

EVALUATION OF THE ANTI-CANCER PROPERTIES OF ETHANOLIC
CRUDE EXTRACTS FROM THAI-ISOLATE BAMBOO MUSHROOM
(*DICTYOPHORA* SP.)

KANYAPAK SAKHEATKARN



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การทดสอบคุณสมบัติการต้านมะเร็งจากสารสกัดหยาดเอทานอล
ของเห็ดเหี่ยวไผ่สายพันธุ์ไทย (*Dictyophora* sp.)



นางสาวกัญญาภัค สาเขตรการณั์

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

Thesis Examining Committee

P. Chantree

(Assoc. Prof. Dr. Pathanin Chantree, D.V.M.)

Chairperson

Sirilak Chumkiew

(Dr. Sirilak Chumkiew)

Member (Thesis Advisor)

Pongsakorn Martviset

(Assoc. Prof. Dr. Pongsakorn Martviset)

Member (Thesis Co-Advisor)

Mantana Jamklang

(Asst. Prof. Dr. Mantana Jamklang)

Member (Thesis Co-Advisor)

Watsana Penkhruae

(Asst. Prof. Dr. Watsana Penkhruae)

Member

Chompunoot Wangboon

(Dr. Chompunoot Wangboon)

Member

Pishyaporn Sritangos

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Member

Santi Maensiri

(Prof. Dr. Santi Maensiri)

Yupaporn Ruksakulpiwat

(Assoc. Prof. Dr. Yupaporn Ruksakulpiwat)

Acting Vice Rector for Academic Affairs
and Quality Assurance

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มะเร็งเป็นสาเหตุหลักของการเสียชีวิตทั่วโลก รวมถึงในประเทศไทย มะเร็งที่พบบ่อยที่สุดในประชากรไทยคือมะเร็งตับและมะเร็งลำไส้ใหญ่ โดยมีอัตราการเกิดมะเร็งเพิ่มขึ้นทุกปี เคมีบำบัดยังคงเป็นวิธีการรักษาหลัก แต่มักมีข้อจำกัดที่สำคัญ เช่น การดื้อยา โดยเฉพาะกับยาเคมีบำบัดแบบเดิม การวิจัยปัจจุบันจึงหันไปศึกษาสารประกอบใหม่ ๆ ที่มีฤทธิ์ต้านมะเร็งอย่างมีประสิทธิภาพและมีผลข้างเคียงน้อยที่สุด *Dictyophora indusiata* ซึ่งรู้จักกันทั่วไปในนามเห็ดเหื่อไผ่ ได้แสดงให้เห็นถึงคุณสมบัติต้านมะเร็งที่ในงานวิจัยต่าง ๆ อย่างไรก็ตาม งานวิจัยส่วนใหญ่เน้นที่เห็ดเหื่อไผ่สายพันธุ์จีน ในขณะที่สายพันธุ์ไทยยังไม่ได้มีการศึกษามากนัก ดังนั้น งานวิจัยนี้จึงมีวัตถุประสงค์เพื่อประเมินศักยภาพในการต้านมะเร็งและผลการกระตุ้นอะพอพโทซิสของสารสกัดจากเห็ดเหื่อไผ่สายพันธุ์ไทยสามส่วนในเซลล์มะเร็งตับและมะเร็งลำไส้ใหญ่ สารสกัดได้รับการเตรียมโดยใช้วิธีที่กลุ่มวิจัยของเราได้รายงานไว้ โดยการสกัดด้วยเอทานอลในอัตราส่วน 1:10 เป็นเวลา 7 วัน ตามด้วยการกรองและการระเหย ความเป็นพิษต่อเซลล์ได้รับการประเมินโดยใช้การทดสอบ MTT สารสกัดที่มีประสิทธิภาพสูงสุดจะได้รับการศึกษาการเหนี่ยวนำอะพอพโทซิสผ่านโพลีไอโตเมทรีและการวิเคราะห์โปรตีนที่เกี่ยวข้องกับอะพอพโทซิส การทดสอบความเป็นพิษของสารสกัดจากเห็ดเหื่อไผ่ทั้งสามส่วนต่อเซลล์มะเร็งตับพบว่าสารสกัดทั้งสามส่วนสามารถยับยั้งการเติบโตของเซลล์มะเร็งได้ โดยเฉพาะอย่างยิ่งเมื่อทดสอบในความเข้มข้นสูง โดยยับยั้งการเจริญของเซลล์มะเร็งได้ร้อยละ 74.71 สำหรับสารสกัดหมวกเห็ด ร้อยละ 87.09 สำหรับดอกเห็ด และร้อยละ 80.47 สำหรับสารสกัดไข่เห็ด อย่างไรก็ตาม แม้ว่าสารสกัดหมวกเห็ดและดอกเห็ดไม่มีฤทธิ์ยับยั้งเซลล์มะเร็งลำไส้ใหญ่ แต่สารสกัดไข่เห็ดกลับมีฤทธิ์เป็นพิษต่อเซลล์มะเร็งลำไส้ใหญ่อย่างมีนัยสำคัญ โดยยับยั้งการเจริญของเซลล์มะเร็งลำไส้ใหญ่ได้ร้อยละ 94.79 เมื่อทดสอบที่ 2,500 ไมโครกรัมต่อมิลลิลิตร ซึ่งเป็นความเข้มข้น

สูงสุด ซึ่งบ่งชี้ว่าสารสกัดไข่เห็ดมีประสิทธิภาพสูงสุดต่อเซลล์มะเร็งลำไส้ใหญ่ นอกจากนี้ สารสกัดทั้งสามส่วนยังพบว่าไม่มีพิษต่อเซลล์ปกติ (เซลล์ไฟโบรบลาสต์) ในการทดลองต่อมาซึ่งศึกษาการเหนี่ยวนำอะพอพโทซิสและการแสดงออกของโปรตีนที่เกี่ยวข้องในเซลล์มะเร็งลำไส้ใหญ่ที่ได้รับสารสกัดไข่เห็ดเยื่อไผ่พบว่าอะพอพโทซิสทั้งในระยะเริ่มต้นและระยะท้ายเพิ่มขึ้นตามความเข้มข้น นอกจากนี้ ระดับการแสดงออกของ Bax และ caspase-9 ซึ่งเป็นโปรตีนหลักที่เกี่ยวข้องกับการตายของเซลล์แบบอะพอพโทซิส ยังเพิ่มขึ้นด้วย ผลการวิจัยเหล่านี้ชี้ให้เห็นว่าสารสกัดจากไข่เห็ดเยื่อไผ่มีแนวโน้มที่จะนำไปใช้เป็นยารักษา มะเร็งลำไส้ใหญ่ได้



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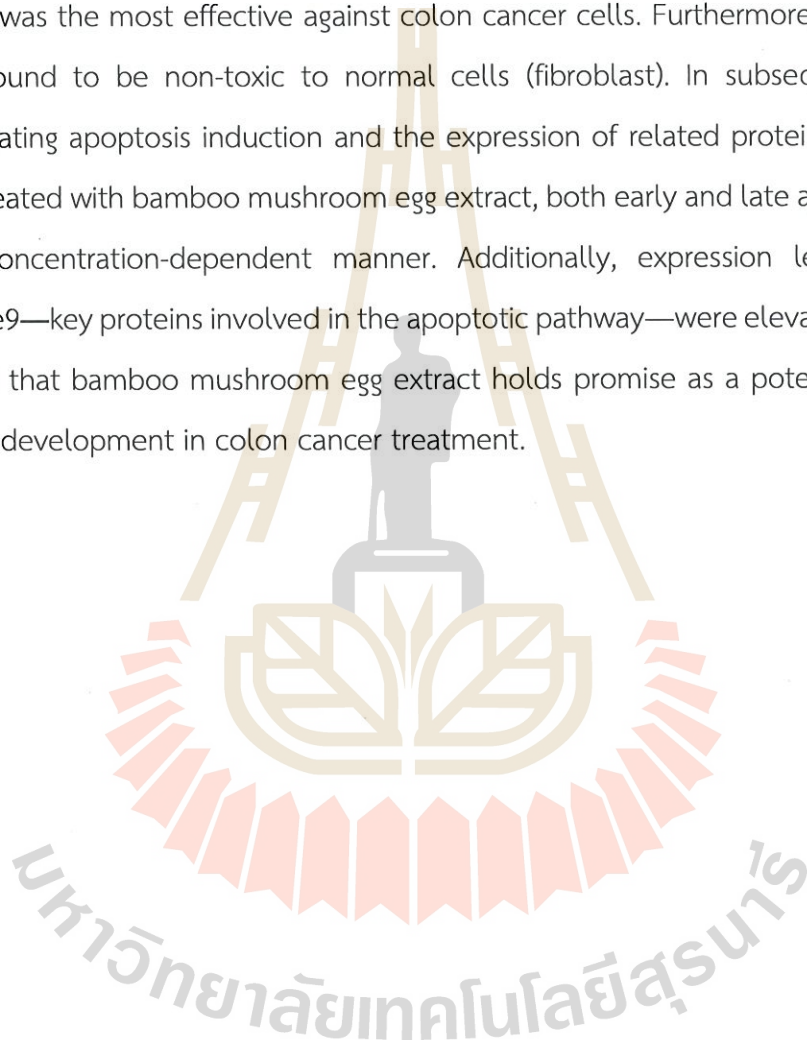
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KANYAPAK SAKHEATKARN : EVALUATION OF THE ANTI-CANCER PROPERTIES OF ETHANOLIC CRUDE EXTRACTS FROM THAI-ISOLATE BAMBOO MUSHROOM (*DICTYOPHORA* SP.). THESIS ADVISOR : SIRILAK CHUMKIEW, Ph.D. 56 PP.

Keyword: Bamboo mushroom; gastrointestinal cancers; apoptosis

Cancer is a leading cause of mortality worldwide, including in Thailand. Among the most prevalent cancers in the Thai population are liver and colon cancers, with incidence rates rising annually. Chemotherapy remains the primary treatment modality; however, it is often associated with significant limitations, such as the development of drug resistance, particularly with conventional chemotherapeutic agents. In response, current research has shifted toward research novel compounds with potent anti-cancer activity and minimal side effects. *Dictyophora indusiata*, commonly known as the bamboo mushroom, has demonstrated promising anti-cancer properties in various studies. However, most of these investigations have focused on Chinese species, while Thai variants remain relatively underexplored. Therefore, this study aims to evaluate the anti-cancer potential and apoptosis-inducing effects of extracts from three parts of the Thai bamboo mushroom in liver and colon cancer models. The extracts were prepared using a method reported by our research group, involving ethanol extraction at a 1:10 ratio over a period of 7 days, followed by filtration and evaporation. Cytotoxicity was assessed using the MTT assay. Extracts demonstrating the highest potency were further analyzed for apoptosis induction through flow cytometry and analysis of apoptosis-associated proteins. Toxicity testing of the three extracts on liver cancer cells revealed that all were capable of inhibiting cancer cell growth at high concentrations. Specifically inhibiting the growth of cancer cells was 74.71% for the cap extract, 87.09% for the fruiting body, and 80.47% for

the mushroom egg extract. However, while the cap and fruiting body extracts showed no inhibitory effect on colon cancer cells, the mushroom egg extract demonstrated significant cytotoxicity on colon cancer, inhibiting the growth of colon cancer cells by 94.79% at 2,500 $\mu\text{g/ml}$, which was the highest concentration. This indicates that the mushroom egg extract was the most effective against colon cancer cells. Furthermore, all three extracts were found to be non-toxic to normal cells (fibroblast). In subsequent experiments investigating apoptosis induction and the expression of related proteins in colon cancer cells treated with bamboo mushroom egg extract, both early and late apoptosis increased in a concentration-dependent manner. Additionally, expression levels of Bax and caspase9—key proteins involved in the apoptotic pathway—were elevated. These findings suggest that bamboo mushroom egg extract holds promise as a potential candidate for further development in colon cancer treatment.



School of Biology

Academic Year 2024

Student's Signature กัญจวิภา
Advisor's Signature [Signature]
Co-advisor's Signature [Signature]
Co-advisor's Signature P. Martviset

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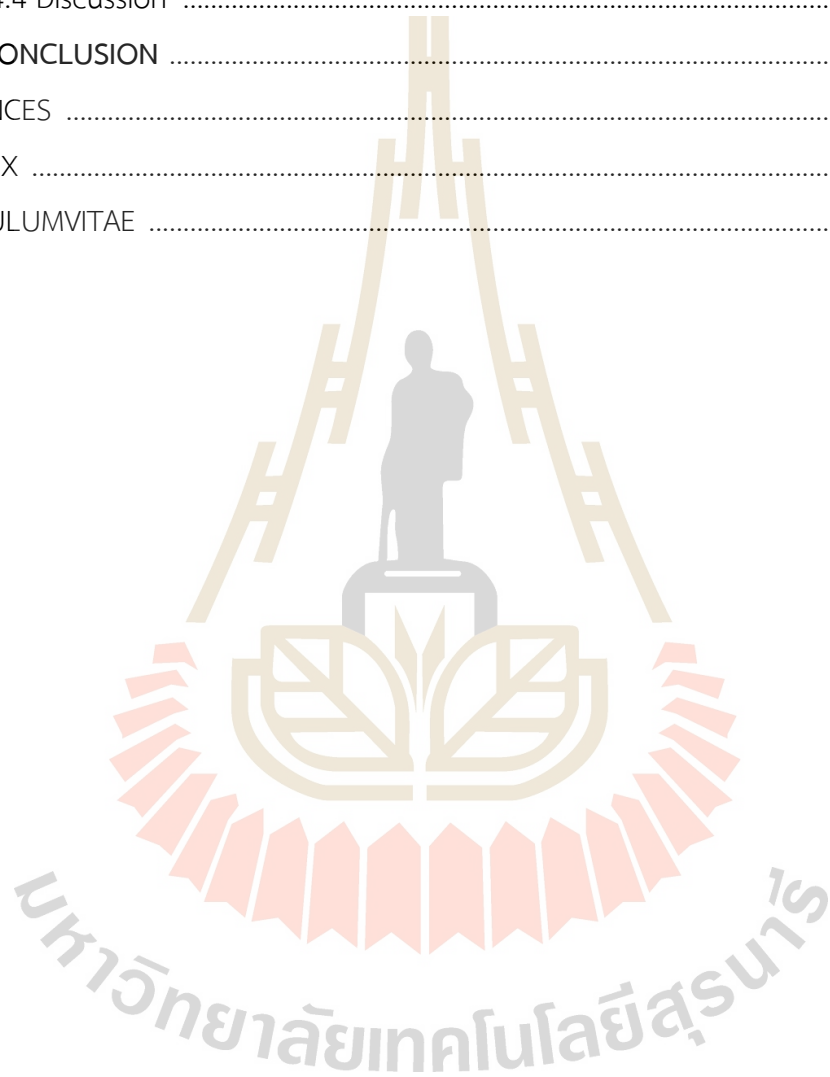
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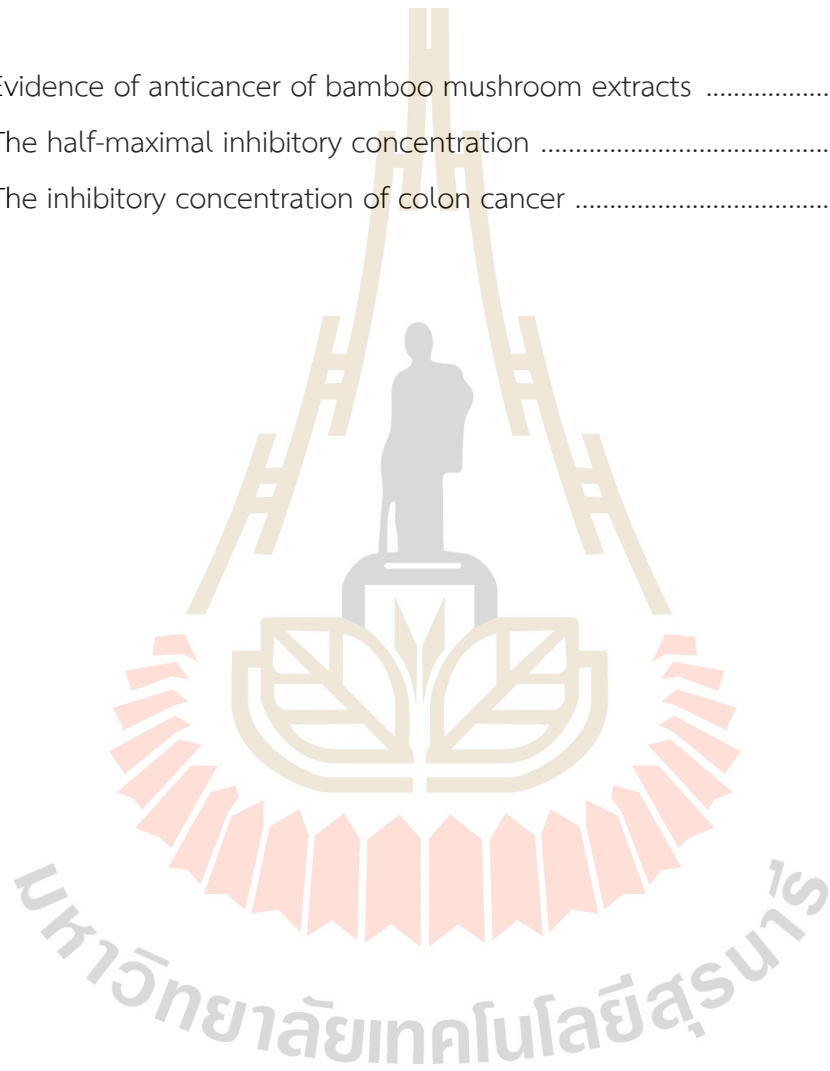
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LIST OF ABBREVIATIONS

<i>Akt</i>	Protein Kinase B
<i>ANOVA</i>	Analysis of Variance
<i>AP</i>	Alkaline Phosphatase
<i>APAF1</i>	Apoptotic Protease Activating Factor 1
<i>Bak</i>	Bcl-2 Antagonist/Killer
<i>Bax</i>	Bcl-2-Associated X Protein
<i>BCA</i>	Bicinchoninic Acid
<i>Bcl-2</i>	B-cell Lymphoma 2
<i>bFGF</i>	Basic Fibroblast Growth Factor
<i>BSA</i>	Bovine serum albumin
<i>C57BL/6</i>	C : Cold Spring Harbor, 57 is the strain number, BL : Black, 6 signifies the Substrain
<i>Caspase</i>	Cysteine-aspartic proteases
<i>c-FLIP</i>	Cellular FLICE-inhibitory Protein
<i>CD4⁺</i>	Helper T cells
<i>CD8⁺</i>	Cytotoxic T cells
<i>CO₂</i>	Carbon Dioxide
<i>DMSO</i>	Dimethyl Sulfoxide
<i>DMEM</i>	Dulbecco's Modified Eagle Medium
<i>DPPH</i>	1,1-diphenyl-2-picrylhydrazyl
<i>ERK</i>	Extracellular Signal-Regulated Kinase
<i>FADD</i>	Fas-associated death domain protein
<i>FBS</i>	Fetal Bovine Serum

