inducible Factor 1 Subunit Alpha |
| **HNPCC** | Hereditary Nonpolyposis Colorectal Cancer |
| **IACUC** | Institutional Animal Care and Use Committee |
| **IAP** | Inhibitor of Apoptosis Proteins |
| **IARC** | International Agency for Research on Cancer |
| **IBD** | Inflammatory Bowel Disease |
| **ICIs** | Immune Checkpoint Inhibitors |
| **IGF1** | Insulin-Like Growth Factor-1 |
| **IKK** | Inhibitory-κB Kinase |
| **IMRT** | Intensity-Modulated Radiation Therapy |
| **$IC_{50}$** | Half-maximal inhibitory concentration |
| **IP** | Intraperitoneal |
| **JAK** | Janus Kinase |
| **$LC_{50}$** | Lethal Concentration 50 |
| **LV** | Leucovorin |
| **MALT** | Mucosa-Associated Lymphoid Tissue |
| **MAM** | Methylazoxymethanol |
| **MAPK/ERK** | Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase |
| **miRNA** | MicroRNA |
| **MMP** | Matrix Metalloproteinase |
| **MMR** | Mismatch Repair |
| **MT** | Masson Trichrome |
| **mTOR** | Mammalian Target of Rapamycin |
| **NCDs** | Noncommunicable Diseases |
| **NEC** | Neuroendocrine Carcinoma |
| **NFκB** | Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells |
| **NQO1** | NAD(P)H-Quinone Oxidoreductase 1 |
| **Nrf2/ARE** | Nuclear Factor Erythroid 2-Related Factor 2/Antioxidant Response Element Pathway |
| **NSAIDs** | Nonsteroidal Anti-Inflammatory Drugs |
| **NSCLC** | Non-Small Cell Lung Cancer |
| **OCT-1** | Organic Cation Transporter 1 |
| **OS** | Overall Survival |
| **PARP** | Polyadenosine Diphosphate-Ribose Polymerase |
| **PDGFRA** | Platelet-Derived Growth Factor Receptor Alpha |
| **PFS** | Progression-Free Survival |
| **PI3K/Akt** | Phosphoinositide 3-Kinase/Protein Kinase B |
| **PI3K/Akt/FoxO** | Phosphoinositide 3-Kinase/Protein Kinase B/Forkhead Box O |
| **PKM2** | Pyruvate Kinase Isoenzyme M2 |

| | |
|---|---|
| **PlGF** | Placental Growth Factor |
| **PTA** | Phosphotungstic Acid |
| **ROS** | Reactive Oxygen Species |
| **RR** | Response Rate |
| **RTK** | Receptor Tyrosine Kinase |
| **SBRT** | Stereotactic Body Radiation Therapy |
| **SD** | Sprague-Dawley |
| **siRNA** | Small Interfering RNA |
| **SIRT-6** | Sirtuin 6 |
| **SMAD** | Suppressor of Mothers Against Decapentaplegic |
| **SRCC** | Signet Ring Cell Carcinoma |
| **SSPs** | Sessile Serrated Polyps |
| **TCF** | Transcription factor 4 |
| **TCR** | T Cell Receptor |
| **TFC** | Total Flavonoid Content |
| **TGF-β** | Transforming Growth Factor-β |
| **TIL** | Tumour-Infiltrating Lymphocyte |
| **TME** | Tumour Microenvironment |
| **TPC** | Total Phenolic Content |
| **TTC** | Total Tannin Content |
| **VEGF** | Vascular Endothelial Growth Factor |
| **XIAP** | X-Linked Inhibitor of Apoptosis Protein |

**EVALUASI EKSTRAK AKUEUS BUNGA E*tlingera elatior* (EEAE) SEBAGAI RAWATAN KANSER KOLON DALAM MODEL TIKUS (KAJIAN RINTIS)**

## ABSTRAK

Kanser kolon adalah salah satu jenis kanser yang berlaku di kolon atau rektum. Oleh itu, ia juga dikenali sebagai kanser kolorektal (CRC). Ia merupakan salah satu punca utama kematian berkaitan kanser di seluruh dunia. Walaupun rawatan untuk kanser kolon tersedia, kesan sampingan yang tidak diingini sering mengiringinya. *Etlingera elatior*, dikenali sebagai *bunga kantan* di Malaysia, merupakan tumbuhan perubatan tradisional yang mempunyai potensi terapeutik yang tinggi, termasuk sifat antimikrob, antioksidan, antikanser, antidiabetik, anti-radang, dan anti-penuaan. Walau bagaimanapun, kajian saintifik mengenai kesan antitumor terhadap kanser kolon masih terhad. Oleh itu, kajian ini menyelidik sifat antitumor ekstrak akueus bunga *E. elatior* (EEAE) *in vivo*. EEAE ini dihasilkan menggunakan kaedah sonikasi. Penilaian toksikologi dilakukan melalui Ujian Kematian Udang Air Garam (BSLA) dengan kepekatan 10 mg/ml, 1 mg/ml, 3 mg/ml, 300 µg/ml, 100 µg/ml, 30 µg/ml, dan 10 µg/ml. Nilai $LC_{50}$ EEAE selepas inkubasi selama 24 jam ditentukan sebagai 2286 ppm (µg/ml), mengklasifikasikan EEAE sebagai tidak toksik mengikut piawaian toksisiti Meyer dan Clarkson. Keberkesanan antitumor EEAE seterusnya dinilai menggunakan model kanser kolon yang teraruh-Azoxymethane pada tikus jantan Sprague-Dawley. Analisis histologi dengan pewarnaan Hematoksilin dan Eosin (H&E) serta Masson-Trichrome (MT) menunjukkan penambahbaikan dalam morfologi selepas rawatan dengan EEAE. Penemuan ini mencadangkan bahawa EEAE merupakan produk semula jadi yang berpotensi sebagai agen antitumor terhadap kanser kolon, dengan tiada kesan toksik yang diperhatikan secara *in vivo*. Penyelidikan lanjut diperlukan untuk meneroka potensi terapeutik dan mekanisme tindakannya.

**EVALUATION OF *Etlingera elatior* FLOWER AQUEOUS EXTRACT (EEAE) AS TREATMENT OF COLON CANCER IN RATS MODEL (PILOT STUDY)**


## ABSTRACT

Colon cancer is a type of cancer that occurs in the colon or rectum. Therefore, sometimes it is also known as colorectal cancer (CRC). It is a leading cause of cancer-related deaths worldwide, even though there are available treatments for colon cancer, undesirable side effects often accompany it. *Etlingera elatior*, known as *bunga kantan* in Malaysia, is a traditional medicinal plant with high potential therapeutic effects with excellent antimicrobial, antioxidant, anticancer, antidiabetic, anti-inflammation, and anti-ageing properties. However, limited scientific research has been conducted on its antitumour effects against colon cancer. Therefore, this study investigates the *in vivo* antitumour effects of *E. elatior* flower aqueous extract (EEAE). EEAE is extracted using the sonication method. Toxicological assessment was performed using the brine shrimp lethality assay (BSLA) with a series of concentrations including concentrations of 10 mg/ml, 1 mg/ml, 3 mg/ml, 300 µg/ml, 100 µg/ml, 30 µg/ml, and 10 µg/ml. The $LC_{50}$ of EEAE determined after the 24-hour incubation period was 2286 ppm (µg/ml), classifying EEAE as non-toxic based on Meyer and Clarkson toxicity standards. The antitumour efficacy of EEAE was further evaluated in an Azoxymethane-induced colon cancer model using male Sprague-Dawley rats. Histological analysis with Hematoxylin and Eosin (H&E) and Masson-Trichrome (MT) staining demonstrated substantial improvements in colon morphology upon treatment with EEAE. These findings suggest that EEAE is a promising natural product with antitumour properties against colon cancer, with no observed toxicity *in vivo*. Further research is warranted to explore its therapeutic potential and underlying mechanisms.

# CHAPTER 1: INTRODUCTION

## 1.1 Research background

Diseases that are not transmitted directly from one person to another are known as noncommunicable diseases (NCDs). Among NCDs, cancer ranked as the second leading cause of death after cardiovascular disease. The most commonly diagnosed cancers in both sexes are breast, lung, colon, prostate, and stomach cancers (excluding non-melanoma skin cancer) (Naeem et al., 2022). Among these cancers, colon cancer is the third most common cancer after lung cancer and breast cancer in women, and prostate cancer in men, and is known as the second most mortal cancer after lung cancer (Menon et al., 2024). According to GLOBOCAN estimates from the International Agency for Research on Cancer (IARC), colon cancer was responsible for approximately 935,173 deaths and an age-standardised mortality rate (ASMR) of 9.0 per 100,000 person-years in 2020. Additionally, the number of new cases of CRC was estimated to be 1,931,590, with an age-standardised incidence rate (ASR) of 19.5 per 100,000 person-years (Roshandel et al., 2024).

Colon cancer is predominantly sporadic, accounting for approximately 70% of cases. However, inherited genetic mutations, such as Lynch syndrome (hereditary nonpolyposis colorectal cancer, HNPCC) and familial adenomatous polyposis (FAP), are responsible for 3% to 5% of cases. According to Menon et al. (2024), the remaining cases often involve a family history of colon cancer without an identifiable inherited mutation. Some factors contribute to the development of colon cancer, including ageing, family history, inherited colon cancer-related mutations, adenomas on screening colonoscopy, history of inflammatory bowel disease (IBD), and environmental and lifestyle factors. Conversely, factors protective against colon cancer development include being physically active, having a healthy diet, consuming garlic and coffee, or taking particular

medications, such as aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs). Clinically, most colon cancers are asymptomatic in the early stages. However, symptoms such as rectal bleeding, abdominal pain, and anaemia may develop as the disease progresses. Advanced disease may present with various symptoms depending on the organs involved. Moreover, the standard treatment of colon cancer includes surgery resection for resectable colon cancer, chemotherapy for advanced colon cancer, and, less commonly, radiotherapy (Menon et al., 2024). However, these therapies can cause multidrug resistance and a range of adverse side effects such as nausea and vomiting, fatigue, decreased appetite, changes in taste, hair loss, dry mouth, and constipation. Given these limitations, there is a growing interest in natural products for cancer therapy due to their potential for effective treatment with fewer side effects. This trend underscores the need to explore and validate natural compounds as complementary or alternative cancer therapies (Altun and Sonkaya, 2018).

Since ancient times, natural products have been a significant source of therapeutic drugs due to their high molecular diversity, novel functionality, and high availability as well as their superior efficacy, low toxicity and, therefore, safe to consume (Naeem et al., 2022). This also makes them an attractive and affordable alternative to synthetic products for cancer treatment. Various natural products propose anticancer properties by affecting multiple pathways, including apoptotic cell death, cell proliferation, migration/invasion, angiogenesis, and metastasis. Not only capable of generating intracellular signals that lead to the death of cancer cells, but they can also enhance chemotherapy in cases where cancer is resistant to treatment (Naeem et al., 2022). For decades, certain natural products, such as *Etlingera elatior,* have been investigated in preclinical studies for their cytotoxic effect as anticancer agents.

*E. elatior,* commonly known as Torch Ginger in English and locally referred to as *bunga kantan* or *bunga siantan* in Malaysia, belongs to the *Zingiberaceae* family under the genus *Etlingera* (Habsah et al., 2005). It is widely distributed across South Asia. In Malaysia, the hearts of young shoots, inflorescences and fruits are consumed by the indigenous communities as condiments and eaten raw or cooked as vegetables. A decoction of fruits can be used to treat earache, while a decoction of leaves can be applied for wound cleaning. Besides, their leaves can also be used by postpartum women in a mixture with other aromatic herbs for bathing to remove body odour (Chan et al., 2011). *E. elatior* has been reported to have various properties, including antioxidant, anticancer, antiproliferative, antibacterial and cytotoxic activity (Ghasemzadeh et al., 2015). These therapeutic attributes highlight its potential for further exploration in developing treatments for various medical conditions.