LIST OF ABBREVIATIONS (Continued)

<i>FGF18</i>	Fibroblast Growth Factor 18
<i>GI</i>	Gastrointestinal
<i>HepG2</i>	Human Liver Cancer Cell Line
<i>HT-29</i>	Human Colorectal Adenocarcinoma Cell Line
<i>HtrA2</i>	High Temperature Requirement Protein A2
<i>IAPs</i>	Inhibitors of Apoptosis Proteins
<i>IC₂₅</i>	25% Inhibitory Concentration
<i>IC₅₀</i>	Half-maximal Inhibitory Concentration
<i>IC₇₅</i>	75% Inhibitory Concentration
<i>KRAS</i>	Kirsten Rat Sarcoma Virus
<i>MDA</i>	Malondialdehyde
<i>mg/mL</i>	Milligrams per Milliliter
<i>ml</i>	Milliliter
<i>MMP</i>	Matrix Metalloproteinase
<i>mTOR</i>	Mechanistic Target of Rapamycin
<i>MTT</i>	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
<i>NF-κB</i>	Nuclear Factor Kappa-light-chain-enhancer of activated B cells
<i>nm</i>	Nanometer
<i>NBT-BCIP</i>	Nitro Blue Tetrazolium and 5-Bromo-4-Chloro-3-Indolyl Phosphate
<i>O₂⁻</i>	Superoxide
<i>OH\cdot</i>	Hydroxyl Radicals
<i>OUMS</i>	Normal Human Embryonic Fibroblasts Cell Line
<i>PBS</i>	Phosphate-Buffered Saline
<i>pH</i>	Potential of Hydrogen
<i>PI</i>	Propidium Iodide

LIST OF ABBREVIATIONS (Continued)

<i>PI3K</i>	Phosphoinositide 3-Kinase
<i>PTEN</i>	Phosphatase and Tensin Homolog
<i>Ras/Raf/MEK/ERK</i>	Rat Sarcoma–Rapidly Accelerated Fibrosarcoma–Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase Pathway
<i>RIPA</i>	Radio Immunoprecipitation Assay
<i>ROS</i>	Reactive Oxygen Species
<i>rpm</i>	Round per Minute
<i>SOD</i>	Superoxide Dismutase
<i>SASP</i>	Senescence-Associated Secretory Phenotype
<i>SD</i>	Standard Deviation
<i>SDS-PAGE</i>	Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis
<i>Smac/Diablo</i>	Second Mitochondria-Derived Activator of Caspases / Direct IAP-binding Protein with Low pI
<i>TBST</i>	Tris-buffered Saline with Tween 20
<i>TBS</i>	Tris-buffered Saline
μg	Micrograms
$\mu\text{g/ml}$	Micrograms per Milliliter
μL	Microliter
<i>USA</i>	United States of America
<i>VEGF</i>	Vascular Endothelial Growth Factor
<i>V-FITC</i>	V conjugated with Fluorescein Isothiocyanate
<i>WHO</i>	World Health Organization
<i>w/v</i>	weight by volume
$^{\circ}\text{C}$	Degree Celsius

CHAPTER I

INTRODUCTION

1.1 Background problem

Gastrointestinal cancers account for more than 20% of all cancer cases and approximately 22.5% of global cancer-related deaths. Colorectal cancer is the third most prevalent disease in the world and the second largest cause of cancer mortality (Kuntz et al., 2021). According to 2020 projections, liver cancer is expected to be the third leading cause of cancer-related mortality and the sixth most commonly diagnosed cancer. Liver cancer has been linked to 1.3 million new cases and fatalities in Southeast Asia (Runggay et al., 2022). while, Thailand has the 13th highest reported new cancer cases and the 12th highest reported cancer deaths in Asia (Huang et al., 2022). Interestingly colorectal and liver cancer are the top common cancers in Thailand (Virani et al., 2017). Liver and bile duct cancers are among the most frequently occurring malignancies in men and rank as the third most prevalent cancers among women. Over the past decade, liver cancer has ranked as the third leading cause of cancer-related mortality in men and the fifth leading cause in women (Chitapanarux and Phornphutkul, 2015). Furthermore, colorectal cancer is the only major cancer in Thailand exhibiting an increasing incidence in both males and females. With over 10,000 new cases diagnosed annually, it currently ranks as the third most common cancer in men and the fourth in women. (Lohsiriwat et al., 2020).

Recently, surgery and chemotherapy are the most popular treatments for treating liver and colorectal cancers diseases (Riesco-Martinez et al., 2022). Many studies reported that chemotherapy becomes the heart of colorectal cancer treatment, due to the increasing of overall survival up to 20 months of colon cancer patients ((Cassidy et al., 2004; Colucci

et al., 2005; Goldberg et al., 2004; Xie et al., 2020). However, chemotherapy has limit on health, age, and severe side effects for the patients such as hair lose, skin changes, and it also destroys normal cells (Bilgin, 2023). Furthermore, traditional chemotherapeutic drugs are the primary cause of cancer resistance and treatment failure (Peng, 2023). Thus, novel drug with high efficiency and minimal side effects are continuing sough (Bilgin, 2023). Research is being conducted to explore the potential of natural extracts for inhibiting cancer. This includes extracts derived from plants such as *Allium sativum* L., *Aloe vera*, *Lepidium meyenii*, and *Rhodiola rosea* L., (Beshbishy et al., 2020; Da Silva Leitão Peres et al., 2020; Gao et al., 2019; Sęczyk et al., 2023) as well as from mushrooms like *Coriolus versicolor*, *Ganoderma lucidum*, *Lentinula edodes*, and *Dictyophora indusiata* (Hu et al., 2020; Panda et al., 2022). The aim is to develop these natural extracts into effective cancer drugs.

The bamboo mushroom (*Dictyophora indusiata*), often referred to as the 'queen of mushrooms' due to its elegant fruiting body and distinctive flavor, is regarded as important for both its nutritional value and medicinal benefits. this mushroom possesses substantial nutritional value and is rich in bioactive compounds, including proteins, minerals, vitamins, amino acids, thiamine, riboflavin, niacin, and polysaccharides (Deng et al., 2016). Accordingly, *D. indusiata* is extensively consumed in various Asian regions, notably in China and Thailand. This mushroom also holds a longstanding role in traditional medicine, where it has been employed to address a variety of ailments. According to ancient Chinese medical texts, it is believed to calm the mind and body, support brain function, moisten the lungs, nourish yin, exhibit anti-inflammatory and analgesic properties, act as a diuretic, and alleviate gastrointestinal and neurological symptoms (J. Wang et al., 2024). Several earlier research claimed that this mushroom possesses albaflavenone, 5-(Hydroxymethyl)-2-furfural, 1,3-glucan polysaccharide, dictyophorines A and B, and dictyoquinazols A, B, and C in the bamboo mushroom extract (M. Huang et al., 2011; I. K. Lee et al., 2002; Lizarme et al., 2016; Sharma et al., 2004). These compounds have antimicrobial, anti-tyrosinase, antioxidant, antitumor, anti-inflammatory, immunomodulatory activities,

encouraging nerve growth factor production, and are neuroprotective (Fu et al., 2019; Habtemariam, 2019; Wang et al., 2021) including have anti-cancer on osteosarcoma, hepatocellular carcinoma, cholangiocarcinoma, and prostate carcinoma (Chantree et al., 2022; Han et al., 2017; Hu et al., 2020; Zhong et al., 2013). Therefore, this mushroom species is widely utilized in research for the investigation of various bioactive compounds.

1.2 Significance of the study

Liver cancer and colon cancer are top cancers with the highest prevalence in Thailand. There are numerous methods for treating cancer diseases. The most popular one is chemotherapy treatment, even though this treatment has many limitations (Bilgin et al., 2023; Peng et al., 2023). Due to the low specificity, this treatment method can destroy normal tissue causing severe side effects for cancer patients. Research is therefore being done extensively to find chemicals that have anti-cancer activity while having little to no impact on normal cells. Numerous document extracts have discussed the beneficial anti-cancer properties of the Chinese bamboo mushroom *Dictyophora indusiata*. Although bamboo mushrooms from Thailand (*Dictyophora* sp.) have been cultivated and sold in the market, they have also occasionally been used as a supplement to food. Interestingly, there are a few reports on this strain's characteristics and bioactive properties (Chantree et al., 2022; Chumkiew et al., 2024). Therefore, this study aims to investigate the anti-cancer properties and apoptotic induction mechanisms of the isolated Thai bamboo mushroom extract in gastrointestinal cancers.

1.3 Research objectives

1.3.1 To study antiproliferative effects of the Thai bamboo mushroom (*Dictyophora* sp.) crude extracts on a human liver cancer cell line (HepG2) and human colorectal adenocarcinoma cell line (HT29).

1.3.2 To study anti-cancer mechanisms of the Thai bamboo mushroom (*Dictyophora* sp.) crude extracts focusing on apoptosis pathway.

1.4 Research hypothesis

Extracts from various parts of the bamboo mushroom exhibited anti-cancer activity against liver and colon cancer cells while sparing normal cells. The mushroom egg extract may be the most potent, possibly due to egg nutrient-accumulating phase. These extracts may induce apoptosis, a programmed form of cell death that minimizes damage to surrounding healthy tissue. This research serves as a preliminary study and may contribute to the development of future cancer therapies.

1.5 Scope of the study

This study aims to evaluate the anti-cancer effects of crude extracts from 3 parts (Cap, fruiting body, and egg) of a Thai isolate bamboo mushroom (*Dictyophora* sp.) on liver cancer (HepG2) and colon cancer (HT-29) cell lines. The growth-inhibitory activity of the crude extracts was assessed using cytotoxicity assays. Apoptotic induction mechanisms were investigated through western blot analysis to examine the expression of apoptosis-related proteins. Crude extract preparation was conducted at Laboratory Building 2 (F2), while cell culture experiments were performed at Laboratory Building 9 (F9) of Suranaree University of Technology and at the RU-NFS Laboratory, Faculty of Medicine, Thammasat University.

CHAPTER II

LITERATURE REVIEW

2.1 Bamboo mushroom

Dictyophora indusiata is a fungus in the phylum Basidiomycetes, class Agaricomycetes, family Phallaceae. Its common names are bamboo mushroom, bamboo pith, long net stinkhorn, stinkhorn basket, and crinoline (Habtemariam, 2019). It grows abundantly in tropical regions of Africa, Asia, Australia, and the Americas, typically on decayed wooden trunks or nutrient-rich soil. In China, it is found growing on the damp roots of bamboo forests as well as in wooded areas. This mushroom's morphologically distinguishing feature is the fruiting body that looks like a white veil (see Figure 2.1C) that hangs from the cap down to cover the stalk.

2.1.1 Bamboo mushroom stage

The growth of *D. indusiata* is divided into 4 stages. The first stage is the egg stage: individuals emerge in the form of eggs, which are covered in a coating of white-brown hue as shown in Figure 2.1A. The next stage, the germination stage: the egg's membrane rends. Then the cap grows out, followed by the stalk and fruiting under the cap (Sitinjak, 2016) as shown in Figure 2.1B. From Sitinjak (2016) the maturity stage: the stalk elongates. The fruiting body expands, a net-like shape surrounds the stalk as shown in Figure 2.1C, almost long touching the ground. The last stage, the wilt stage: Fruiting body shriveled and turned from white to golden yellow as shown in Figure 2.1D. The cap and stalk slowly hung down to the ground.

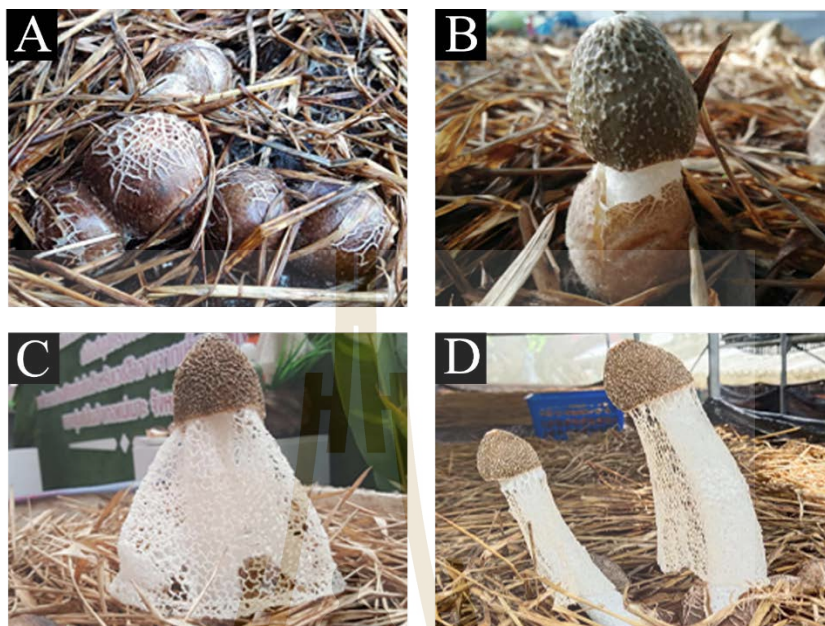


Figure 2.1 Morphology of *D. indusiata* in 4 stages. (A) egg stage, (B) germination stage, (C) maturity stage, and (D) wilt stage.

2.1.2 Bioactive compound in bamboo mushroom

D. indusiata is the popular bamboo mushroom isolated for doing research and well known as a substantial health benefit. Most extracted substances were polysaccharides, which have variable total sugar contents based on extraction methods (Deng et al., 2016; Zhang et al., 2016). Analysis using various techniques revealed that the primary polysaccharide components include arabinose, fucose, galactose, glucosamine, glucose, glucuronic acid, mannose, ribose, rhamnose, and xylose (Hu et al., 2020; Wang et al., 2019; Zhang et al., 2016). The main polysaccharide in mushrooms is 1,3-glucan that has side branches 1,6- β -glucosyl units as shown in Figure 2.2. This polysaccharide has antioxidant, anti-inflammatory, antitumor, anticancer, and immunomodulatory activities (Fu et al., 2019). In addition, albaflavenone, 5-(Hydroxymethyl)-2-furfural, dictyophorines A and B, and dictyoquinazols A, B, and C, (see Figure 2.3) are all present from extraction.

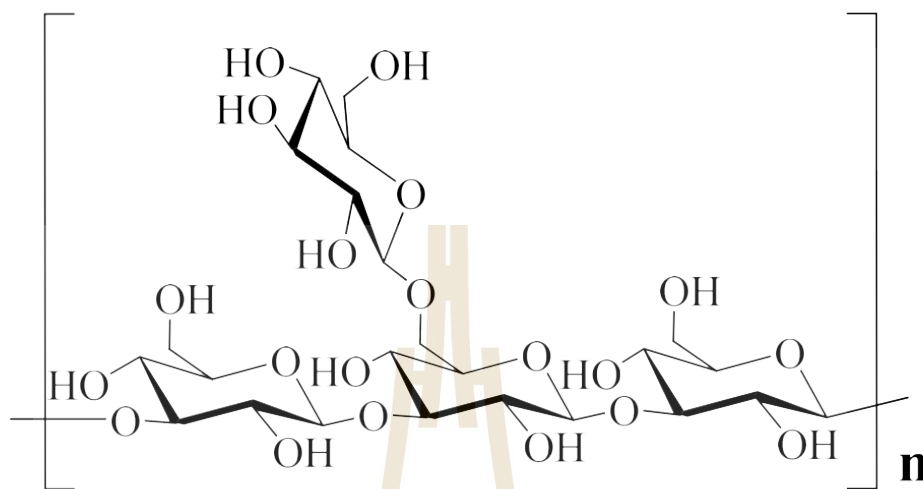


Figure 2.2 Structure of polysaccharide (Habtemariam, 2019).

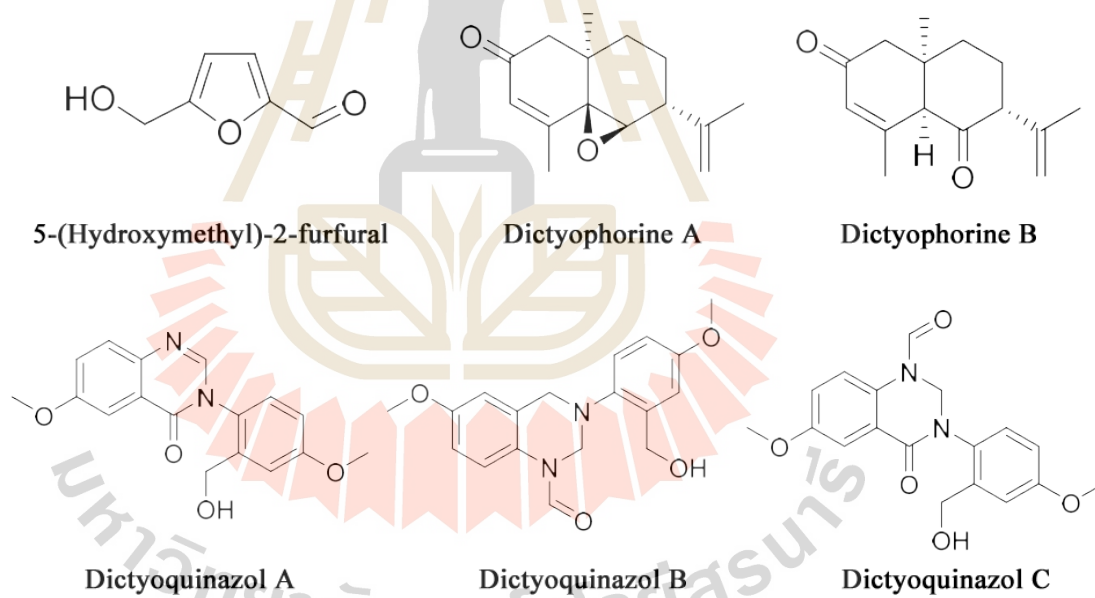


Figure 2.3 Structure of bioactive compound from *D. indusiata* (Habtemariam, 2019).

2.1.3 Activity of bioactive compound from bamboo mushroom

Albaflavenone, 5-(Hydroxymethyl)-2-furfural, dictyophorines A and B, and dictyochinazols A, B, and C, have antimicrobial, anti-tyrosinase—inhibits tyrosinase, which is an enzyme that stimulates the production of melanin and pigment—, encouraging nerve growth factor production, and are neuroprotective (Wang et al., 2021). Researchers can quantify antioxidant capacities via malondialdehyde (MDA) content, superoxide dismutase (SOD) activities, and reactive oxygen species (ROS) levels, which measure 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide (O_2^-), and hydroxyl radicals (OH^\bullet). In *Caenorhabditis elegans*—a free-living transparent nematode—the polysaccharide of *D. indusiata* decreases ROS and MDA levels and increases SOD activity (Zhang et al., 2016). *In vitro* polysaccharides have high reduced capacity and scavenging action on 1,1-diphenyl-2-picrylhydrazyl, superoxide, and hydroxyl radicals (X. Liu et al., 2017). Obese mice *in vivo* experiments revealed polysaccharides improved antioxidant capacities (Wang(e) et al., 2019). From studying anti-inflammatory effect mechanisms of macrophages, the polysaccharide can inhibit NF- κ B signaling pathway, and decreased NLRP3 expression in the cytoplasm reduced inflammasome activation (Wang(d) et al., 2019). Such findings are consistent with colitis study in C57BL/6 mice (Wang(b) et al., 2019). From Wang(b) et al. (2019) the polysaccharide modulating macrophage polarization and restoring intestinal barrier function renders reduced colitis inflammation. The studying molecular mechanisms in immunostimulatory action in RAW264.7 cells, researchers observed the polysaccharide from *D. indusiata* can stimulate macrophages to produce greater nitric oxide, interleukin-1, interleukin-6, and tumor necrosis factor (Deng et al., 2016). Such findings are also consistent with function of prostate cancer-associated fibroblasts study (Han et al., 2017). Han et al., 2017 reported found that the polysaccharide from *D. indusiata* stimulates the proliferation of immune cell and decrease prostate cancer-associated fibroblast functions by inhibiting $CD4^+/CD8^+$ T cells growth. This research may lead to the development of new cancer-fighting techniques.

Furthermore, the *D. indusiata* extract has anti-hyperlipidemic and anti-cancer. For anti-hyperlipidemic activity, during an *in vitro* experiment, *D. indusiata* polysaccharides

demonstrated the ability to bind with fat, cholesterol, and bile acid, as well as inhibit lipase activity (Wu et al., 2021). Accordingly *in vivo* investigation on obese mice, showed that polysaccharide reduced serum enzyme activity and hepatic lipid levels, reduced insulin and leptin resistance (Wang(e) et al., 2019). The alkali-extractable and enzyme-extractable *D. indusiata* polysaccharide has protective action against hyperlipidemia—the blood has too many lipids— by reducing lipid in liver (Wang(a) et al., 2019) and acidic-extractable. *D. indusiata* Polysaccharides reduce lipid peroxide levels, helping restore normal serum lipid concentrations and protect the liver and kidneys, as indicated by decreased serum enzyme activities (Wang(c) et al., 2019). In anti-cancer activity as shown in Table 2.1, *D. indusiata* water extract can inhibit the proliferation of human hepatocellular carcinoma cells and increase the expression of Bax and caspase-3, proteins involved in programmed cell death (Hu et al., 2020) including can inhibit osteosarcoma S180 cell growth (Zhong et al., 2013). Furthermore, ethanolic crude extracts of *Dictyophora* sp. can kill cholangiocarcinoma (Chantree et al., 2022).

Table 2.1 Evidence of anticancer of bamboo mushroom extracts.

Extraction methods	Bioactive activity	Conclusion	Reference
Water extract at 100 °C	Anti-cancer and Induction of apoptosis in osteosarcoma	Inhibit growth of osteosarcoma S180 cells and increase caspase 3.	(Zhong et al., 2013)
Water extract at 100 °C	Inhibition of immunosuppressive function of prostate cancer-associated fibroblast	Stimulate the proliferation of immune cells and decrease prostate cancer-associated fibroblasts functions which inhibit	(Han et al., 2017)

Table 2.1 (Continued) Evidence of anticancer of bamboo mushroom extracts.

Extraction methods	Bioactive activity	Conclusion	Reference
		CD4 ⁺ /CD8 ⁺ T cells growth.	
Water extract at 70°C with high-pressure ultrasonic	Anti-cancer	Inhibit cell proliferation of human hepatocellular carcinoma cell line and enhance expression of Bax and caspase-3 which are proteins associated with program cell death.	(Hu et al., 2020)
95% ethanol extract	Cytotoxic activities	Kill three type of cholangiocarcinoma (CL-6, HuCCT1, HuH28) and changes morphology disclose apoptotic induction.	(Chantree et al., 2022)

2.2 Gastrointestinal cancer

Gastrointestinal cancer ranks among the leading causes of death globally, comprising several types such as esophageal, gastric, hepatic, pancreatic, and colorectal cancer (Tong et al., 2021). In 2018, the World Health Organization (WHO) reported around 3.5 million new cases of gastrointestinal cancer, comprising 20% of all cancer diagnoses and responsible for 22.5% of cancer-related deaths (Kuntz et al., 2021).

2.2.1 Colorectal cancer

Colorectal cancer has notably surged, now ranking as the third most prevalent gastrointestinal cancer in Asia. Research has highlighted smoking, alcohol use, obesity, and

lack of physical activity as major risk factors. In Thailand, colorectal cancer represented about 10.3% of newly diagnosed cancer cases from 2000 to 2012, with its incidence rising among both men and women nationwide (Tiankanon et al., 2021). At present, it is the third most frequently diagnosed cancer in Thai men and the fourth in women (Lohsiriwat et al., 2020).

2.2.2 Liver cancer

Liver cancer ranks as the second most common gastrointestinal cancer in Asia, with a high occurrence of hepatocellular carcinoma linked to hepatitis B and C infections, alcohol consumption, and smoking (Ko et al., 2018). In Thailand, predictive models forecast a rise in liver cancer incidence rates in both men and women to approximately 6.7 and 1.5 cases per 100,000 people per year, respectively, by 2030 (Yeesoonsang et al., 2018). Notably, mortality rates of liver cancer in males also increase with age in northeastern provinces, while in the North and the South, incidence rates for both sexes rise with age (Sriwattanapongse and Prasitwattanaseree, 2013).

2.3 Pathways of cancers cell

Cancer cells are caused by genetic mutations that cause abnormal growth. This mutation can occur in many genes. Mutations in genes involved in signaling pathways that regulate cellular senescence are among the causes of cancer. When the cells do not senescent, they can divide indefinitely. In Addition, senescent cancer cells can adopt a Senescence-associated secretory phenotype (SASP), which can foster cancer progression (Sikora et al., 2018). There are reported abnormal activations of the Wnt/ β -catenin pathway as a risk factor in several types of cancer. Liver cancer is one of them, according to the study Wnt/ β -catenin pathway ligand enhanced RPS15A—ribosomal protein mediates the nuclear transcription of FGF18— levels. Also, the Wnt/ β -catenin pathway regulates the expression of several angiogenic proteins, including MMP-2, MMP-9, VEGF-A, VEGF-C, and bFGF (Taciak et al., 2018).

The phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) pathway, which regulates key cellular processes such as transcription, translation, proliferation, growth, and survival, is activated by various cellular signals. Overactivation of the PI3K/Akt pathway has been linked to a variety of clinical diseases (see Figure 2.4) (Asati et al., 2016). PTEN is an essential molecule that acts downstream of the PI3K/Akt signaling pathway. This phosphatase, with both lipid and protein functions, serves as a tumor suppressor by inhibiting cell growth and enhancing the likelihood of programmed cell death. Alternatively, PTEN acts to suppress the PI3K/Akt signaling pathway. Consequently, when PTEN function is lost, the PI3K/Akt pathway remains continuously active, resulting in reduced apoptosis and promoting cancer cell proliferation (Porta et al., 2014).

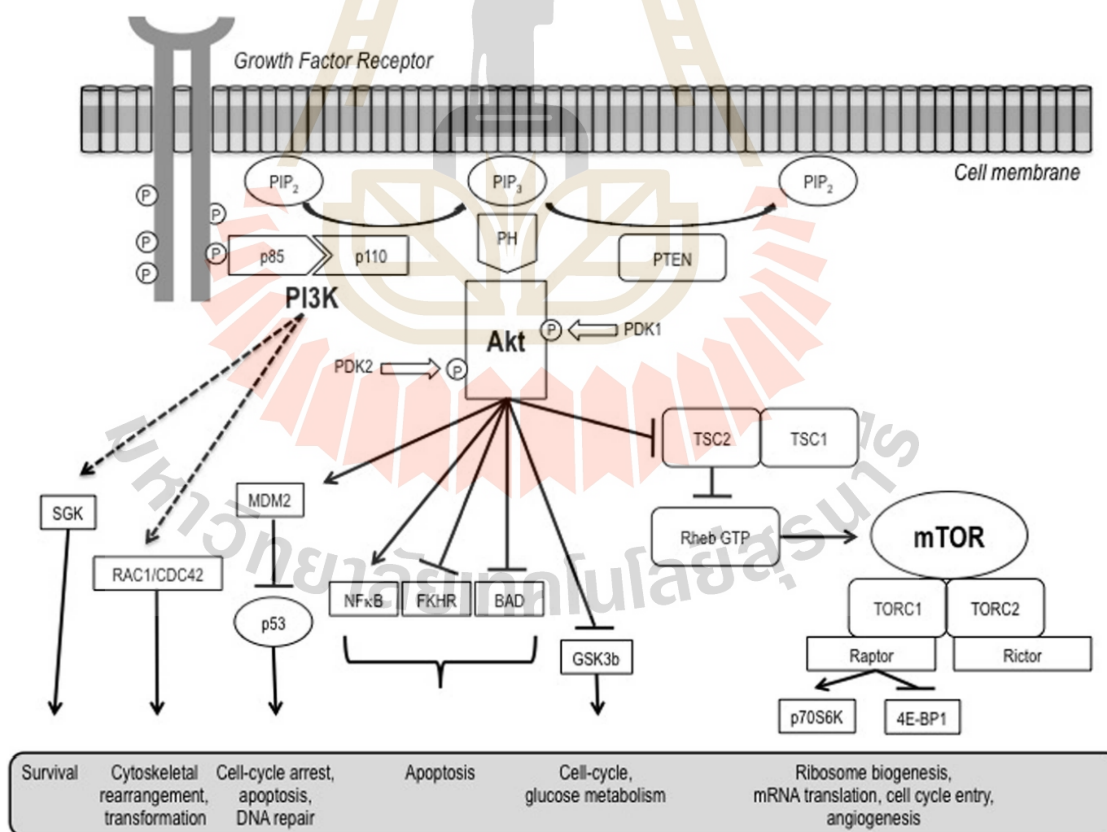


Figure 2.4 PI3K/Akt/mTOR pathway (Porta et al., 2014).

The Ras/Raf/MEK/ERK pathway also plays a crucial role in supporting cell survival throughout different stages of cancer development. Its growth factor receptor promotes cell proliferation and cell survival. Including vital function in signal transmission from growth factor receptors to control gene expression and thereby avoid apoptosis (Asati et al., 2016). Abnormal regulation of the Ras-Raf-MEK-ERK pathway (see Figure 2.5) has been associated with the development of human cancers, as it can drive tumor growth, enhance cell survival, facilitate invasion and metastasis, promote extracellular matrix degradation, and stimulate angiogenesis. Mutations in *RAS* genes, found in approximately 30% of all cancers, lead to aberrant activation of the Raf-MEK-ERK signaling pathway. Among the *RAS* isoforms, *KRAS* is the most frequently mutated and is commonly associated with pancreatic adenocarcinoma, colorectal cancer, lung cancer, and biliary tract cancer. Mutations in components of the Ras-Raf-MEK-ERK signaling pathway have been associated with various types of cancer. Due to its critical role in tumor development and progression, this pathway has become a key target for the development of anti-cancer therapies (Ullah et al., 2022).

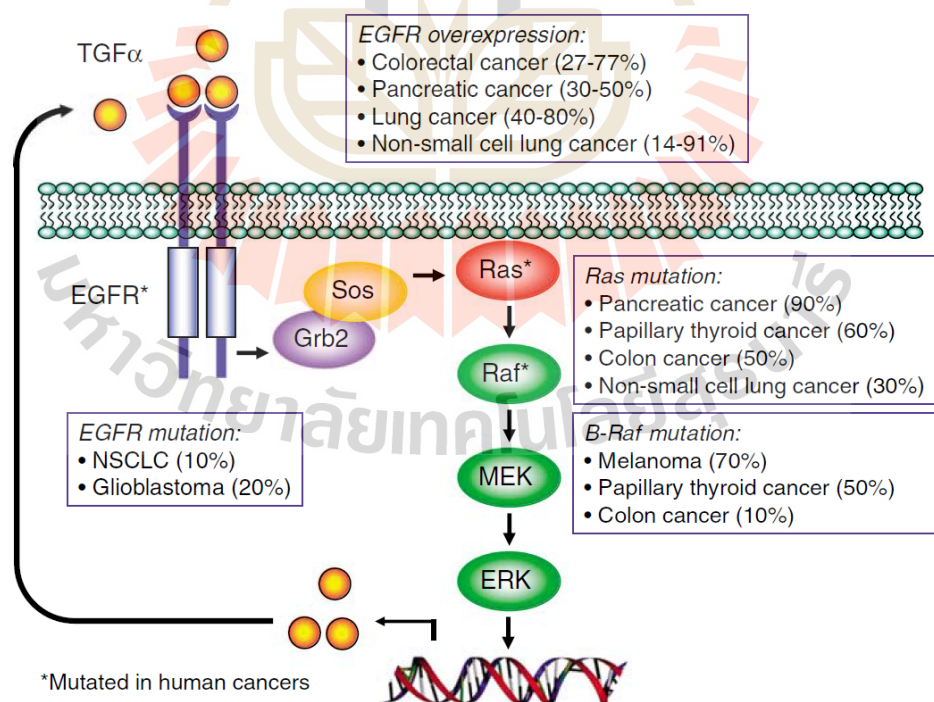


Figure 2.5 RAS-RAF-MEK-ERK pathway (Roberts and Der, 2007).

2.3.1 Pathway of liver cancer

The development and progression of hepatocellular carcinoma (HCC) are regulated by multiple signaling pathways, including Wnt/ β -catenin, Hippo-Yes-associated protein signaling pathway (Hippo-YAP), and PI3K/AKT. These pathways are frequently dysregulated through genetic mutations, epigenetic modifications, or external stimuli. The Wnt/ β -catenin pathway is commonly activated by mutations in key regulatory genes, such as catenin beta 1 (CTNNB1) (which encodes β -catenin), leading to uncontrolled cell proliferation and inhibition of apoptosis (Aqib et al., 2022). In the PI3K/AKT/GSK-3 β signaling cascade, glycogen synthase kinase-3 beta (GSK-3 β) functions as a downstream effector. Its activation has been shown to promote tumorigenesis by enhancing HCC cell proliferation, migration, chemoresistance, and metastasis, whereas its inhibition induces apoptosis (Guo et al., 2024). Similarly, dysregulation of the Hippo-YAP pathway, particularly the overactivation of Yes-associated protein (YAP), drives hepatocarcinogenesis by promoting aberrant cell growth, suppressing apoptosis, and facilitating tumor cell migration and invasion (Ji et al., 2018).

2.3.2 Pathway of colon cancer

In approximately 93% of colorectal cancer cases, mutations in the Wnt signaling pathway are among the most frequently observed. Alterations in the *Adenomatous Polyposis Coli* (APC) gene prevent the degradation of β -catenin, resulting in its accumulation and constitutive activation of the Wnt pathway, thereby promoting abnormal cell proliferation. Mutations in the *Kirsten rat sarcoma viral oncogene homolog* (KRAS) are also common, leading to persistent activation of RAS signaling and uncontrolled cellular division. Within the Transforming Growth Factor- β (TGF- β) signaling pathway, mutations in *SMAD2* and *SMAD4* impair downstream signaling, contributing to unregulated cell proliferation and metastasis (Brown and Ried, 2022). Additionally, the PI3K/AKT/mTOR signaling pathway is frequently altered in colorectal cancer. Mutations in *PIK3CA* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha) and *AKT* genes enhance tumor growth, invasiveness, and resistance to apoptosis. Moreover, mutations in the *TP53* gene, which encodes the tumor suppressor protein p53, compromise the cell's

ability to halt the cell cycle or initiate apoptosis following DNA damage, leading to genomic instability and further promoting tumorigenesis (Y. J. Lee et al., 2024).

2.4 Apoptosis pathway

Apoptosis is a programmed cell death, which is a cellular self-destruction mechanism. Apoptosis has two pathways that is the extrinsic and intrinsic pathways. The mitochondrion is the primary organelle-regulated intrinsic pathway and extrinsic has cell surface receptors and death ligand as a controller (Wang et al., 2009). Transmembrane receptor-mediated interactions are involved in the extrinsic signaling pathways that start apoptosis. To begin with death ligand binding with death receptor results in adaptor proteins binding to pro-caspase 8, 10 becoming to death-inducing signaling complex. This complex activates pro-caspase 8, 10. Then, caspase 8, 10 activate caspase-3, 6, and 7 leading to apoptosis. The protein c-FLIP can inhibit this pathway by binding to Fas-associated death domain protein (FADD), an adaptor protein, and caspase-8, thereby preventing effective activation as see shown in Figure 2.6 (Elmore, 2007).

The intrinsic mechanism of apoptosis, also known as the mitochondrial pathway, is triggered by a range of stimuli that operate on various sites inside the cell. This type of apoptosis is triggered by mitochondrial factors and can occur via either a positive or negative route. When receiving positive or negative stimuli, the mitochondrial membrane will change. BAX and BAK cause pores in the mitochondria. Because of pores in mitochondria, pro-apoptosis protein, cytochrome c, Smac/Diablo, and HtrA2/Omi leaking into the cytoplasm. Cytochrome c binding with Apoptotic Protease Activating Factor 1 (APAF1) becomes apoptosome. Apoptosomes activate pro-caspase 9. Then, caspase 9 activates caspase-3,6,7 to induce apoptosis. Moreover, Smac/Diablo and HtrA2/ Omi inhibit inhibitors of apoptosis proteins (IAPs) which inhibit apoptosis as shown in Figure 2.6 (D'Arcy, 2019).