**1.2 Problem statement**

Colon cancer is having a trend of increasing prevalence among younger generations nowadays. The typical treatment of colon cancer includes surgery, radiotherapy, chemotherapy, and immunotherapy. However, these therapies have side effects and limitations in therapeutic approaches. Natural products, such as *E. elatior*, possess antitumour properties. Though research has been conducted on its use against colon cancer, most studies have been limited to *in vitro* experiments. Given the lack of *in vivo* studies specifically focusing on the *E. elatior* flower aqueous extract (EEAE), there is a pressing need to evaluate its anticancer effects *in vivo* to understand its therapeutic potential better.

## 1.3 Research Questions

1. Is *E. elatior* flower Aqueous Extract (EEAE) toxic?

2. Does EEAE improve Body Mass Index (BMI) among colon cancer rats (CCRs)?

3. Does EEAE improve the structural changes among CCRs?

## 1.4 Objectives

### General objective

To determine the effects of *E. elatior* flower Aqueous Extract (EEAE) as a treatment of colon cancer in colon cancer rats (CCRs).

### Specific objectives

1. To determine the $LC_{50}$ (lethal concentration) of EEAE via brine shrimp lethality assay (BSLA).
2. To evaluate the effect of EEAE on Body Mass Index (BMI) in CCRs.
3. To evaluate the effect of EEAE in improving microscopic structural changes of the colon among CCRs.

## 1.5 Hypothesis

1. *E. elatior* flower Aqueous Extract (EEAE) is considered toxic.
2. EEAE will not improve the Body Mass Index (BMI) among colon cancer rats (CCRs).
3. EEAE) will not improve the microscopic structural changes among CCRs.

### 1.6 Significance of study

Colon cancer is a leading cause of cancer-related deaths worldwide; despite there being available treatment for colon cancer, undesirable side effects always accompany it. Therefore, there is a demand for the exploration of effective natural therapies. Natural products, such as *E. elatior*, present promising alternatives but remain underexplored due to limited research. It has shown significant potential as an anticancer agent across various cell lines such as breast cancer cell lines like MCF-7 and MDA-MB-231, human T4-lymphoblastoid (CEM-SS), B16 melanoma cells, HeLa cervical cell lines, human hepatocellular carcinoma tissue Hep-G2 cell lines, and HT-29 colon cancer cell lines with minimal toxicity on non-cancerous cells (Al-Mansoub et al., 2021; Habsah et al., 2005; Herni et al., 2021; Hueh Zan et al., 2011; Krajarng et al., 2017). These studies suggest that *E. elatior* may possess *in vitro* potential as a natural treatment for colon cancer. Hence, this pilot study aims to evaluate the *in vivo* anticancer properties of EEAE against colon cancer and assess the extract's safety through toxicological testing to enhance the understanding of *E. elatior* as a non-toxic natural treatment for colon cancer in rat models and support its potential use in complementary cancer therapies.

# CHAPTER 2: LITERATURE REVIEW

## 2.1 Cancer

According to Cooper (2000), a tumour refers to the abnormal proliferation of cells that can be divided into benign tumours or malignant tumours based on their invasiveness and metastatic states. A benign tumour is a tumour that remains at the original site and does not spread to other sites, while a malignant tumour, or cancer, refers to the uncontrolled proliferated cells that invade normal tissue and metastasize to other body sites (Alzahrani et al., 2021). Most cancers can be divided into 3: carcinomas, sarcomas, and leukaemias or lymphomas. The four most common cancers are those of the breast, prostate, lung, and colon/rectum (Alzahrani et al., 2021).

Cancer development usually starts with tumour initiation, a process in which abnormal proliferation occurs because of genetic alteration. Cancer can be induced by many exposures, including radiation, chemicals, or viruses (Basu, 2018). These agents are generally referred to as initiating agents to induce mutation in key target genes (Basu, 2018). In addition, other carcinogens, such as phorbol esters, can contribute to cancer development by promoting cell proliferation in the early stage instead of inducing mutation. Therefore, they are also known as tumour promoters. Cancer cells are clonal and continue to divide, eventually leading to the outgrowth of clonally derived tumour cells. Tumour progression continues with additional mutations occurring. In this step, clonal selection occurs, and the tumour evolves its survival rate, invasiveness, and metastasis to rapidly grow and become malignant (Tuo et al., 2024).

Cancer can be differentiated from normal cells in that they have fewer requirements for extracellular growth factors compared to normal cells (Cooper, 2002). For instance, they can secrete growth factors to stimulate their proliferation, known as autocrine growth stimulation (Cooper, 2002). Moreover, most cancers are less likely to

express the cell surface adhesion molecules, such as E-cadherin. The lack of adhesion molecules makes them free to interact with surrounding cells and tissue components, allowing for invasion and metastasis and resulting in morphological (rounder shape) and cytoskeletal alterations (Rossetti et al., 2015). Besides, cancer cells also lack contact inhibition; they grow in disordered, multilayered patterns and migrate over adjacent cells. Moreover, in terms of invasion and metastasis, cancer cells generally secrete proteases to digest extracellular matrix components, allowing the cancer cells to invade nearby normal tissues and secrete growth factors to promote angiogenesis for the supply of oxygen and nutrients needed for proliferation, and helping the spreading of cancer cells via the circulatory system (Cooper, 2000). In addition, cancer can evade apoptosis by either inactivating pro-apoptotic genes or by destabilizing them (Fernald and Kurokawa, 2013). Another way to escape apoptosis is by releasing anti-apoptotic proteins, the anti-apoptotic members of the B-cell lymphoma-2 (Bcl-2) family proteins, and the inhibitor of apoptosis proteins (IAP) (Shahar and Larisch, 2020). It can also inhibit apoptosis by changing the function of pro-apoptotic genes via post-translational modification (Fernald and Kurokawa, 2013).