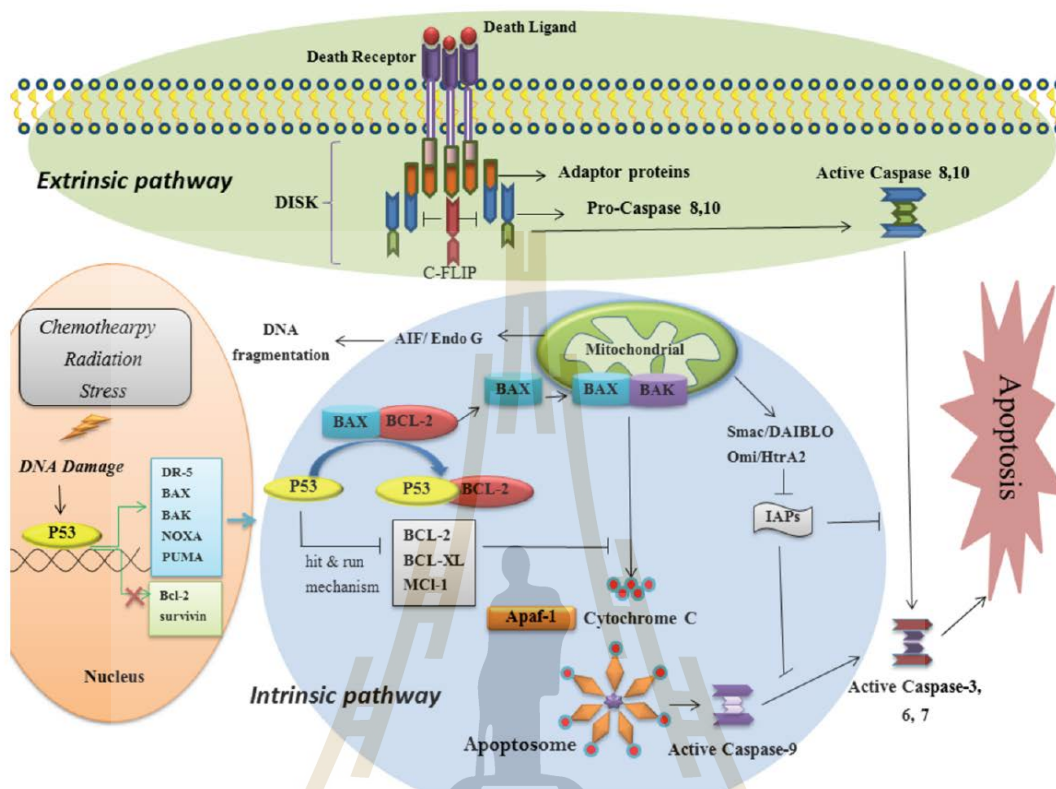


Figure 2.6 The extrinsic and intrinsic pathways of apoptosis (Goldar et al., 2015).

Appropriate apoptotic signaling is vital to maintaining a healthy balance between cell death and cell survival, therefore apoptosis evasion is a key characteristic of cancer. In cancer cell, expression of Bcl-2, which is an anti-apoptotic protein is higher than in normal cells. Furthermore, the down-regulation of caspase leads to many types of cancer. For instance, a decrease in caspase-9 leads to colorectal cancer, and a decrease in caspase-3 leads to ovarian, breast, and cervical cancers. Consequently, the substance that induces apoptosis is one of the attractive features in cancer drug development (Goldar et al., 2015).

CHAPTER III

MATERIALS AND METHODOLOGY

3.1 Preparation of ethanolic crude extract of Thai-isolate bamboo mushroom

3.1.1 Cap and fruiting body extract

The fruiting body and cap were crushed into a fine powder after being dried for 24 hours at 50°C in a hot air oven. The resultant powder was immersed in 70% ethanol at a 1:10 (w/v) ratio for 7 days at 25°C while being continuously shaken at 180 rpm. The extract was then obtained by filtering the mixture and evaporating the solvent. The extract was kept at -20°C until it was examined further.

3.1.2 Egg extract

Likewise, once the mucus was removed, the egg was dried for 24 hours at 50°C in a hot air oven and pounded into a powder. After that, the powder was soaked in 70% ethanol at a 1:10 (w/v) ratio for 7 days at 25°C while being shaken at 180 rpm. Before being used in further investigations, the extract was held at -20°C after the resultant mixture was filtered and evaporated.

3.2 Cell culture

Three cell lines were used in this study: two gastrointestinal (GI) cancer cell lines—HT-29 (colon) and HepG2 (liver)—and a normal human fibroblast cell line, which served as a normal control.

3.2.1 Colon cancer cell line

HT-29 cells were cultured in complete McCoy's 5A medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic. Cells were maintained at 37°C in an incubator that was humidified and had 5% CO₂. Cells were sub-cultured after confluence reached around 80%. The old medium was removed, and cells were washed with phosphate-buffered saline (PBS, pH 7.4%). Trypsin was then added, and the cells were incubated at 37°C and 5% CO₂ for 5 minutes to promote detachment. The trypsin activity was neutralized by adding three volumes of complete medium. After centrifuging the cells for 5 minutes at 1,500 rpm and discarding the supernatant, the cell pellet was resuspended in new complete media and then moved to a different culture flask.

3.2.2 Liver cancer cell line

HepG2 cells were cultured in complete Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS and 1% antibiotic-antimycotic, maintained at 37°C in a 5% CO₂ incubator. When cells reached 80% confluence, subculturing was performed using the same protocol as described for HT-29 cells, with the exception that trypsinization was carried out for 2 minutes under the same incubation conditions.

3.2.3 Fibroblast cells

The normal human embryonic fibroblasts (OUMS) cell line, derived from fetal connective tissue, was used as the normal control in this study. The cells were cultured in complete DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic and maintained at 37°C in a humidified incubator with 5% CO₂. Subculturing was performed using the same protocol as described for the HepG2 cell line.

3.3 Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test was used to evaluate cell viability. In brief, a 96-well plate was seeded with 10,000 cells per well, and the cells were then incubated for 24 hours at 37°C with 5% CO₂. Following treatment with varying doses of the crude extracts (0-2,500 µg/ml), cells were cultured for a further 48 hours under the same circumstances. Following treatment, 20 µL of MTT

solution (5 mg/mL) was added to each well and incubated for 3 hours. The mitochondrial enzyme succinate dehydrogenase in viable cells catalyzes the conversion of MTT, a yellow tetrazolium salt, into insoluble purple formazan crystals. After removing the supernatant, 100 μ L of dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals. A microplate reader was used to detect absorbance at 562 nm. 25% inhibitory concentration (IC_{25}), the half-maximal inhibitory concentration (IC_{50}), 75% inhibitory concentration (IC_{75}), and the proportion of viable cells were computed. The percentage of cell viability was calculated using the following equation: $\text{Percentage of cell viability (\%)} = \left(\frac{OD_{\text{sample}} - OD_{\text{blank}}}{OD_{\text{control}} - OD_{\text{blank}}} \right) \times 100$. The IC_{25} , IC_{50} , and IC_{75} value was calculated from the slope of the linear regression between the logarithm of the extract concentration and the percentage of cell growth inhibition (calculated as 100 - the percentage of cell viability).

3.4 Apoptosis

To assess the crude extracts' apoptotic effects, HT-29 cells were exposed to egg-derived crude extracts for 48 hours at 37°C with 5% CO_2 . Following treatment, 3×10^5 cells were collected and washed twice with PBS. The cells were then stained using the Annexin V-FITC Apoptosis Detection Kit (Invitrogen, Thermo Fisher Scientific, USA) for 10 minutes at room temperature in the dark. Staining was performed by adding 1 μ L of FITC-conjugated Annexin V to 100 μ L of binding buffer. After incubation, 100 μ L of binding buffer and 1 μ L of propidium iodide (PI; 100 μ g/mL) were added to the cells. The distribution of apoptotic cells was subsequently analyzed by flow cytometry.

3.5 Western blot analysis

Crude egg extracts were treated with HT-29 cells for 48 hours at 37°C with 5% CO_2 . Subsequently, cells were harvested using a cell scraper, centrifuged at 1,500 rpm for 5 minutes at 4°C, and washed twice with PBS. Cellular proteins were then extracted using 300 μ L of RIPA lysis buffer supplemented with protease inhibitors. Protein concentrations

were determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, IL, USA). A standard curve was generated using bovine serum albumin (BSA) at various concentrations, and the protein concentration of the samples was calculated based on the slope of the resulting linear equation. Proteins in equal quantities (30 μ g) were separated on a 12.5% SDS-PAGE gel and then put onto a nitrocellulose membrane. Using 5% Skimmed milk in tris-buffered saline (TBS, pH 7.5) with stirring, membranes were blocked for 1 hour at room temperature. After that, primary antibodies (1:1000 dilution in 5% Skimmed milk in TBS with 0.1% Tween20) including rabbit anti- β -actin, anti-Bax, anti-caspase-8, anti-caspase-9, and anti-cleaved caspase-3 (Cell Signaling Technology, USA) were added to the membranes and incubated for a whole night at 4°C. Following three TBST washes, membranes were incubated for 1 hour at room temperature with a goat anti-rabbit secondary antibody (1:10,000 dilution in 1% Skimmed milk in TBS) coupled with alkaline phosphatase (AP) (Invitrogen, Thermo Fisher Scientific, USA). Nitro Blue Tetrazolium and 5-Bromo-4-Chloro-3-Indolyl Phosphate (NBT-BCIP) (Thermo Fisher Scientific, USA) was used to view the protein bands, and ImageJ software was used to measure the band intensities.

3.6 Statistical analysis

The data are shown as mean \pm standard deviation (SD), and each experiment was conducted in triplicate. One-way analysis of variance (ANOVA) was employed for statistical analysis of normally distributed data, whereas the Kruskal–Wallis test was applied to non-normally distributed data. The p -value was deemed statistically significant if it was less than 0.05 ($p < 0.05$)

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Cytotoxicity

4.1.1 Cytotoxicity of Liver cancer

The viability of liver cancer cells (HepG2) decreased in a dose-dependent manner following 48 hours of treatment with the cap extract of Thai bamboo mushroom. At the highest tested concentration of 2,500 $\mu\text{g}/\text{mL}$, cell viability was reduced to 25.29% (± 26.35), showing a statistically significant difference compared to the control group (p -value < 0.001). At concentrations of 2,000 $\mu\text{g}/\text{mL}$ and 1,500 $\mu\text{g}/\text{mL}$, cell viability was 35.32% (± 30.59) and 59.47% (± 20.55), respectively, both of which were also significantly different from the control group, with p -values of < 0.01 and < 0.05 , respectively (Figure 4.1A). Similarly, treatment with the fruiting body (Figure 4.1B) and egg extracts (Figure 4.1C) resulted in a concentration-dependent decrease in liver cancer cell viability. The egg extract at a concentration of 2,500 $\mu\text{g}/\text{mL}$ reduced cell viability to 19.53% (± 29.07), which was significantly different from the control group (p -value < 0.01). At concentrations of 2,000 $\mu\text{g}/\text{mL}$ and 1,500 $\mu\text{g}/\text{mL}$, cell viability was reduced to 22.25% (± 28.63) and 47.17% (± 25.12), respectively, both showing statistically significant differences compared to the control group (p -value < 0.05).

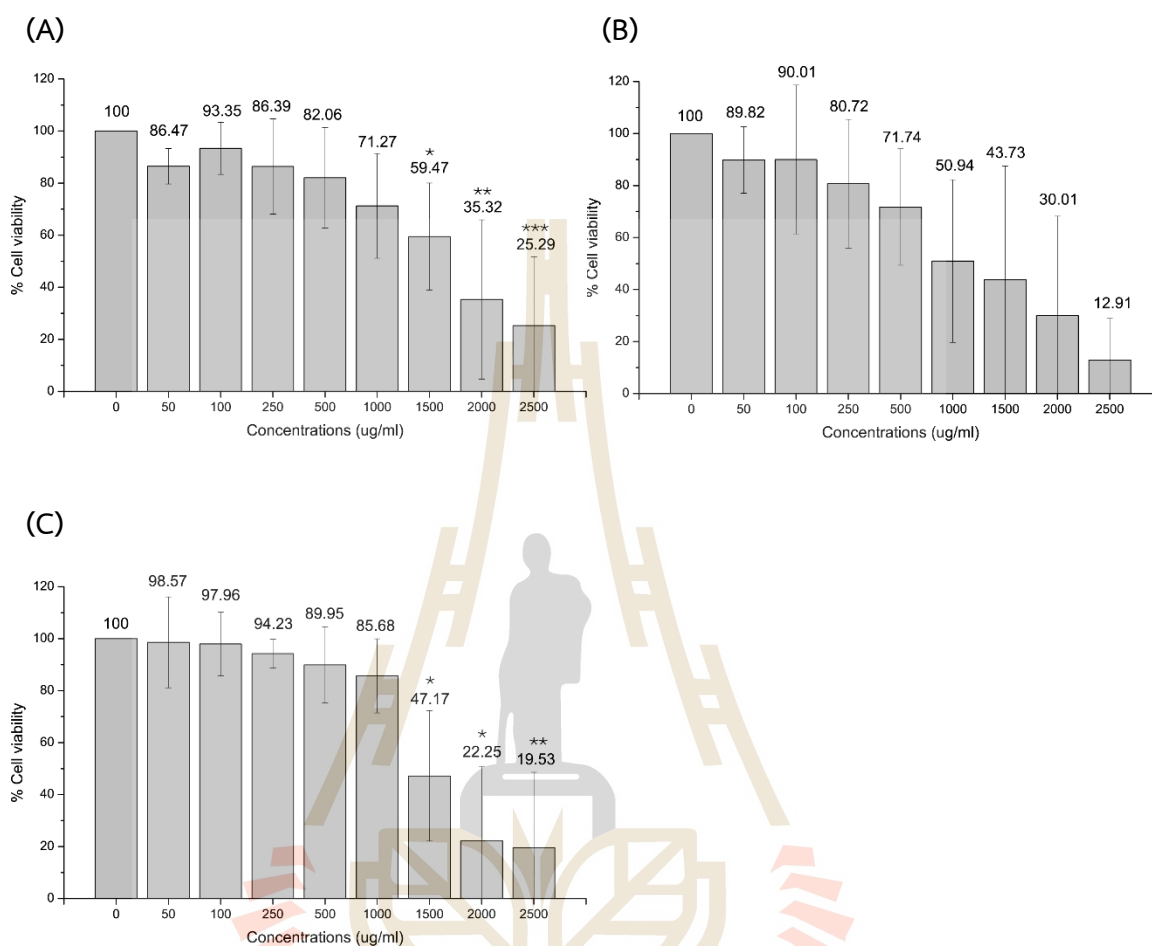


Figure 4.1 The percentage of cell viability of HepG2 after treatment with extract for 48h. (A) Cap extract, (B) Fruiting body extract, and (C) Egg extract. All data were statistically compared to the control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.1.2 Cytotoxicity of colon cancer

The cell viability of colon cancer cells (HT-29) treated with cap and fruiting body extracts of bamboo mushroom ranged from 60% to 100%, showing no significant differences from the control group at any concentration (Figure 4.2A-B). In contrast, treatment with the egg extract of bamboo mushroom resulted in a concentration-dependent decrease in cell viability (Figure 4.2C), with survival rates of 5.21% (± 4.32) and

18.54% (± 3.59) observed at concentrations of 2,000 and 1,500, respectively. These reductions were statistically significant, with P -values of <0.01 and <0.05 respectively.

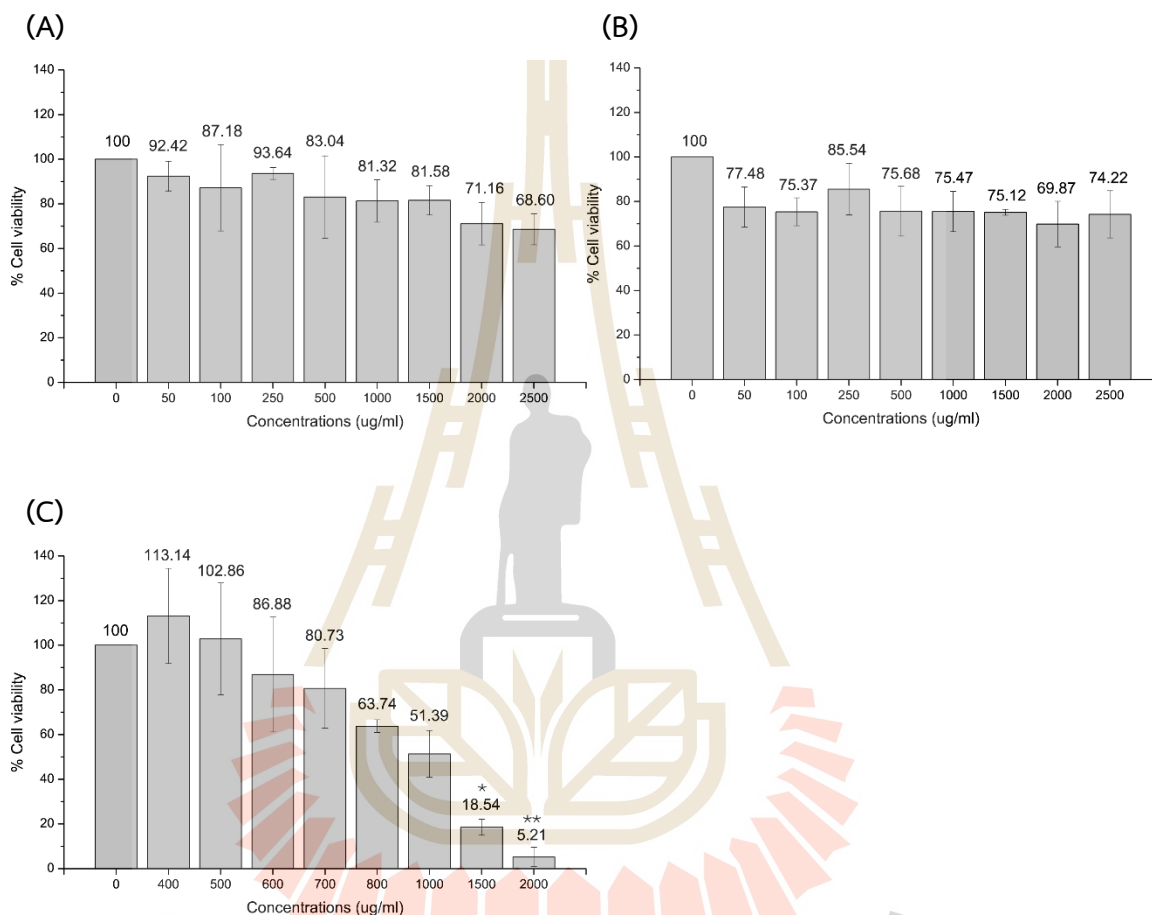


Figure 4.2 The percentage of cell viability of HT-29 after treatment with extract for 48h. (A) Cap extract, (B) Fruiting body extract, and (C) Egg extract. All data were statistically compared to the control group. * $p < 0.05$, ** $p < 0.01$

4.1.3 Cytotoxicity of fibroblast

Following treatment of fibroblasts (OUMS), which are normal cells, with extracts from all three parts of the bamboo mushroom, cell viability remained high with no significant differences compared to the control group (Figure 4.3A-C). These results indicate

that the extracts from all three parts of the bamboo mushroom had no cytotoxic effect on normal cells.