## 2.2 Colon cancer

### 2.2.1 Colon cancer risk factors

Colon cancer, sometimes also known as colorectal cancer (CRC), is due to the aberrant proliferation of glandular epithelial cells of the colon (Hossain et al., 2022). There are many factors contributing to CRC. The most significant factors include a family history of CRC and a medical history of IBD, such as ulcerative colitis or Crohn's disease (Sawicki et al., 2021). These can increase the chance of developing colon cancer. Moreover, the risk of developing colon cancer is 2-3 times higher in individuals with

diabetes compared to those without the condition. Besides, some studies have found excess body weight or obesity may raise the colon cancer risk (Alrushaid et al., 2023). Another factor that contributes to colon cancer is lifestyle habits. For example, physical activity, increased consumption of vegetables and fruits, foods with calcium and vitamin D, or dairy products can reduce the risk of developing colon cancer (Sawicki et al., 2021). Conversely, high consumption of red and processed meats, alcohol, and smoking significantly increase the risk of CRC (Sawicki et al., 2021). Age is another important factor. The risk of developing CRC increases considerably with age, particularly after 40 (Alrushaid et al., 2023). In addition, males have a 30% higher risk of developing colon cancer compared to women. They also face a worse prognosis, with a 40% higher mortality rate compared to women, highlighting a gender disparity in both risk and prognosis.

### 2.2.2 Types of colon cancer

Most colonic cancers are adenocarcinoma (98%), making it the most common primary colon carcinoma. According to Gonzalez (2021), there are 9 WHO-recognised subtypes of adenocarcinoma: adenoma-like, adenosquamous, carcinoma with sarcomatoid components, medullary, micropapillary, mucinous, serrated, signet ring cell, and undifferentiated adenocarcinoma.

Adenoma-like carcinoma is a very well-differentiated subtype of colon cancer that mimics the tubulovillous adenoma. However, it usually has an excellent prognosis and metastasis is rarely seen. The next is adenosquamous carcinoma, which is rare and characterised by epithelial malignancy of the colon with glandular and squamous elements. Opposite to adenoma-like carcinoma, these subtypes have worse prognoses and higher metastatic rates. Also, medullary carcinoma is another subtype of adenocarcinoma

with solid growth patterns. It is relatively rare, with less than 1% of colorectal neoplasms. It is associated with a high degree of microsatellite instability (MSI), and usually no nodal metastasis. This tumour is characterised by sheets of epithelioid neoplastic cells with large vesicular nuclei, prominent nucleoli, and abundant cytoplasm. It typically has a pushing border on resection specimens and has marked tumour-infiltrating lymphocytes (Fleming et al., 2012). As for micropapillary carcinoma, this adenocarcinoma is uncommon and has distinctive behaviour. It is usually seen as a small cluster of malignant cells with abundant eosinophilic cytoplasm and pleomorphic nuclei under microscopy. It also has a poor prognosis, and the rate of metastasis to lymph nodes is high; this happens even at the early stage of cancer development. Next, mucinous adenocarcinoma is the most common subtype of colorectal carcinoma, which represents 10% of colorectal carcinoma. This type of tumour has more than 50% extracellular mucin content (Fleming et al., 2012). Therefore, mucin can be easily observed during histology examination. In addition, signet ring cells may be seen (Fleming et al., 2012). Signet ring cells may become carcinoma as well, and it is a rare subtype of colon cancer with a poor prognosis at a high stage. It forms a diffuse lesion and frequently spreads to lymph nodes, peritoneal surface and ovary, but less commonly to the liver. Signet ring cell carcinoma (SRCC) is characterised by tumour cells with intracytoplasmic mucin vacuoles that displace the nucleus to the cell periphery, creating a distinctive signet ring appearance. To meet the diagnostic criteria, these cells must constitute more than 50% of the tumour. This may show an infiltrative growth pattern or are present within the pools of extracellular mucin (Fleming et al., 2012).

Besides adenocarcinoma, there are other rare non-adenocarcinoma types of colon cancer, such as neuroendocrine carcinoma (NEC), gastrointestinal stromal tumours (GISTs), and lymphoma. NEC of the colon and rectum is rare and only accounts for less than 1% of all colorectal malignancies (Yoshida et al., 2019). They have highly aggressive

growth and a high tendency for metastasis (Yoshida et al., 2019). Further, GISTs are relatively rare under the age of 40, and only less than 1% occur below age 21 (Miettinen and Lasota, 2013). It is developed from the intestinal cells of Cajal, sometimes called the "pacemaker cells" of the digestive tract, to move food through the body, and it was recognised as a tumour driven by mutation of KIT or platelet-derived growth factor receptor alpha (PDGFRA) (Miettinen and Lasota, 2013). GISTs can occur anywhere in the GI tract but are most common in the stomach and small intestine. Mutations in tyrosine kinase receptors are the genetic basis of GISTs. It is similar to sarcoma in that both are soft tissue, and both are aggressive cancers that are unresponsive to traditional chemo/radiation therapies (Dutta, 2018).

Next, colorectal lymphoma makes up just 0.9 % to 1.2 % of all colon cancers (Times, 2011). The diffuse large cell B-cell lymphoma (DLBCL) grows and spreads quickly. The most common subtype in the colon is diffuse large B-cell lymphoma (60%), followed by mucosa-associated lymphoid tissue (MALT) (15%) and Burkitt's lymphoma (15%) (Times, 2011). The sporadic primary malignant colorectal melanomas comprise about 2% of all mucosal melanomas. As of 2018, only 36 cases of primary colonic melanoma have been reported, with the most common location being ascending colon and cecum (Foley et al., 2023).

### 2.2.3 Colon cancer development and pathophysiology

Most colon cancer is sporadic, and approximately 5% are due to an inherited genetic mutation, primarily due to Lynch syndrome (HNPCC and FAP) (Menon et al., 2024). Colon cancer development involves histological, morphological, and genetic changes (Simon, 2016). Typically, colon cancer develops from focal changes within benign,

precancerous polyps. Adenomas and sessile serrated polyps (SSPs) are the two main polyps that tend to turn malignant. Most adenomas generally have tubular histology with small, roundish, atypical glands and are present as villous or tubulovillous. By definition, adenomas are characterised by a low degree of cellular and structural atypia, which is known as dysplasia. SSPs are flat and carpet-like, with serrated or saw-toothed glands. Sessile serrated adenomas, traditional serrated adenomas, and mixed polyps are all included in SSPs, and are associated with CRC development (Conteduca et al., 2013; Yamane, 2014).