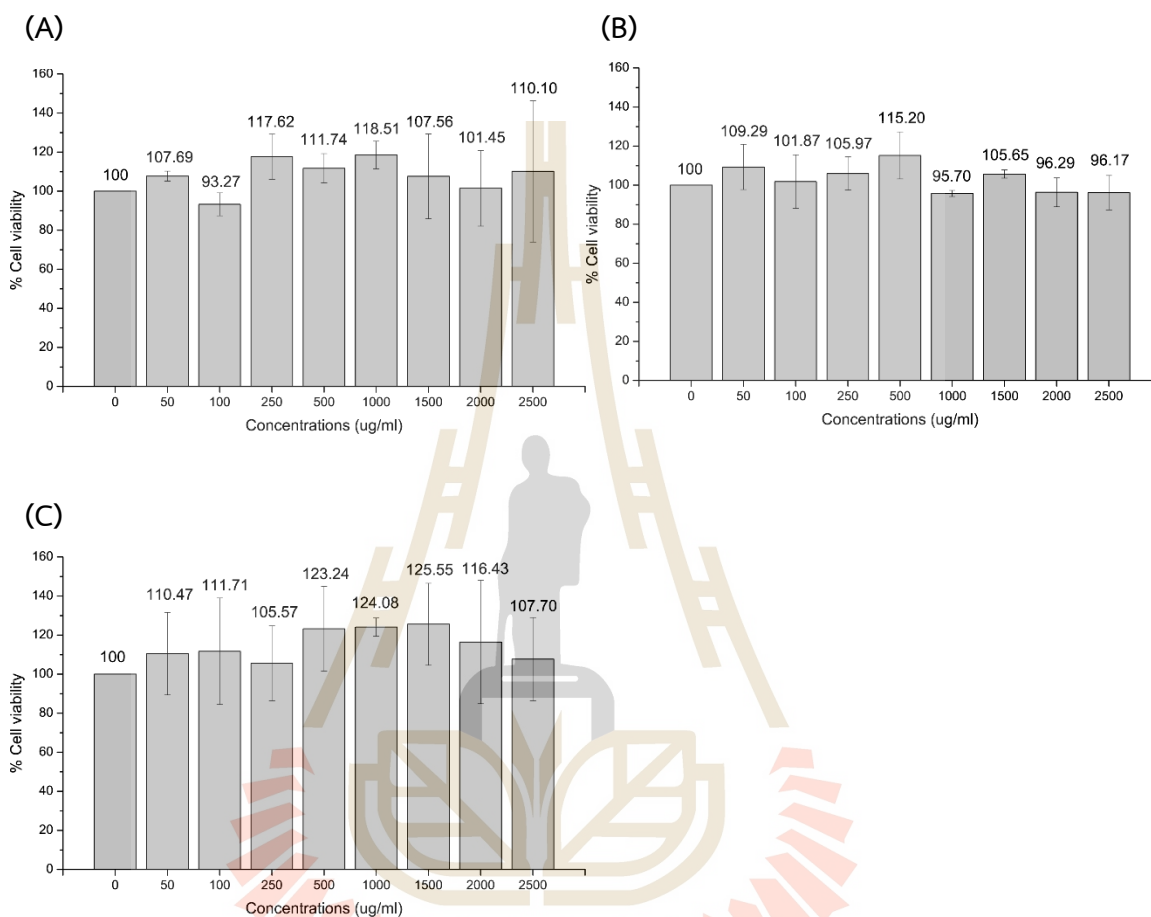


Figure 4.3 The percentage of cell viability of OUMS after treatment with extract for 48h. (A) Cap extract, (B) Fruiting body extract, and (C) Egg extract.

4.1.4 The half-maximal inhibitory concentration

The half-maximal inhibitory concentration (IC_{50}) was calculated based on the cytotoxicity assay results. The IC_{50} value of the fruiting body extract for liver cancer cells was $1,013.44 \pm 1067.78 \mu\text{g/mL}$, which was lower than those of the egg and cap extracts, calculated at $1,672.19 \pm 606.49 \mu\text{g/mL}$ and $1,920.71 \pm 1296.90 \mu\text{g/mL}$, respectively (Table 4.1). In contrast, the IC_{50} values of the fruiting body and cap extracts for colon

cancer cells and fibroblasts exceeded 2,500 $\mu\text{g/mL}$, the highest concentration tested in this study. Similarly, the IC_{50} value of the egg extract on fibroblasts also exceeded 2,500 $\mu\text{g/mL}$. However, the IC_{50} value of the egg extract on colon cancer cells was $1,005.75 \pm 94.09 \mu\text{g/mL}$ (Table 4.1), indicating the most potent cytotoxic effect observed. Furthermore, the high IC_{50} values on fibroblasts across all three extracts confirmed the non-toxic of bamboo mushroom extracts on normal cells.

Table 4.1 The half-maximal inhibitory concentration.

Extract	IC_{50} ($\mu\text{g/mL}$)		
	HepG2	HT-29	OUMS
Cap extract	1920.71 ± 1296.90	>2500	>2500
Fruiting body extract	1013.44 ± 1067.78	>2500	>2500
Egg extract	1672.19 ± 606.49	1005.75 ± 94.09	>2500

4.1.5 The inhibitory concentration

Based on the IC_{50} results, the egg extract exhibited the lowest IC_{50} value for colon cancer cells, indicating the strongest cytotoxic effect among all extracts tested. Consequently, further experiments were conducted to evaluate apoptosis induction and the expression of apoptosis-related proteins using the bamboo mushroom egg extract in colon cancer cells. The extract was tested at concentrations corresponding to its IC_{25} , IC_{50} , and IC_{75} values. Specifically, the IC_{25} and IC_{75} values were $698.14 \pm 128.51 \mu\text{g/mL}$ and $1,458.54 \pm 72.29 \mu\text{g/mL}$, respectively (Table 4.2). For practical purposes in extract preparation, concentrations of 700, 1,000, and 1,450 $\mu\text{g/mL}$ —corresponding approximately to IC_{25} , IC_{50} , and IC_{75} —were used in subsequent apoptosis and protein expression assays.

Table 4.2 The inhibitory concentration of colon cancer.

Extract	Concentrations ($\mu\text{g/mL}$)		
	IC ₂₅	IC ₅₀	IC ₇₅
Egg extract	698.14 \pm 128.51	1005.75 \pm 94.09	1458 \pm 72.29

4.2 Apoptosis by flow cytometry

Apoptosis was assessed by Annexin V staining and analyzed via flow cytometry (Figure 4.4B–E). After 48 hours of treatment with mushroom egg extract, the percentage of live (Annexin V-negative) HT-29 cells decreased in a concentration-dependent manner. At IC₅₀ (1,000 $\mu\text{g/mL}$), the percentage of live cells was significantly reduced compared to the control ($P < 0.05$), while treatment with IC₇₅ (1,450 $\mu\text{g/mL}$) resulted in an even more significant reduction ($P < 0.001$) (Figure 4.4A). Concurrently, the percentages of early and late apoptotic cells increased with higher extract concentrations. Notably, at IC₇₅, early apoptosis showed a significant increase ($P < 0.01$), and late apoptosis was also significantly elevated ($P < 0.05$).

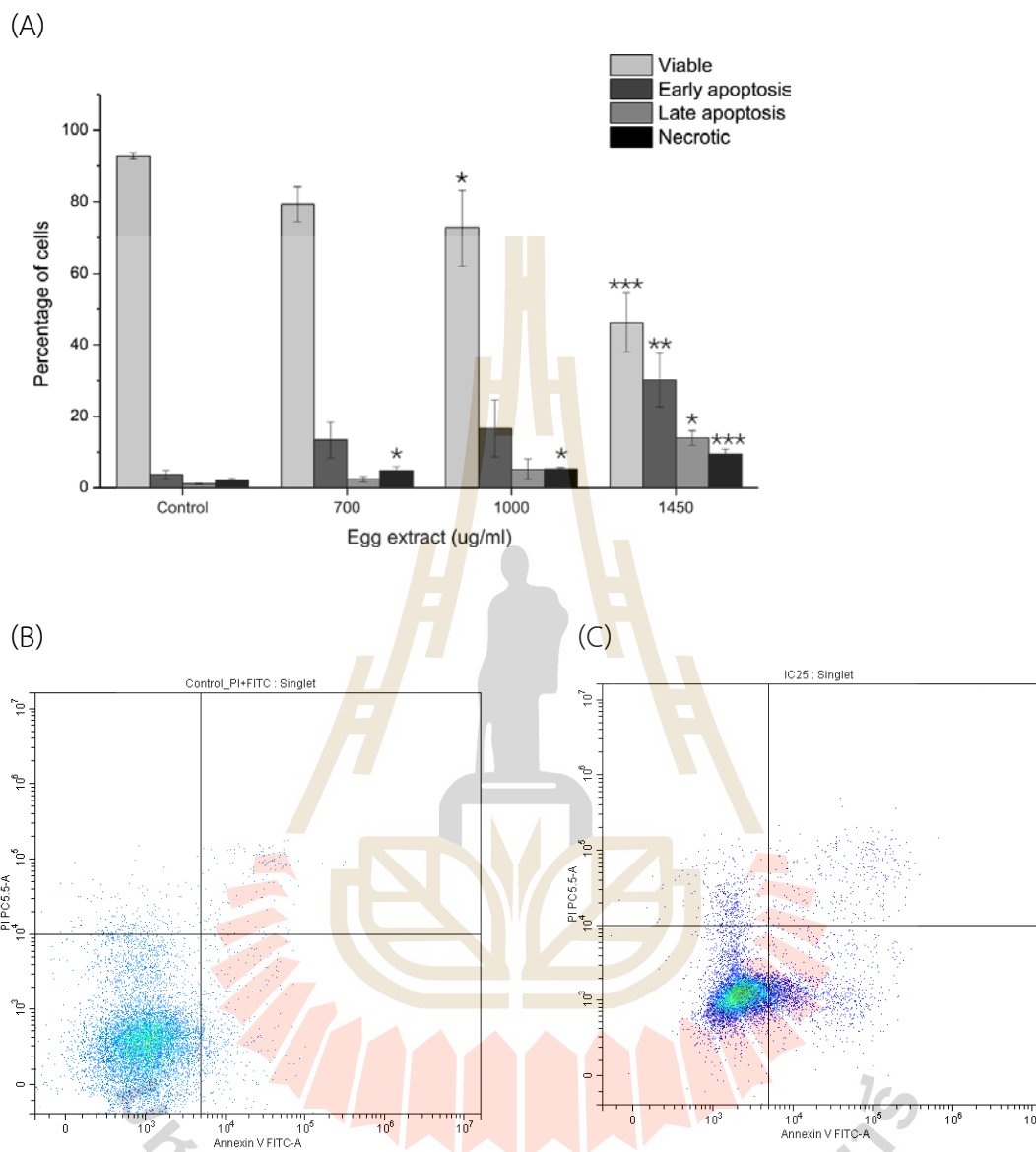


Figure 4.4 The percentage distribution of HT-29 cells across various cell apoptosis stages after 48 hours of treatment with egg extract was analyzed using flow cytometry. (A) Graph illustrating the comparative results across different extract concentrations and cell apoptosis stages, (B) Untreated control, (C) Treatment with IC25 concentration; 700 µg/ml, (D) Treatment with IC50 concentration; 1,000 µg/ml, and (E) Treatment with IC75 concentration; 1,450 µg/ml. All data were statistically compared to the control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

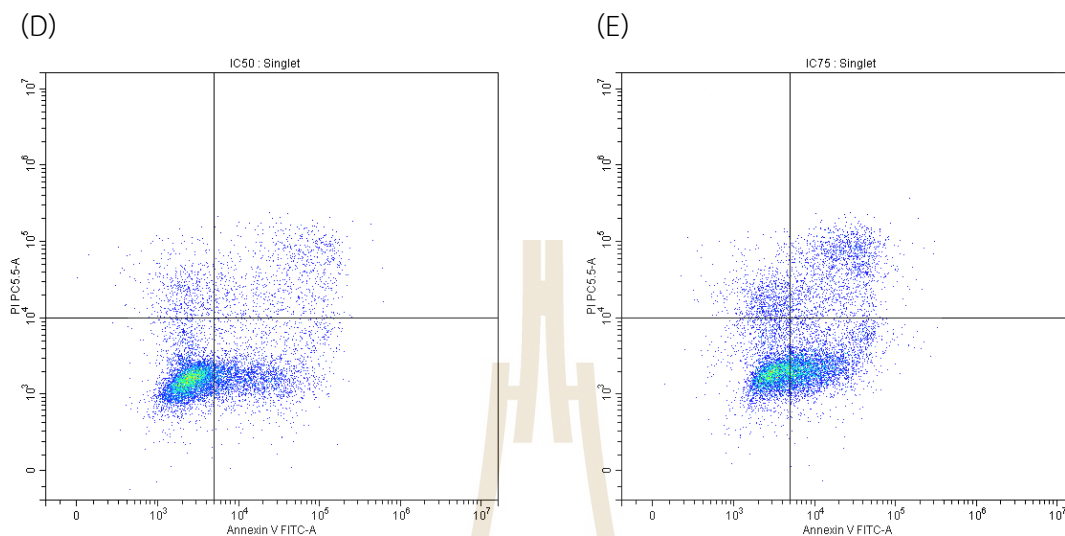
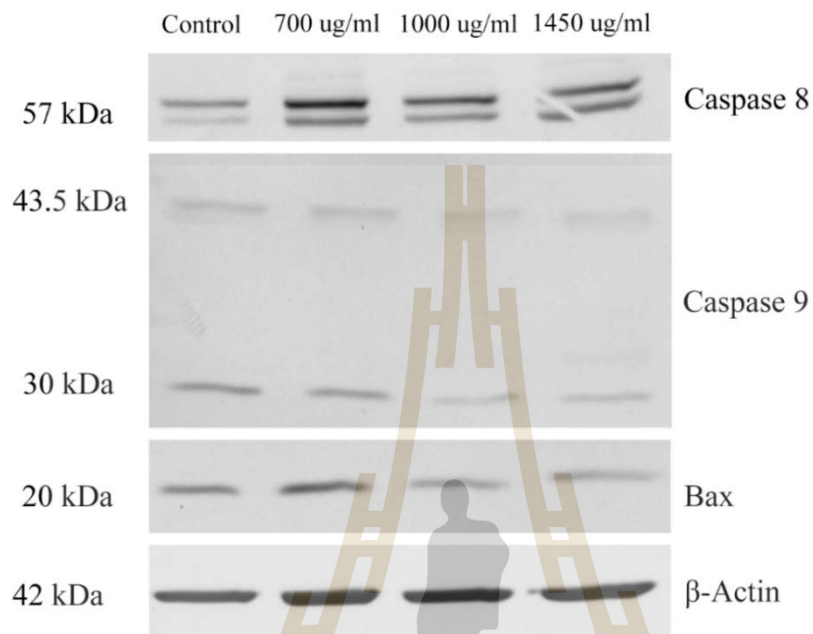


Figure 4.4 (Continued) The percentage distribution of HT-29 cells across various cell apoptosis stages after 48 hours of treatment with egg extract was analyzed using flow cytometry. (A) Graph illustrating the comparative results across different extract concentrations and cell apoptosis stages, (B) Untreated control, (C) Treatment with IC₂₅ concentration; 700 µg/ml, (D) Treatment with IC₅₀ concentration; 1,000 µg/ml, and (E) Treatment with IC₇₅ concentration; 1,450 µg/ml. All data were statistically compared to the control group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

4.3 Apoptosis-associated proteins by western blot

The expression levels of apoptosis-related proteins were evaluated by Western blot analysis (Figure 4.5A). Bax expression increased following treatment with the mushroom egg extract, with the highest expression observed at IC₂₅, followed by IC₇₅ and IC₅₀ (Figure 4.5B). Caspase-8 expression also increased at IC₂₅ but showed a slight decrease at IC₅₀ and IC₇₅ (Figure 4.5C). In the case of Caspase-9, expression levels were elevated at both IC₂₅ and IC₇₅, while a slight reduction was observed at IC₅₀ (Figure 4.5D).

(A)



(B)

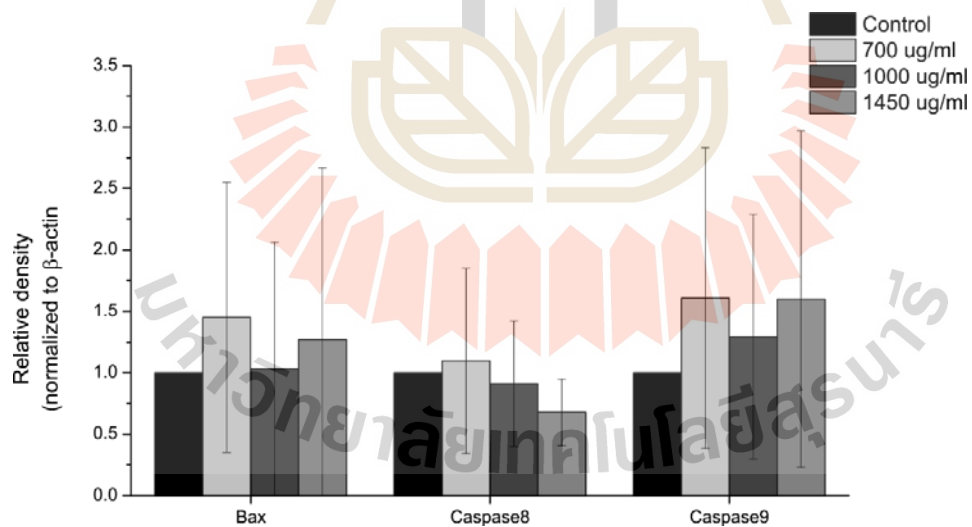


Figure 4.5 Western blot analysis of protein expression following treatment with mushroom egg extract for 48 hours. (A) Western blot analysis (B) Graph showing the relative expression levels of proteins.

4.4 Discussion

The cytotoxicity assay results demonstrated that extracts from all three parts of the bamboo mushroom—cap, fruiting body, and egg—could inhibit the growth of liver cancer cells. Notably, only the egg extract showed inhibitory activity against colon cancer cells. These findings are consistent with previous studies reporting that polysaccharides extracted from the fruiting bodies of *Dictyophora indusiata* suppressed liver cancer cell proliferation in a time- and dose-dependent manner (Hu et al., 2020). Additionally, ethanolic crude extracts of *Dictyophora* sp. have been shown to inhibit cholangiocarcinoma cell growth at high concentrations (Chantree et al., 2022). Moreover, the findings demonstrated that the egg extract of the bamboo mushroom showed the highest anticancer potency compared to the cap and fruiting body extracts. The mushroom egg extract is rich in bioactive compounds, including polyphenols, flavonoids, monosaccharides, mucopolysaccharides, allantoin, and alkaloids. These constituents are known for their significant anti-inflammatory and antioxidant properties, which may contribute to the enhanced therapeutic potency of the extract (Nazir et al., 2021; Ruksiriwanich et al., 2022).