The risk for adenomas to develop into CRC is higher when the size of the polyp is larger (Conteduca et al., 2013). As the cells within the polyp proliferate, the size of the polyp increases, and genetic mutations and epigenetic changes may accumulate (Lochhead et al., 2014). Tubulovillous and villous adenomas are typically larger and have a greater potential for harbouring cancerous cells. Statistically, 60% to 70% of CRCs develop from adenomas, while 25% to 35% develop from SSPs (Yamane, 2014).

Three main genetic pathways that lead to the development of CRC are chromosomal instability (CIN), CpG island methylator phenotype (CIMP), and Mismatch repair (MMR) (Worthley and Leggett, 2010). Typically, the adenomatous polyposis coli (*APC*) genes were first mutated in the CIN pathway, also described as the adenoma-carcinoma sequence, affecting chromosome segregation during cell division (Menon et al., 2024). Mutations follow this in the *KRAS* oncogene, influencing processes like cell growth, differentiation, motility, and survival. Eventually, these changes can lead to the inactivation of the *p53* gene, a crucial regulator of transcription and apoptosis, driving cancer formation.

The CIMP pathway, which tends to arise in serrated polyps rather than adenomas, begins with mutations in the *BRAF* gene, altering growth signalling and inhibiting apoptosis (Bateman, 2014; Kang, 2011; Yamane, 2014). Although *KRAS* mutations can also occur in SSPs, it is less frequent (Bateman, 2014; Kang, 2011). This pathway is also characterised by epigenetic changes such as promoter hypermethylation, which silences genes by preventing transcription. This gene silencing impacts many regulatory genes, including those that promote growth (Kang, 2011). Genes like *BMP3* and *NDRG4* are often hypermethylated in CRC cases associated with the CpG island methylator phenotype, contributing to cancer progression (Loh et al., 2008; Melotte et al., 2009).

Another pathway that contributes to colon cancer is the MMR pathway, involving gene mutations of *MLH1*, *MSH2*, *MSH6*, or *PMS2*, resulting in DNA replication error accumulation. MSI is the byproduct of MMR, resulting from uneven replication of repetitive DNA sequences, known as microsatellites, within noncoding regions, increasing the likelihood of further genetic mutations (Bateman, 2014; Boland and Goel, 2010; Kang, 2011; Yamane, 2014). This phenomenon can occur in both adenomatous and serrated polyps. MSI is associated with germline mutations in DNA mismatch repair genes, such as those linked to HNPCC. Additionally, MSI can result from sporadic mutations, often caused by abnormal methylation of the MLH1 promoter regions, a hallmark of the CpG island methylator phenotype (Boland and Goel, 2010; Kang, 2011; Yamane, 2014).

### 2.2.4 Colon cancer staging

According to the American Joint Committee on Cancer (AJCC) TNM staging system, colon cancer can be divided into several stages based on 3 characteristics: the size of the tumour and its locations, the number of nearby lymph nodes it spreads to and the spreading to distant sites (Hari et. al., 2013). At stage 0 (Tis, N0, M0), the tumour is confined to the colon's inner lining. Known as in situ carcinoma. In stage I (T1 or T2, N0, M0), the tumour has invaded the submucosa or lamina propria but has not spread to the lymph nodes or distant sites. If the colon cancer cells invade the outer layer of the colon, it is in stage II. Stage II colon cancer involves the invasion of the colon wall without spreading to lymph nodes. It is divided into three subcategories. In stage IIa (T3, N0, M0), cancer has penetrated the muscularis propria and reached the outermost layer of the colon (serosa) while in stage IIb (T4a, N0, M0), cancer has grown through the entire colon wall but has not invaded nearby tissues or organs and in stage IIc (T4b, N0, M0), cancer has extended through the colon wall into nearby structures or tissues.

At stage III, the cancer has progressed to involve nearby lymph nodes, but it has not metastasised to distant parts of the body (Hari et. al., 2013). Stage 3 is further divided into several sub-stages based on the number of lymph nodes invaded. Stage IIIa includes the spreading of the mucosa into the submucosa or lamina propria and has spread to 1 to 3 nearby lymph nodes or into areas of fat near the lymph nodes but not the nodes themselves (T1 or T2, N1/N1c, M0); or spreading of colon cancer from mucosa to submucosa and spreading to 4 to 6 lymph nodes (T1, N2a, M0). Stage IIIb refers to the cancer stage when growing into serosa through the wall of the colon and spreading to 1 to 3 nearby lymph nodes or areas of fat near the lymph nodes (T3 or T4a, N1/N1c, M0); or spreading from muscularis propria to serosa and involving 4 to 6 nearby lymph nodes (T2 or T3, N2a, M0) or from muscularis mucosa to submucosa or lamina propria and

involve 7 or more lymph nodes (T1 or T2, N2b, M0). Stage IIIc is the spreading of cancer through the wall of the colon but not nearby organs, and spread to 4 to 6 nearby lymph nodes (T4a, N2a, M0); or cancer has grown into the serosa or through the wall of the colon, and spread to 7 or more nearby lymph nodes (T3 or T4a, N2b, M0); or cancer has grown through the colon wall and/or has spread into other nearby tissues or organs and involves at least 1 nearby lymph node or area near the lymph nodes (T4b, N1 or N2, M0).

Colon cancer eventually develops into stage IV colon cancer; this advanced stage signifies a significant progression of the disease, with cancer cells metastasising to distant organs and tissues, most frequently involving the liver and other organs such as the lungs, brain, peritoneum, or distant lymph nodes. It can be divided into IVa, IVb, and IVc (Hari et. al., 2013). Stage IVa (Any T, Any N, M1a) signifies the cancer has spread to one distant organ or lymph node group but not the peritoneum. Stage IVb (Any T, Any N, M1b) involves metastasis to more than one distant organ or site, while stage IVc (Any T, Any N, M1c) is characterised by the spread to the peritoneal surface, with or without additional distant organ involvement.

### 2.2.5 Colon cancer signs and symptoms

Colon cancer may be unnoticeable as it may not cause any early-stage symptoms. Even when symptoms are present, they are typically vague, such as fatigue or dull abdominal pain. Sometimes, it may cause infections, haemorrhoids, or IBD (swelling in the digestive tract). Certain lower gastrointestinal symptoms may raise suspicion of CRC. Generally, the most common colon cancer symptoms include cramping or stomach pain, dark or bloody stools, weight loss, irregular bowel activity (diarrhoea or constipation), rectal bleeding, vomiting, fatigue, iron deficiency anaemia, and jaundice (Sawicki et al., 2021).

### 2.2.6 Colon cancer treatment

Colon cancer treatment includes surgical removal for resectable colon cancer, which is for early-stage cancer (stage I to III), while chemotherapy, radiotherapy, and immunotherapy are for unresectable, metastatic colon cancer (stage IV). However, these therapies are non-specific and cytotoxic to normal cells (Schuell et al., 2005). Although some therapies can be utilised in combinations, for example, surgery followed by chemotherapy, multidrug resistance CRC can develop in patients (Zhou et al., 2021). Recently, emerging therapies such as immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T cell therapy, T cell receptor (TCR) alterations, and cytokine therapy have been effective in CRC treatment. Also, recent research on the use of probiotics, RNA-based therapies including small interfering RNA (siRNA), microRNA (miRNA), and RNA aptamer, as well as oncolytic viral therapies, and natural products used for colon cancer treatment have shown improvement in colon cancer as well (Liu and Guo, 2020).

Specifically, the treatment can be classified based on different stages of cancer according to Kumar et al. (2023). For cancer stage 0 that does not spread, a colonoscope can be used to remove the polyp, while a partial colectomy can be used when the cancer is too large and cannot be removed by local removal. For cancer stage I that has not spread, no extra treatment is needed except the removal of polyps. For high-grade polyps, surgery may be more suitable. Also, the standard treatment is partial colectomy for colon cancer that does not develop from a polyp. Next, stage II colon cancer requires partial colectomy and adjuvant chemotherapy (AC) to avoid cancer relapse. If the cancer develops into stage III, the standard treatment is partial colectomy followed by AC. Lastly, surgery accompanied by partial colectomy may work for metastatic stage IV colon cancer that spreads to small areas. However, if many organs are involved, the primary treatment is

chemotherapy. In addition, surgery may be an option if the tumour size shrinks (Kumar et al., 2023).

#### 2.2.6.1 Surgery

The purpose of surgical resection for colon cancer is to remove visible malignant tissue, resect the affected intestinal segment, and excise draining lymph nodes through vascular ligation while preserving mesocolon integrity (Lorenzon et al., 2018). In cases without synchronous lesions, the surgeon should inspect the abdominal cavity for other disease evidence and plan the resection based on tumour location and lymphovascular drainage. This ensures that a margin of 5-7 cm of colon proximal and distal to the tumour is removed *en bloc* with the mesentery and the corresponding primary blood vessel (Vogel et al., 2017). When feasible, bowel continuity is restored via anastomosis of the resected margins. Via surgery, the 5-year disease-free survival (DFS) rate for Stage I, Stage II, and Stage III colon cancer, is 95%, 82% to 88%, and 45% to 50%, respectively (Chakrabarti et al., 2020).

In addition, the concept of complete mesocolic excision (CME) has gained traction in recent years. This involves a sharp dissection along embryological planes to isolate the mesocolon and angiolymphatic drainage at its central origin (Hameed et al., 2019). Studies suggest a 2% to 3% occurrence of central nodal metastasis even when pericolic or intermediate lymph nodes are metastasis-free. By reducing recurrence risk and optimizing lymph node harvest, CME may be as effective as AC for low-risk Stage II patients (Chakrabarti et al., 2020). However, aggressive lymphatic clearance near the aortic vessels can damage surrounding nerve plexuses, potentially leading to

complications such as diarrhoea, delayed gastric emptying, and urologic or sexual dysfunction (Prevost et al., 2018).

#### 2.2.6.2 Radiation therapy

Radiation therapy uses high-energy rays (such as X-rays) or particles to kill cancer cells or slow their growth by damaging their DNA (Hutchinson et al., 2020). The damage of DNA beyond repair causes cancer cells to stop dividing or die. They are used for a small number of tumours and if surgery cannot be performed. It can be delivered using an external source (external beam radiotherapy/EBRT), or an internal source (brachytherapy). EBRT is more frequently used for people with colon cancer and is delivered via a linear accelerator. These machines use sophisticated software to direct X-rays (photons) or electrons to a target. A typical total dose ranges from 40 to 80 Gy and is given over 3 to 8 weeks. Advancements in EBRT techniques, such as three-dimensional conformal radiation therapy (3D-CRT), intensity modulated radiation therapy (IMRT), and stereotactic body radiation therapy (SBRT), have been shown to treat metastatic colon cancers more accurately while reducing the exposure to normal tissues. In brachytherapy, instead of using a machine, seeds, ribbons, or capsules are used to transfer the radiation source by placing them in or near the tumour (Skowronek, 2017).

Radiotherapy is applied to prevent local recurrence and improve overall survival. In terms of local recurrence, preoperative radiotherapy is better, though it does not improve overall survival (Fallowfield et al., 2021). Moreover, radiation and adjuvant radiation therapy bring a better prognosis for CRC patients in stages II and III. However, there may be long-term toxicity effects, particularly on the gastrointestinal tract (e.g.,

bowel obstruction or diarrhoea), urogenital system, or surrounding soft tissues, depending on the radiation field and dose (Fallowfield et al., 2021).

#### 2.2.6.3 Chemotherapy

Chemotherapy is used as a neoadjuvant or adjuvant treatment before or after surgery to slow down disease progression and reduce the tumour significantly. It is also the treatment for stage IV colon cancer. This can increase an individual's lifespan by eliminating cancer cells or stopping proliferation. The medications approved include fluoropyrimidines, irinotecan, oxaliplatin, trifluridine-tipiracil, capecitabine, and 5-fluorouracil (5-FU), which is most commonly used as a chemotherapeutic agent for curing colon cancer. The use of adjuvant fluoropyrimidine-based (capecitabine, floxuridine, or 5-FU) AC after surgery has been a standard treatment to reduce cancer relapse and improve patients' survival.