Apoptosis is an active and tightly regulated form of programmed cell death governed by specific genes and signaling pathways. It is primarily mediated through a caspase-dependent mechanism, initiated by either intrinsic mitochondrial signals or extrinsic death receptor pathways (Chen et al., 2024). Bax is a critical regulator of apoptosis, functioning through multiple mechanisms including the intrinsic (mitochondrial) and extrinsic (death receptor) pathways. Consequently, Bax activation represents a promising therapeutic approach for enhancing the effectiveness of cancer treatments (Z. Liu et al., 2016). In this study, flow cytometry analysis confirmed the induction of apoptotic cell death following treatment with mushroom egg extract, while western blot analysis demonstrated a trend toward upregulation of Bax expression, supporting its role in the apoptotic response. Caspase-8 is primarily associated with activation through the extrinsic apoptotic pathway, whereas caspase-9 is predominantly involved in the intrinsic, mitochondria-mediated pathway (Sharifi et al., 2014). The extrinsic pathway

is typically initiated by ligand–receptor interactions at the cell surface, with key members of the TNF receptor superfamily—such as TNFR1 and Fas—recruiting adaptor proteins like TRADD and FADD to activate caspase-8 and trigger cell death (Cho and Choi, 2002). In contrast, the intrinsic pathway is activated by intracellular stress signals, leading to the release of cytochrome c from the mitochondrial intermembrane space into the cytoplasm. This event promotes the assembly of the apoptosome complex with Apaf-1 and caspase-9, ultimately activating downstream executioner caspases and initiating apoptosis (Cho and Choi, 2002). In the present study, Western blot analysis demonstrated an overall increase in caspase expression compared to the control. Notably, caspase-8 levels showed only a modest and inconsistent increase across treatment concentrations, while caspase-9 levels were elevated, suggesting that mushroom egg extract primarily induces apoptosis via the intrinsic mitochondrial pathway. However, due to the relatively high standard deviation observed in the Western blot results, the experiment should be repeated, and the expression of the target gene at the mRNA level should be evaluated to confirm the findings



CHAPTER V

CONCLUSION

The results from this study indicate that all three bamboo mushroom extracts could inhibit the growth of liver cancer cells at high concentrations. However, only the egg extract exhibited inhibitory effects against colon cancer cells. Among the three, the egg extract demonstrated the most potent anticancer activity, particularly against colon cancer cells. Including, the extracts showed no cytotoxicity toward normal fibroblast cells. Furthermore, the mushroom egg extract was shown to induce apoptosis in colorectal cancer cells, as evidenced by the upregulated expression of Bax and caspase-9—key proteins involved in the intrinsic apoptotic pathway. These findings suggest the preliminary potential of the mushroom egg extract as an alternative therapeutic agent for colon cancer. We sincerely hope that these findings contribute to future advancements in cancer therapy and offer an additional treatment option for patients.

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REFERENCES

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REFERENCES

- Aqib, M., Usman, M., and Nazir, A. (2022). *Etiology, mechanism and treatment of liver cancer* (M. A. Feitelson & A. Arzumanyan (eds.)). IntechOpen. doi:10.5772/intechopen.106020
- Asati, V., Mahapatra, D. K., and Bharti, S. K. (2016). PI3K/Akt/mTOR and Ras/Raf/MEK/ERK signaling pathways inhibitors as anticancer agents: Structural and pharmacological perspectives. *European Journal of Medicinal Chemistry*, 109, 314–341. doi:10.1016/j.ejmech.2016.01.012
- Beshbishy, A., Wasef, L., Elewa, Y., Al-Sagan, A., Abd El-Hack, M., Taha, A., and Abd-Elhakim, Y. (2020). Chemical constituents and pharmacological activities of garlic (*Allium sativum* L.): A Review. *Nutrients*, 12(3), 872. doi:10.3390/nu12030872
- Bilgin, S., Erden Tayhan, S., Yıldırım, A., and Koç, E. (2023). Investigation of the effects of isoeugenol-based phenolic compounds on migration and proliferation of HT29 colon cancer cells at cellular and molecular level. *Bioorganic Chemistry*, 130(October 2022), 106230. doi:10.1016/j.bioorg.2022.106230
- Brown, M. A., and Ried, T. (2022). Shifting the focus of signaling abnormalities in colon cancer. *Cancers*, 14(3). doi:10.3390/cancers14030784
- Cassidy, J., Tabernero, J., Twelves, C., Brunei, R., Butts, C., Conroy, T., Debrand, F., Figer, A., Grossmann, J., Sawada, N., Schöffski, P., Sobrero, A., Van Cutsein, E., and Díaz-Rubio, E. (2004). XELOX (capecitabine plus oxaliplatin): Active first-line therapy for patients with metastatic colorectal cancer. *Journal of Clinical Oncology*, 22(11), 2084–2091. doi:10.1200/JCO.2004.11.069

- Chantree, P., Chumkiew, S., Jamklang, M., and Martviset, P. (2022). Cytotoxic activities of ethanolic crude extracts from fruiting bodies of bamboo mushrooms (*Dictyophora* spp.) against cholangiocarcinoma cells. *Research Results in Pharmacology*, 8(1), 33–41. doi:10.3897/rrpharmacology.8.72098
- Chen, Y., Li, X., Yang, M., and Liu, S. B. (2024). Research progress on morphology and mechanism of programmed cell death. *Cell Death and Disease*, 15(5). doi:10.1038/s41419-024-06712-8
- Chitapanarux, T., and Phornphutkul, K. (2015). Risk factors for the development of hepatocellular carcinoma in thailand. *Journal of Clinical and Translational Hepatology*, 3(3), 182–188. doi:10.14218/JCTH.2015.00025
- Cho, S. G., and Choi, E. J. (2002). Apoptotic signaling pathways: Caspases and stress-activated protein kinases. *Journal of Biochemistry and Molecular Biology*, 35(1), 24–27. doi:10.5483/bmbrep.2002.35.1.024
- Chumkiew, S., Jamklang, M., Wangboon, C., Penkhruue, W., Mangmee, N., Sakheatkarn, K., Martviset, P., Chantree, P., Chaimon, S., Sanannam, B., Thanee, M., Suksawat, M., and Phetcharaburanin, J. (2024). Morphological characterization, molecular identification, and metabolic profiles of two novel isolated bamboo mushrooms (*Phallus* sp.) from Thailand. *PLoS One*, 19(10), e0307157. doi:10.1371/journal.pone.0307157
- D’Arcy, M. S. (2019). Cell death: a review of the major forms of apoptosis, necrosis and autophagy. *Cell Biology International*, 43(6), 582–592. doi:10.1002/cbin.11137
- Da Silva Leitão Peres, N., Cabrera Parra Bortoluzzi, L., Medeiros Marques, L. L., Formigoni, M., Fuchs, R. H. B., Droval, A. A., and Reitz Cardoso, F. A. (2020). Medicinal effects of Peruvian maca (: *Lepidium meyenii*): A review. *Food and Function*, 11(1), 83–92. doi:10.1039/c9fo02732g

- Deng, C., Shang, J., Fu, H., Chen, J., Liu, H., and Chen, J. (2016). Mechanism of the immunostimulatory activity by a polysaccharide from *Dictyophora indusiata*. *International Journal of Biological Macromolecules*, 91, 752–759. doi:10.1016/j.ijbiomac.2016.06.024
- Elmore, S. (2007). Apoptosis: A review of programmed cell death. *Toxicologic Pathology*, 35(4), 495–516. doi:10.1080/01926230701320337
- Fu, Y., Lin, S., Lu, M., Wei, S. Y., Zhou, J., Zhao, L., Zhang, Q., Lin, D. R., Liu, Y. T., Chen, H., Qin, W., and Wu, D. T. (2019). Quantitative evaluation of ultrasound-assisted extraction of 1,3- β -glucans from *Dictyophora indusiata* using an improved fluorometric assay. *Polymers*, 11(5), 5–8. doi:10.3390/polym11050864
- Gao, Y., Kuok, K. I., Jin, Y., and Wang, R. (2019). Biomedical applications of Aloe vera. *Critical Reviews in Food Science and Nutrition*, 59(0), S244–S256. doi:10.1080/10408398.2018.1496320
- Goldar, S., Khaniani, M. S., Derakhshan, S. M., and Baradaran, B. (2015). Molecular mechanisms of apoptosis and roles in cancer development and treatment. *Asian Pacific Journal of Cancer Prevention*, 16(6), 2129–2144. doi:10.7314/APJCP.2015.16.6.2129
- Guo, J., Jiang, X., Lian, J., Li, H., Zhang, F., Xie, J., Deng, J., Hou, X., Du, Z., and Hao, E. (2024). Evaluation of the effect of GSK-3 β on liver cancer based on the PI3K/AKT pathway. *Frontiers in Cell and Developmental Biology*, 12(August), 1–19. doi:10.3389/fcell.2024.1431423
- Habtemariam, S. (2019). The chemistry, pharmacology and therapeutic potential of the edible mushroom *Dictyophora indusiata* (Vent ex. Pers.) Fischer (Synn. Phallus indusiatus). *Biomedicines*, 7(4). doi:10.3390/biomedicines7040098

- Han, S., Ma, C., Hu, M., Wang, Y., Ma, F., Tao, N., and Qin, Z. (2017). A polysaccharide from *Dictyophora indusiata* inhibits the immunosuppressive function of cancer-associated fibroblasts. *Cell Biochemistry and Function*, 35(7), 414–419. doi:10.1002/cbf.3290
- Hu, T., Zhang, K., Pan, D., Pan, X., Yang, H., Xiao, J., Shen, X., and Luo, P. (2020). Inhibition effect of dictyophora polysaccharides on human hepatocellular carcinoma cell line HCC-LM3. *Medical Science Monitor*, 26, 1–9. doi:10.12659/MSM.918870
- Huang, J., Ngai, C. H., Deng, Y., Tin, M. S., Lok, V., Zhang, L., Yuan, J., Xu, W., Zheng, Z.-J., and Wong, M. C. S. (2022). Cancer incidence and mortality in Asian countries: A trend analysis. *Cancer Control*, 29, 10732748221095956. doi:10.1177/10732748221095955
- Huang, M., Chen, X., Tian, H., Sun, B., and Chen, H. (2011). Isolation and identification of antibiotic albaflavenone from *Dictyophora indusiata* (Vent:Pers.) Fischer. *Journal of Chemical Research*, 35(11), 659–660. doi:10.3184/174751911X13202334527264
- Ji, Y. B., Yu, W. J., Xin, G. S., and He, J. X. (2018). Correlation between Hippo-YAP signaling pathway and liver cancer. *Iczbe*, 17–20. doi:10.25236/iczbe.2018.004
- Ko, K. P., Shin, A., Cho, S., Park, S. K., and Yoo, K. Y. (2018). Environmental contributions to gastrointestinal and liver cancer in the Asia-Pacific region. *Journal of Gastroenterology and Hepatology (Australia)*, 33(1), 111–120. doi:10.1111/jgh.14005
- Kuntz, S., Kriehoff-Henning, E., Kather, J. N., Jutzi, T., Höhn, J., Kiehl, L., Hekler, A., Alwers, E., von Kalle, C., Fröhling, S., Utikal, J. S., Brenner, H., Hoffmeister, M., and Brinker, T. J. (2021). Gastrointestinal cancer classification and prognostication from histology using deep learning: Systematic review. *European Journal of Cancer*, 155(2021), 200–215. doi:10.1016/j.ejca.2021.07.012
- Lee, I. K., Yun, B. S., Han, G., Cho, D. H., Kim, Y. H., and Yoo, I. D. (2002). Dictyoquinazols A, B, and C, new neuroprotective compounds from the mushroom *Dictyophora indusiata*. *Journal of Natural Products*, 65(12), 1769–1772. doi:10.1021/np020163w

- Lee, Y. J., Kim, W. R., Park, E. G., Lee, D. H., Kim, J. M., Shin, H. J., Jeong, H. S., Roh, H. Y., and Kim, H. S. (2024). Exploring the key signaling pathways and ncRNAs in colorectal cancer. *International Journal of Molecular Sciences*, 25(8). doi:10.3390/ijms25084548
- Liu, X., Chen, Y., Wu, L., Wu, X. Q., Huang, Y., and Liu, B. (2017). Optimization of polysaccharides extraction from *Dictyophora indusiata* and determination of its antioxidant activity. *International Journal of Biological Macromolecules*, 103, 175–181. doi:10.1016/j.ijbiomac.2017.04.125
- Liu, Z., Ding, Y., Ye, N., Wild, C., Chen, H., and Zhou, J. (2016). Direct activation of Bax protein for cancer therapy. *Medicinal Research Reviews*, 36(2), 313–341. doi:10.1002/med.21379
- Lizarme, Y., Wangsahardja, J., Marcolin, G. M., Morris, J. C., Jones, N. M., and Hunter, L. (2016). Synthesis and neuroprotective activity of dictyoquinazol A and analogues. *Bioorganic and Medicinal Chemistry*, 24(7), 1480–1487. doi:10.1016/j.bmc.2016.02.016
- Lohsiriwat, V., Chaisomboon, N., and Pattana-Arun, J. (2020). Current colorectal cancer in Thailand. *Annals of Coloproctology*, 36(2), 78–82. doi:10.3393/ac.2020.01.07
- Nazir, Y., Linsaenkart, P., Khantham, C., Chaitep, T., Jantrawut, P., Chittasupho, C., Rachtanapun, P., Jantanasakulwong, K., Phimolsiripol, Y., Sommano, S. R., Tocharus, J., Mingmalairak, S., Wongsa, A., Arjin, C., Sringarm, K., Berrada, H., Barba, F. J., and Ruksiriwanich, W. (2021). High efficiency in vitro wound healing of *Dictyophora indusiata* extracts via anti-inflammatory and collagen stimulating (MMP-2 inhibition) mechanisms. *Journal of Fungi*, 7(12). doi:10.3390/jof7121100
- Panda, S. K., Sahoo, G., Swain, S. S., and Luyten, W. (2022). Anticancer activities of mushrooms: A neglected source for drug discovery. *Pharmaceuticals*, 15(2). doi:10.3390/ph15020176

- Peng, B.-Y., Singh, A. K., Chan, C.-H., Deng, Y.-H., Li, P.-Y., Su, C.-W., Wu, C.-Y., and Deng, W.-P. (2023). AGA induces sub-G1 cell cycle arrest and apoptosis in human colon cancer cells through p53-independent/p53-dependent pathway. *BMC Cancer*, 23(1), 1. doi:10.1186/s12885-022-10466-x
- Porta, C., Paglino, C., and Mosca, A. (2014). Targeting PI3K/Akt/mTOR signaling in cancer. *Frontiers in Oncology*, 4 APR(April), 1–11. doi:10.3389/fonc.2014.00064
- Riesco-Martinez, M. C., Modrego, A., Espinosa-Olarte, P., La Salvia, A., and Garcia-Carbonero, R. (2022). Perioperative chemotherapy for liver metastasis of colorectal cancer: Lessons learned and future perspectives. *Current Treatment Options in Oncology*, 23(9), 1320–1337. doi:10.1007/s11864-022-01008-5
- Roberts, P. J., and Der, C. J. (2007). Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. *Oncogene*, 26(22), 3291–3310. doi:10.1038/sj.onc.1210422
- Ruksiriwanich, W., Khantham, C., Linsaenkart, P., Chaitep, T., Rachtanapun, P., Jantanasakulwong, K., Phimolsiripol, Y., Režek Jambrak, A., Nazir, Y., Yoojin, W., Sommano, S. R., Jantrawut, P., Sainakham, M., Tocharus, J., Mingmalairak, S., and Sringarm, K. (2022). Anti-inflammation of bioactive compounds from ethanolic extracts of edible bamboo mushroom (*Dictyophora indusiata*) as functional health promoting food ingredients. *International Journal of Food Science and Technology*, 57(1), 110–122. doi:10.1111/ijfs.15338
- Rumgay, H., Arnold, M., Ferlay, J., Lesi, O., Cabasag, C. J., Vignat, J., Laversanne, M., McGlynn, K. A., and Soerjomataram, I. (2022). Global burden of primary liver cancer in 2020 and predictions to 2040. *Journal of Hepatology*, 77(6), 1598–1606. doi:10.1016/j.jhep.2022.08.021

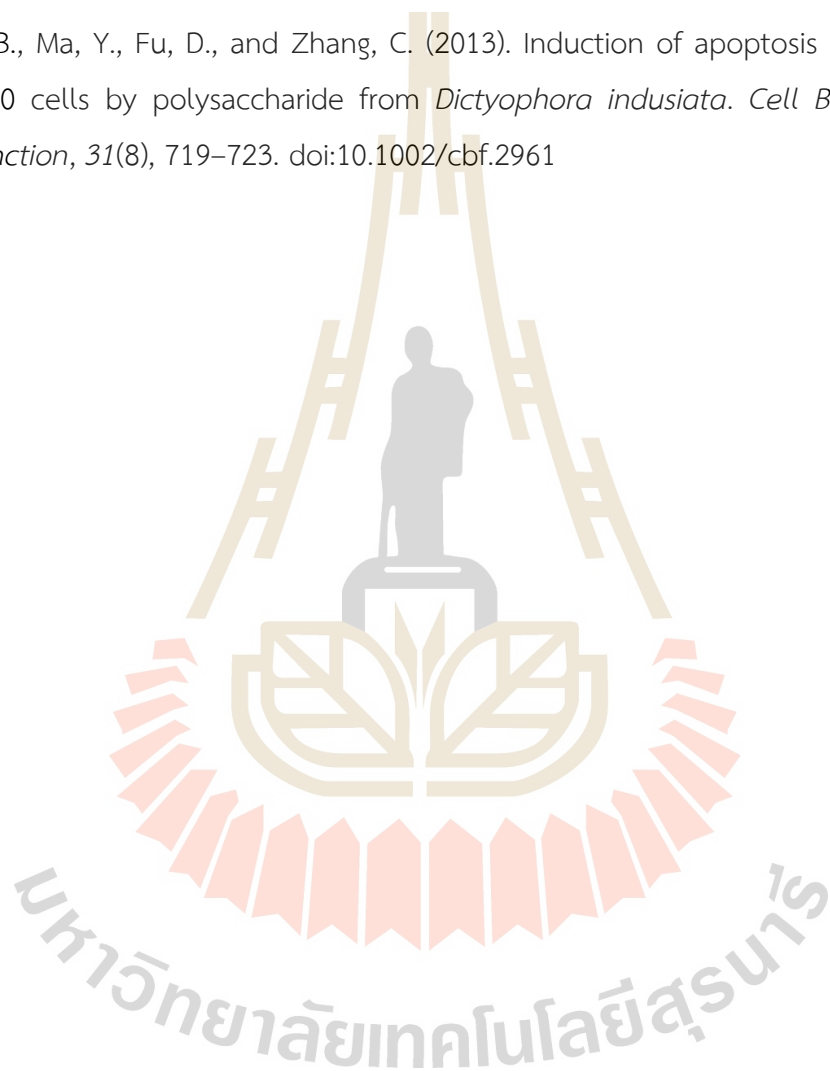
- Seczyk, E., Sugier, D., Dervişoğlu, G., Özdemir, F. A., and Kołodziej, B. (2023). Phytochemical profile, in vitro bioaccessibility, and anticancer potential of golden root (*Rhodiola rosea* L.) extracts. *Food Chemistry*, 404(August 2022). doi:10.1016/j.foodchem.2022.134779
- Sharifi, S., Barar, J., Hejazi, M. S., and Samadi, N. (2014). Roles of the Bcl-2/Bax ratio, caspase-8 and 9 in resistance of breast cancer cells to paclitaxel. *Asian Pacific Journal of Cancer Prevention*, 15(20), 8617–8622. doi:10.7314/APJCP.2014.15.20.8617
- Sharma, V. K., Choi, J., Sharma, N., Choi, M., and Seo, S. Y. (2004). In vitro anti-tyrosinase activity of 5-(hydroxymethyl)-2-furfural isolated from *Dictyophora indusiata*. *Phytotherapy Research*, 18(10), 841–844. doi:10.1002/ptr.1428
- Sikora, E., Bielak-Zmijewska, A., and Mosieniak, G. (2018). What is and what is not cell senescence. *Advances in Biochemistry*, 64(2), 110–118. doi:10.18388/pb.2018_120
- Sitinjak, R. R. (2016). Analysis of the morphology and growth of the fungus *Phallus indusiatus* Vent. in Cocoa Plantation, Gaperta-Ujung Medan. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 7(6), 442–449.
- Sriwattanapongse, W., and Prasitwattanaseree, S. (2013). Liver cancer mortality rate model in Thailand. *AIP Conference Proceedings*, 1557, 455–461. doi:10.1063/1.4823955
- Taciak, B., Pruszyńska, I., Kiraga, L., Bialasek, M., and Krol, M. (2018). Wnt signaling pathway in development and cancer. *Journal of Physiology and Pharmacology*, 69(2), 185–196. doi:10.26402/JPP.2018.2.07
- Tiankanon, K., Aniwan, S., and Rerknimitr, R. (2021). Current status of colorectal cancer and its public health burden in Thailand. *Clinical Endoscopy*, 54(4), 499–504. doi:10.5946/ce.2020.245-IDEN
- Tong, Y., Gao, H., Qi, Q., Liu, X., Li, J., Gao, J., Li, P., Wang, Y., Du, L., and Wang, C. (2021). High fat diet, gut microbiome and gastrointestinal cancer. *Theranostics*, 11(12), 5889–5910. doi:10.7150/thno.56157

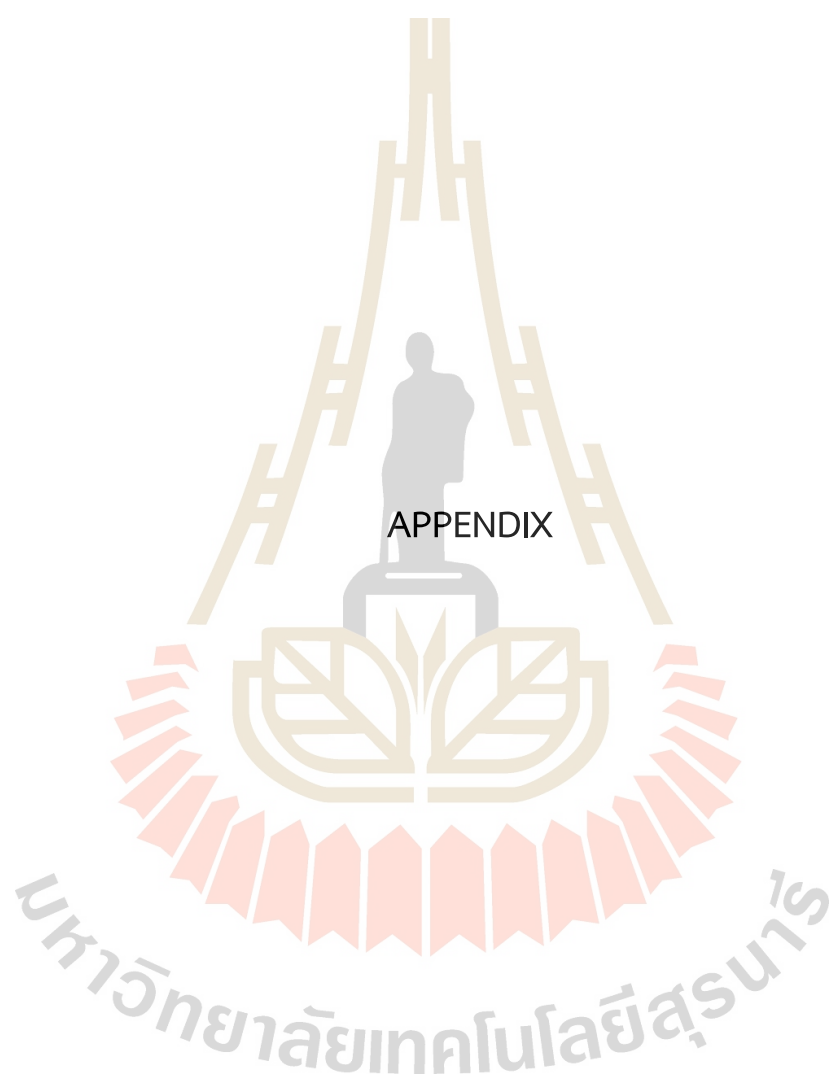
- Ullah, R., Yin, Q., Snell, A. H., and Wan, L. (2022). RAF-MEK-ERK pathway in cancer evolution and treatment. *Seminars in Cancer Biology*, 85(May 2021), 123–154. doi:10.1016/j.semcancer.2021.05.010
- Virani, S., Bilheem, S., Chansaard, W., Chitapanarux, I., Daoprasert, K., Khuanchana, S., Leklob, A., Pongnikorn, D., Rozek, L. S., Siriarechakul, S., Suwanrungruang, K., Tassanasunthornwong, S., Vatanasapt, P., and Sriplung, H. (2017). National and subnational population-based incidence of cancer in Thailand: Assessing cancers with the highest burdens. *Cancers*, 9(8). doi:10.3390/cancers9080108
- Wang(a), W., Liu, H., Zhang, Y., Feng, Y., Yuan, F., Song, X., Gao, Z., Zhang, J., Song, Z., and Jia, L. (2019). Antihyperlipidemic and hepatoprotective properties of alkali- and enzyme-extractable polysaccharides by *Dictyophora indusiata*. *Scientific Reports*, 9(1), 1–12. doi:10.1038/s41598-019-50717-9
- Wang(b), Y., Ji, X., Yan, M., Chen, X., Kang, M., Teng, L., Wu, X., Chen, J., and Deng, C. (2019). Protective effect and mechanism of polysaccharide from *Dictyophora indusiata* on dextran sodium sulfate-induced colitis in C57BL/6 mice. *International Journal of Biological Macromolecules*, 140, 973–984. doi:10.1016/j.ijbiomac.2019.08.198
- Wang(c), W., Song, X., Gao, Z., Zhao, H., Wang, X., Liu, M., and Jia, L. (2019). Anti-hyperlipidemic, antioxidant and organic protection effects of acidic-extractable polysaccharides from *Dictyophora indusiata*. *International Journal of Biological Macromolecules*, 129, 281–292. doi:10.1016/j.ijbiomac.2019.01.182
- Wang(d), Y., Lai, L., Teng, L., Li, Y., Cheng, J., Chen, J., and Deng, C. (2019). Mechanism of the anti-inflammatory activity by a polysaccharide from *Dictyophora indusiata* in lipopolysaccharide-stimulated macrophages. *International Journal of Biological Macromolecules*, 126, 1158–1166. doi:10.1016/j.ijbiomac.2019.01.022

- Wang(e), W., Song, X., Zhang, J., Li, H., Liu, M., Gao, Z., Wang, X., and Jia, L. (2019). Antioxidation, hepatic- and renal-protection of water-extractable polysaccharides by *Dictyophora indusiata* on obese mice. *International Journal of Biological Macromolecules*, 134, 290–301. doi:10.1016/j.ijbiomac.2019.05.028
- Wang, C., and Youle, R. J. (2009). The role of mitochondria in apoptosis. *Annual Review of Genetics*, 43, 95–118. doi:10.1146/annurev-genet-102108-134850
- Wang, J., Pan, J., Zou, J., Shi, Y., Guo, D., Zhai, B., Zhao, C., Luan, F., Zhang, X., and Sun, J. (2024). Isolation, structures, bioactivities, and utilizations of polysaccharides from *Dictyophora* species: A review. *International Journal of Biological Macromolecules*, 278(P1), 134566. doi:10.1016/j.ijbiomac.2024.134566
- Wang, J., Wen, X., Zhang, Y., Zou, P., Cheng, L., Gan, R., Li, X., Liu, D., and Geng, F. (2021). Quantitative proteomic and metabolomic analysis of *Dictyophora indusiata* fruiting bodies during post-harvest morphological development. *Food Chemistry*, 339(June 2020), 127884. doi:10.1016/j.foodchem.2020.127884
- Wu, D. T., Zhao, Y. X., Guo, H., Gan, R. Y., Peng, L. X., Zhao, G., and Zou, L. (2021). Physicochemical and biological properties of polysaccharides from *Dictyophora indusiata* prepared by different extraction techniques. *Polymers*, 13(14). doi:10.3390/polym13142357
- Yeesoonsang, S., McNeil, E., Virani, S., Bilheem, S., Pittayawonganon, C., Jiraphongsa, C., and Sriplung, H. (2018). Trends in incidence of two major subtypes of liver and bile duct cancer: Hepatocellular carcinoma and cholangiocarcinoma in Songkhla, Southern Thailand, 1989-2030. *Journal of Cancer Epidemiology*, 2018. doi:10.1155/2018/8267059

Zhang, J., Shi, R., Li, H., Xiang, Y., Xiao, L., Hu, M., Ma, F., Ma, C. W., and Huang, Z. (2016). Antioxidant and neuroprotective effects of *Dictyophora indusiata* polysaccharide in *Caenorhabditis elegans*. *Journal of Ethnopharmacology*, 192, 413–422. doi:10.1016/j.jep.2016.09.031

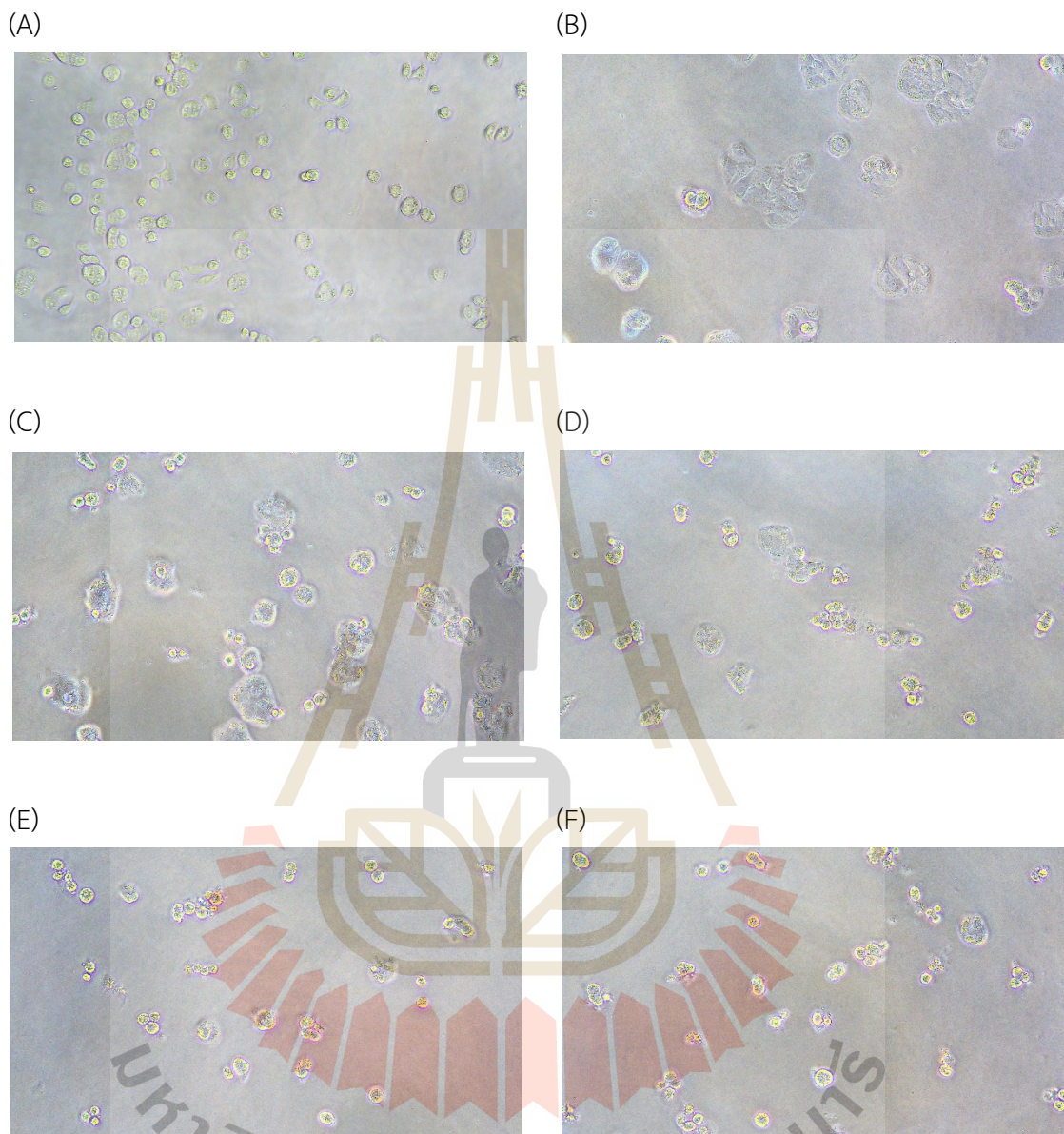
Zhong, B., Ma, Y., Fu, D., and Zhang, C. (2013). Induction of apoptosis in osteosarcoma s180 cells by polysaccharide from *Dictyophora indusiata*. *Cell Biochemistry and Function*, 31(8), 719–723. doi:10.1002/cbf.2961





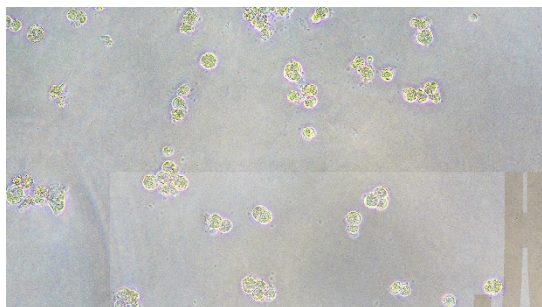
APPENDIX

Name	Source
6-well plate and 96-well plate	Thermo scientific Nunc, Korea
Amersham ImageQuant™ 800 systems	Cytiva, UK
Amersham Pharmacia Electrophoresis Power Supply	Cytiva, UK
Autoclave	Hirayama, Japan
Autopipettes (multi-channel, 10, 100, 1000 µL.)	Thermo Fisher Scientific, USA
CO ₂ incubator	Thermo scientific, USA
Conical tube (15, 50 ml.)	Thermo scientific, USA
DxFLEX Flow Cytometer	Beckman Coulter, USA
Easyflask 25 cm ² and 75 cm ² filter	Thermo scientific Nunc, Korea
Hot Air Oven	Binder, Germany
Incubator shaker	New Brunswick Innova, USA
Light microscope Olympus DP20	Olympus, Japan
Media bottle (250, 500, 1000 ml.)	Duran, Germany
Microplate reader Multiskan GO	Thermo Fisher Scientific, USA
MiniVE Vertical Electrophoresis System	Cytiva, UK
PES Bottle Top Filter	Thermo scientific Nunc, Korea
Refrigerated centrifuge	Hettich, Germany
Rotary Evaporator	Biobase, USA
Safety Cabinet Class II	Thermo Fisher Scientific, USA
Serological pipette (5, 10 ml.)	Thermo scientific Nunc, Korea
Tips (200 µL.)	Genaxy, India
Tips (10, 1000 µL.)	Thermo scientific, Mexico
Vortex-Genie 2	Scientific Industries, UK

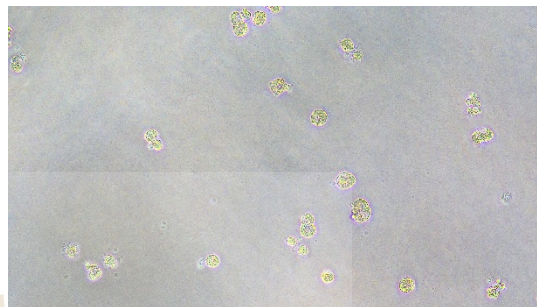


HT-29 cells observed under a microscope at 400x magnification (A) Before treat (B)-(J) after treat with egg extract for 48 h in different concentrations. (B) control (C) 400 µg/ml (D) 500 µg/ml (E) 600 µg/ml (F) 700 µg/ml (G) 800 µg/ml (H) 1000 µg/ml (I) 1500 µg/ml (J) 2000 µg/ml

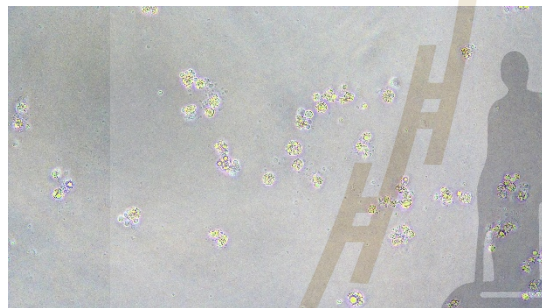
(G)



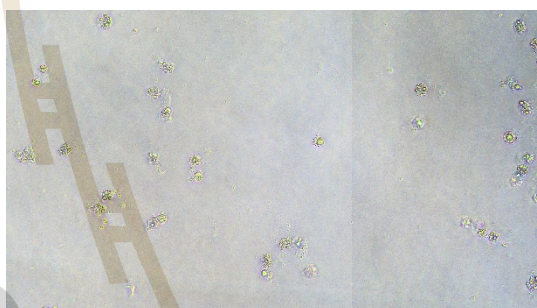
(H)



(I)