Based on Kumar et al. (2023), 5-FU is considered one of the safest chemotherapeutic agents. However, it brings side effects, including fever, mucositis, stomatitis, leukopenia, and thrombocytopenia and may cause cerebellar ataxia and other neurological diseases in 1% of patients. Therefore, all 5-FU-based regimens must include Leucovorin (LV, folinic acid), which functions in chemoprotection that potentiates the activity of 5-FU via the formation of complex forming and preventing its adverse effects (Kumar et al., 2023). The patient survival and tumour response rate (RR) have successfully improved with the use of fluorouracil in combination with the L-folinic acid (FU/LV) regimen (Gustavsson et al., 2015). This regimen resulted in a 15% reduced risk of death at 5 years when given daily for 5 days in 6 cycles (Kumar et al., 2023).

As a prodrug of 5-FU, capecitabine has a bioavailability of 100% with Cmax and the area under the curve increases linearly with dosage. Its RR is as efficient as 5-FU/LV in two phase III randomised studies in patients with metastatic colon cancer (Comella, 2007). Its combination with oxaliplatin is known as CAPOX. Oxaliplatin, which is a platinum derivative from the diaminocyclohexane platinum family, has the potential to generate DNA adducts and hinder DNA repair, thereby leading to a cytotoxic effect in colon cancer (Latchman et al., 2014). It has an overall response rate of only 10% to 24% if given alone. In CAPOX, the RR is 48% and with progression-free survival (PFS) of 7.1 months (Porschen et al., 2007).

Moreover, irinotecan and oxaliplatin are also used in combination therapy with 5-FU/LV to enhance its efficacy, known as FOLFIRI (5-FU+ irinotecan) and FOLFOX (5-FU/LV + oxaliplatin). In FOLFOX, the RR reached 56% and had a PFS of 8 months (Kumar et al., 2023). While, irinotecan, a specific inhibitor of topoisomerase I, when added to the standard regimen of 5FU/LV (FOLFIRI), provides an overall RR of 54% and PFS of 8.4 months (Kumar et al., 2023). Anyway, FOLFOX is more commonly been prescribed as there are fewer patients with serious adverse events (neuropathy), more patients amenable to surgery for metastases, fewer chemotherapy cycles, and lower cost per life year to achieve the same results (Neugut et al., 2019).

In evaluating the efficacy of chemotherapy, the 5-year overall survival (OS) rate is the benchmark, while the 3-year DFS rate serves as a reliable surrogate marker (Sargent et al., 2007). The initiation of AC is recommended within 6–8 weeks after surgery; however, delayed initiation up to 24 weeks may still provide some benefits (Turner et al., 2018). The duration of AC depends on the regimen and patient risk stratification. According to Chakrabarti et al., (2020), 3 months of CAPOX has shown non-inferiority to 6 months of therapy, with reduced neurotoxicity for stage III low-risk patients. In

contrast, high-risk stage III patients achieve better outcomes with 6 months of CAPOX or FOLFOX. For high-risk stage II patients, 3 months of CAPOX is an option, but 6 months of FOLFOX provides superior efficacy. High-risk stage II patients may achieve greater benefits from 6 months of FOLFOX compared to CAPOX, though patient tolerance and side effect profiles should guide decision-making. Generally, CAPOX is often preferred due to its lower incidence of neurotoxicity compared to FOLFOX, especially in shorter treatment durations (Chakrabarti et al., 2020).

#### 2.2.6.4 Immunotherapy

Cancer immunotherapy aims to activate the immune system to produce antitumour effects. This provides a more specific anti-tumour therapy compared to chemotherapy, radiotherapy, and other approaches. Moreover, an increasing number of monoclonal antibodies other immunotherapeutic drugs have been approved by the Food and Drug Administration (FDA), making immunotherapy a potent treatment option (Hemmatzadeh et al., 2018), immunotherapy is based on the principle of actively targeting a specific tumour antigen or enhancing the host's immune system (Sahu and Suryawanshi, 2021). There are several cancer immunotherapies, including adoptive cell therapy (ACT), immune checkpoint inhibitors, targeted monoclonal antibodies, oncolytic virus therapy, cancer vaccines, and cytokines (Sahu and Suryawanshi, 2021).

In ACT, T cells are vital to eliminate cancer cells and abundant T cells are required to promote anti-tumour immunity (Menon et al., 2016). Hence, tumour-infiltrating lymphocyte (TIL)- derived T cells or T cells are genetically engineered to express tumour-recognizing receptors (Met et al., 2019). There are several types of ACT, the first is TIL therapy. The TILs are isolated from cancer, cultured to a greater number, and reintroduced

to patients who are under a lymphocyte-depleting preparatory regimen. The large number of TIL cells, therefore, enhances the anti-tumour immune response. The second type is engineered T-cell receptor therapy, in which the T cell is engineered with specific antigen receptors to recognize tumour-specific antigens in a major histocompatibility complex-dependent manner. The third type is CAR T-cell therapy. CARs are specialised structures that function in antigen binding as well as intracellular signalling apparatus. It can be introduced into T lymphocytes aided by a viral receptor. They can recognize antigens without antigen-presenting cells (APCs) and drive cellular activation to kill tumour cells (Menon et al., 2016).

The next is immune checkpoint inhibitors. Immune checkpoints are normal components of the immune system. Antigen presentation along with many co-stimulatory and co-inhibitory signals are required to activate T-cells. When co-inhibitory signals are present, the stimulatory signal fails, leading to T-cell anergy or apoptosis and failing to induce cytotoxicity (Moy et al., 2017). Co-inhibitory (checkpoint) signals prevent exaggerated immune responses and maintain immune tolerance under normal conditions. In the tumour microenvironment (TME), these inhibitory checkpoints are overexpressed, causing tumour-promoting immunosuppression. Immune checkpoint inhibitors (MoAbs) may be used to block the corresponding checkpoints, allowing the T cells to kill tumour cells. For example, anti-cytotoxic T lymphocyte-associated molecule 4 (anti-CTLA4) antibodies such as Ipilimumab, anti-programmed cell death receptor 1 (anti-PD1) antibodies like Nivolumab and Pembrolizumab, and anti-programmed cell death ligand 1 antibodies.

Besides, the targeted monoclonal antibodies can target specific antigens of cancer cells. They are created in a lab, are more specific than chemotherapy, and have fewer side effects. Tumours capture nutrients by forming new blood vessels through angiogenesis,

which is stimulated by the release of a substance called vascular endothelial growth factor (VEGF). Angiogenesis can be shut down by monoclonal antibodies that are classified into anti-angiogenic drugs or angiogenesis inhibitors. For example, Bevacizumab (Avastin), Ramucirumab (Cyramza) and ziv-aflibercept (Zaltrap). Another example is Cetuximab (Erbitux) and panitumumab (Vectibix) which slow cancer growth by targeting a protein found on the surface of some cells called the epidermal growth factor receptor (EGFR) that plays a role in regulating cell growth and is present in about 75% of colon cancers (García-Foncillas et al., 2019).

Moreover, in oncolytic virus therapy, it was found that virus-induced cytopathic effects can be used to destroy tumour cells. Hence, genetically modified viruses are used to infect tumour cells, for example, herpes simplex virus type 1 (HSV-1) has been genetically modified for oncolytic virus therapy. The cytopathic effects of the virus not only stimulate a pro-inflammatory environment to produce systemic antitumour immunity but also promote inherent tumour immunity in cancer patients. These viruses are termed “oncolytic viruses” as they specifically target tumour cells (Yura and Hamada, 2017).

Next, cancer vaccines are also one of the cancer immunotherapies. This works by triggering an intense immune response to specific antigens of cancer cells, promoting local TIL infiltration, and destroying cancer cells. Cancer vaccine for colorectal cancer is still under development. There are several types of colorectal cancer vaccines in the trial phase: molecular-based, cancer cell vaccine, dendritic cell vaccine, vector-based, and viral vector-based vaccine (Jia et al., 2022). However, these cancer vaccines for colon cancer are still under clinical trial and require further investigation before they can be used in real-life settings.

### 2.3 The need for EEAE as an alternative treatment for colon cancer

Despite the availability of multiple colon cancer treatments, they often come with significant limitations and side effects. Colon cancer removal may come with complications after surgery, such as adhesion and small bowel obstruction, thrombosis, infection, anastomotic leakage, colonic ischaemia, or other systemic complications (Pak et al., 2020). Moreover, even though chemotherapy and radiotherapy are effective in killing cancer cells, they cause damage to normal cells and lead to side effects. For instance, the side effects are nausea, vomiting, fatigue, decreased appetite, changes in taste, hair loss, dry mouth, skin changes, memory problems, hearing loss and constipation (Altun and Sonkaya, 2018). On the other hand, the therapies also cause distress, anxiety, and depression (El Kheir and Ibrahim, 2019). In immunotherapy, for patients receiving immunotherapy drugs intravenously, the most common side effects include skin reactions at the site of the injection, such as pain, swelling, and soreness. In rare cases, some immunotherapy drugs may cause severe or even fatal allergic reactions. In addition, most of the mCRC patients are microsatellite stable (MSS) and resistant to immunotherapy, making it not an ideal therapy (Gandini et al., 2023). Moreover, this method is expensive. Therefore, natural product therapy may be used for the public as it causes fewer side effects and is less costly.

The usage of natural products has been raised since a growing amount of research has proven their value in treating or preventing some diseases, depending on the compounds they carry. *E. elatior* was believed to contain potent anti-tumour agents. In a previous study, *E. elatior* was observed to have a potent antitumour activity (Habsah et al., 2005). Moreover, Ghasemzadeh et al. (2015) demonstrated its effectiveness against breast cancer cell lines MCF-7 and MDA-MB-231. A study by Habsah et al. (2005) showed the half-maximal inhibitory concentration ($IC_{50}$) of ethyl acetate extracted *E.*

*elatior* on MCF-7 and CEM-SS of 4 µg/ml and 6.25 µg/ml, respectively. In addition, Krajarng et al. (2017) reported selective cytotoxicity of the extract on B16 melanoma cells, with no harmful effects on non-cancerous cells, suggesting its potential as a chemopreventive agent. Moreover, it exhibits inhibitory activity on HeLa cervical cancer cell line growth with an $IC_{50}$ of 127.98 µg/ml (Herni et al., 2021). In addition, in a study conducted by Hueh Zan et al. (2011), *E. elatior* showed $IC_{50}$ of 97.5 µg/ml towards Hep-G2 cell lines and more than 100 µg/ml towards MDA-MB-231, MCF-7, and HeLa cell lines. Notably, its anti-cancer effectiveness against colon cancer has been proven by a study carried out by Al-Mansoub et al. (2021) that EEAE exhibited selective cytotoxicity against the HT-29 colon cancer cell line, with $IC_{50}$ values of 19.82, 37.001, 50.49, and 53.29 µg/mL against HT-29, HCT 116, Hela, and MCF-7 tumour cell lines respectively, highlighting its potential role as a natural treatment for colon cancer.

In plants, the most valuable compounds are the phytochemical content, phenolic compounds and flavonoid compounds. According to Sulaiman and Balachandran (2012), approximately, there are about 8000 plant phenolics, and about half of them are flavonoids. Phenolics have properties like antioxidant, antimutagenic, and anticarcinogenic and are also able to edit gene expression. On the other hand, flavonoids are grouped under phenolic compounds and can exist in both free states and as glycosides in different plant parts. They are found to have many biological activities, including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, and protein kinase inhibition (Sulaiman and Balachandran, 2012). *E. elatior*, as a natural product, also contains many phenolic and flavonoid compounds like gallic acid, tannins, chlorogenic acid, caffeic acid, coumarin, quinones, stigmastane, ergostanoid, anthocyanin, quercetin, apigenin, kaempferol, luteolin, myricetin, and also anthocyanin (Ghasemzadeh