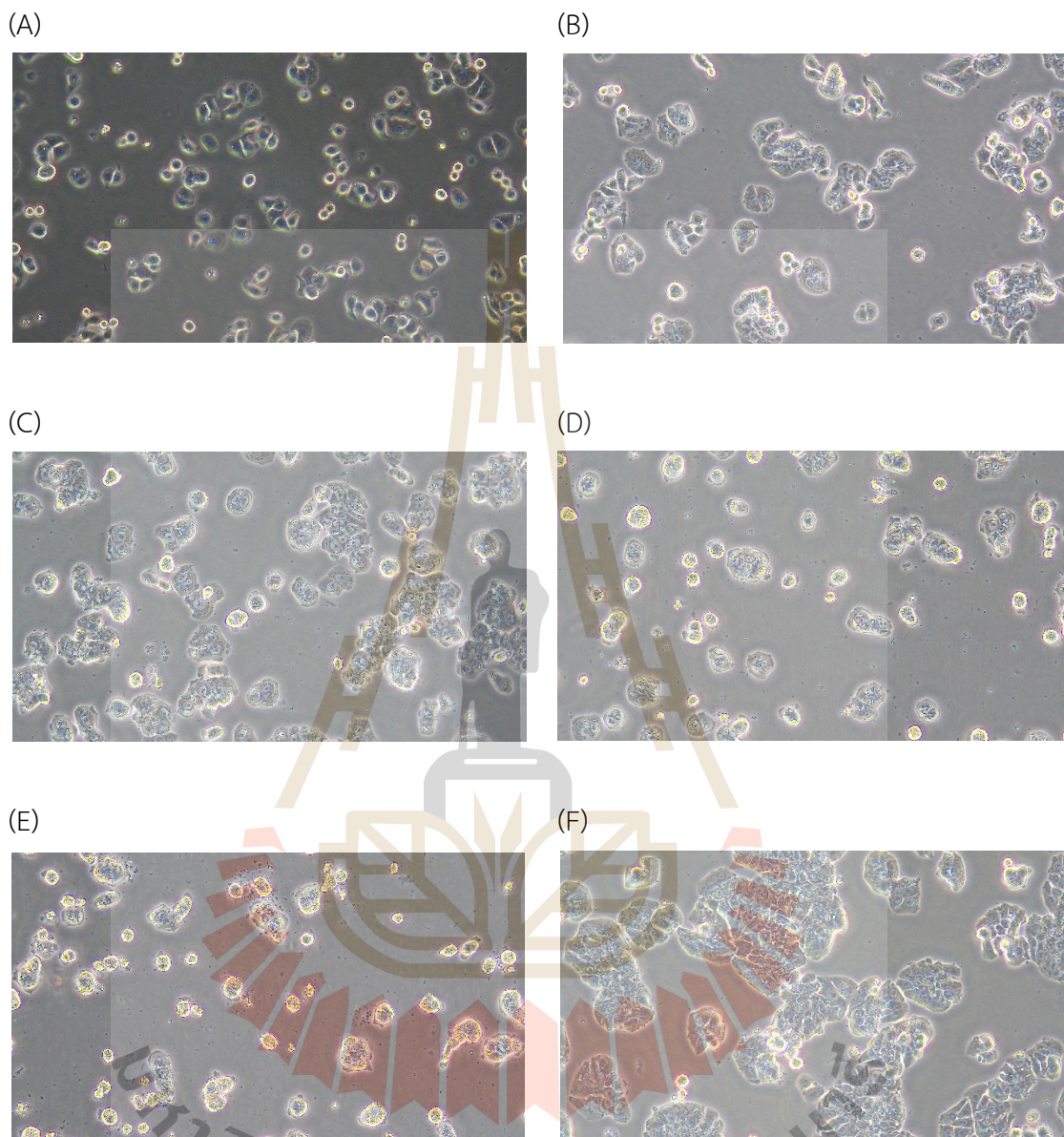


(J)



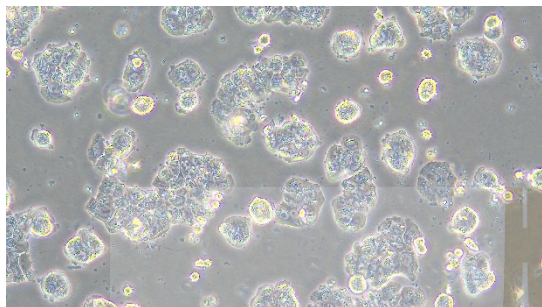
HT-29 cells observed under a microscope at 400x magnification (A) Before treat (B)-(J) after treat with egg extract for 48 h in different concentrations. (B) control (C) 400 µg/ml (D) 500 µg/ml (E) 600 µg/ml (F) 700 µg/ml (G) 800 µg/ml (H) 1000 µg/ml (I) 1500 µg/ml (J) 2000 µg/ml (Continued).

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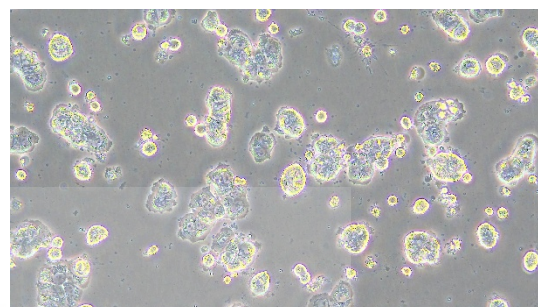


HT-29 cells observed under a microscope at 400x magnification (A) Before treat (B)-(E) after treat with egg extract for 24 h in different concentrations. (B) control (C) IC25; 700 µg/ml (D) IC50; 1000µg/ml (E) IC75; 1450 µg/ml (F)-(I) after treat with egg extract for 48 h in different concentrations. (F) control (G) IC25; 700 µg/ml (H) IC50; 1000µg/ml (I) IC75; 1450 µg/ml

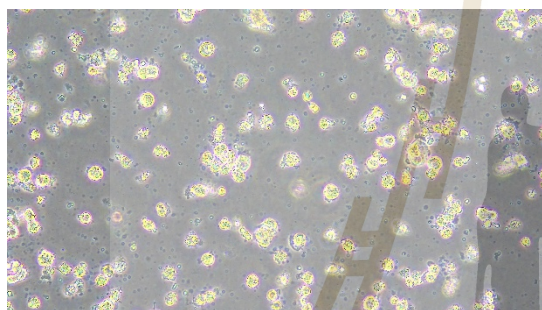
(G)



(H)



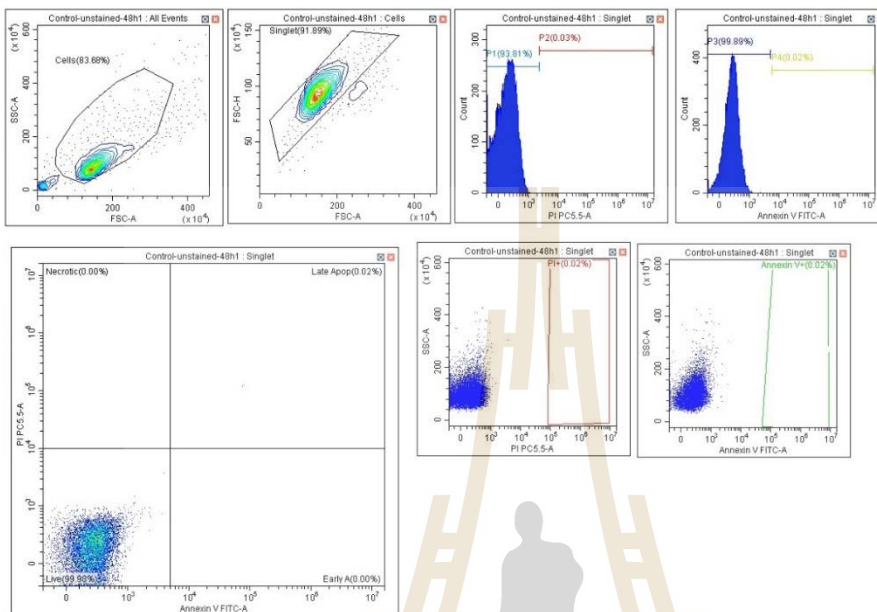
(I)



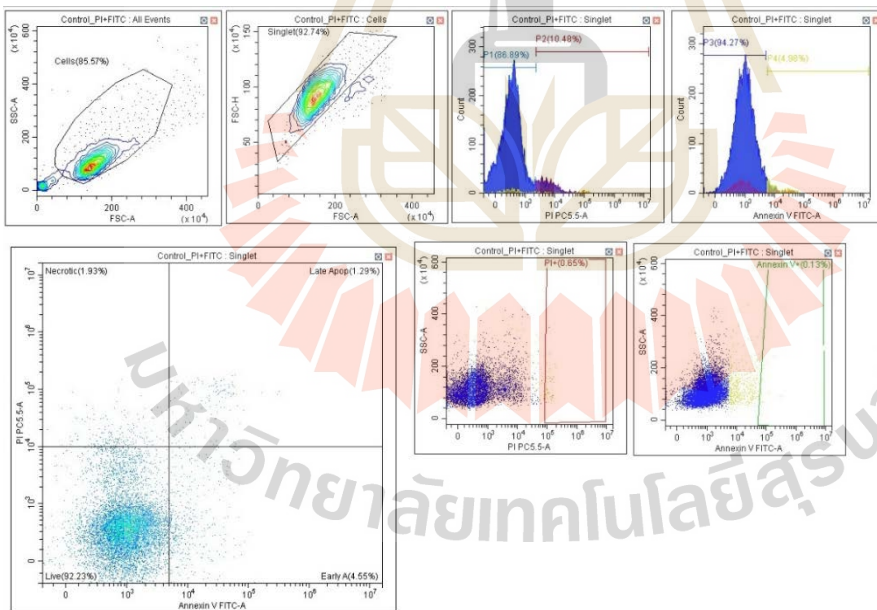
HT-29 cells observed under a microscope at 400x magnification (A) Before treat (B)-(E) after treat with egg extract for 24 h in different concentrations. (B) control (C) IC₂₅; 700 µg/ml (D) IC₅₀; 1000µg/ml (E) IC₇₅; 1450 µg/ml (F)-(I) after treat with egg extract for 48 h in different concentrations. (F) control (G) IC₂₅; 700 µg/ml (H) IC₅₀; 1000µg/ml (I) IC₇₅; 1450 µg/ml (Continued).

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(A)

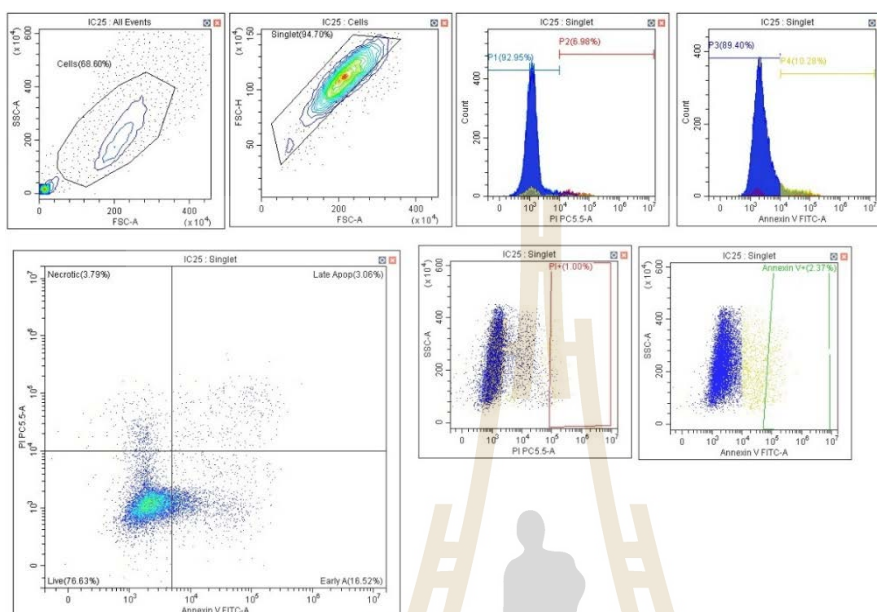


(B)

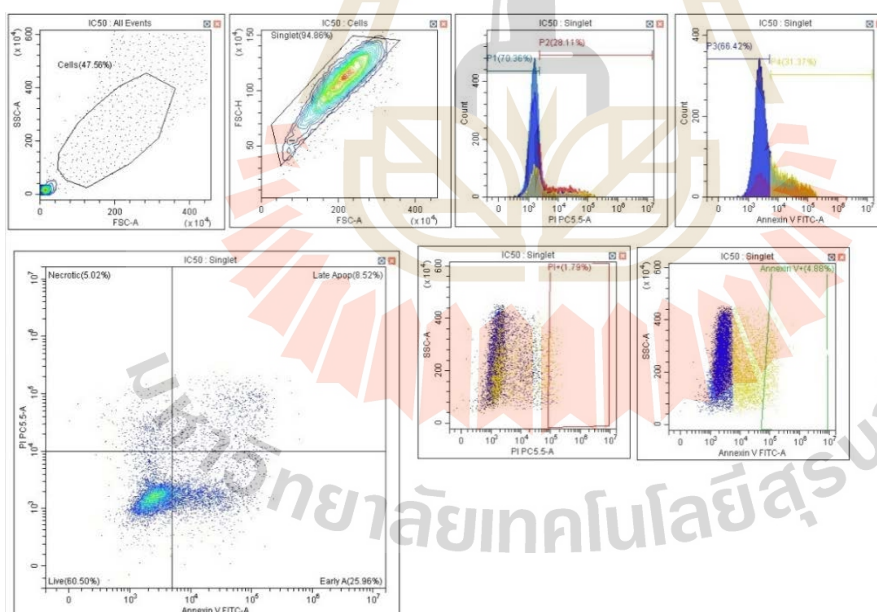


Assessment of apoptosis by flow cytometry (A) Unstained, (B) control, (C) IC₂₅; 700 $\mu\text{g/ml}$, (D) IC₅₀; 1000 $\mu\text{g/ml}$, (E) IC₇₅; 1450 $\mu\text{g/ml}$, (F) Negative control: FITC-only staining without apoptotic induction, (G) Negative control: PI-only staining without apoptotic induction (H) setting.

(C)

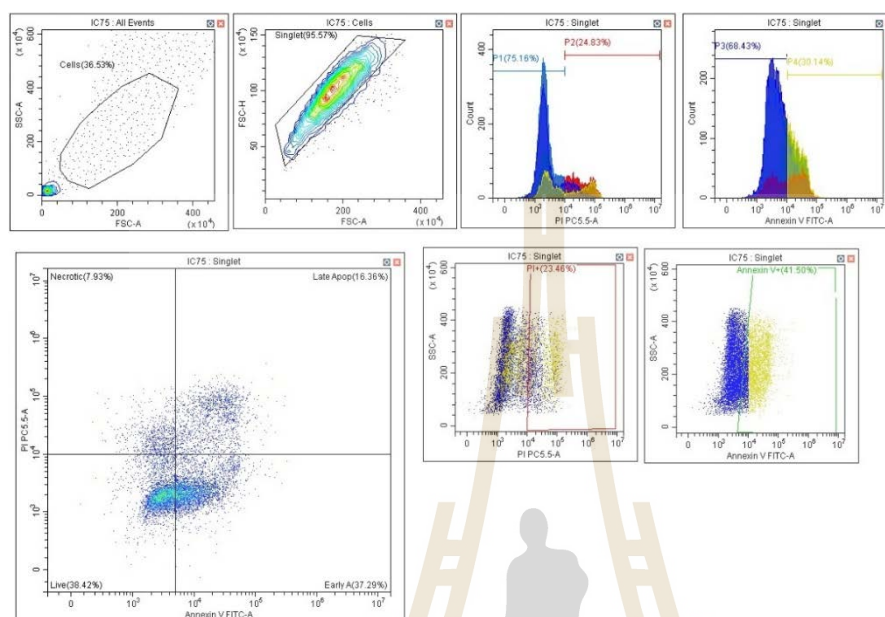


(D)

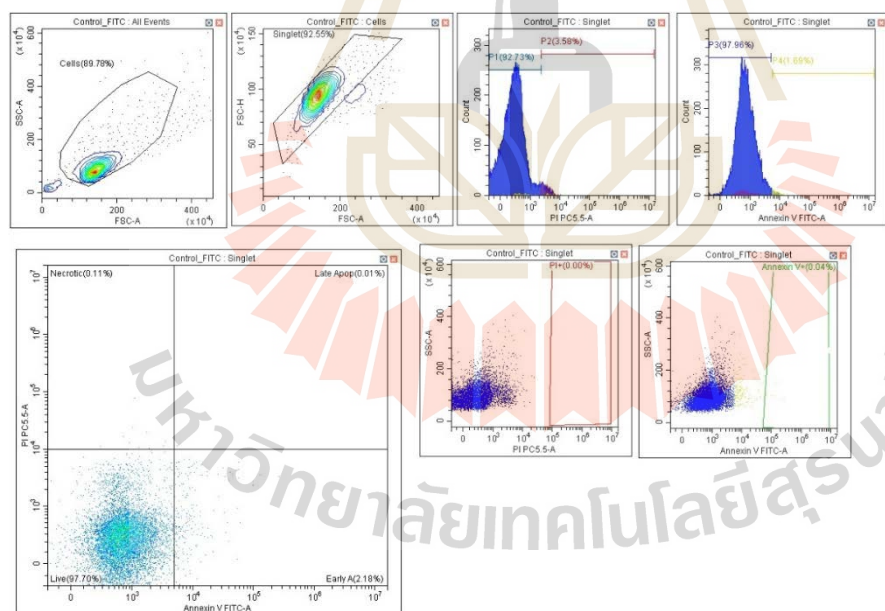


Assessment of apoptosis by flow cytometry (A) Unstained, (B) control, (C) IC25; 700 $\mu\text{g}/\text{ml}$, (D) IC50; 1000 $\mu\text{g}/\text{ml}$, (E) IC75; 1450 $\mu\text{g}/\text{ml}$, (F) Negative control: FITC-only staining without apoptotic induction, (G) Negative control: PI-only staining without apoptotic induction (H) setting (Continued).

(E)

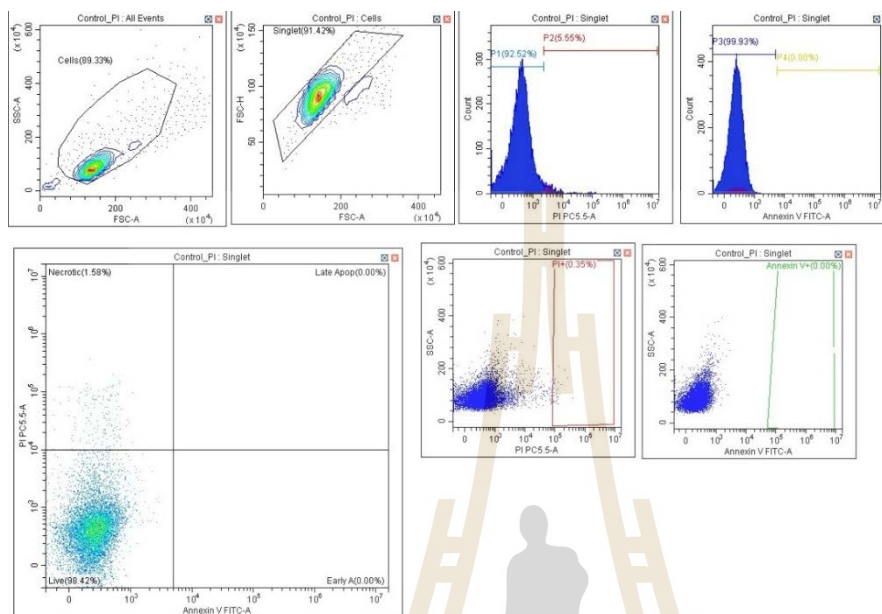


(F)



Assessment of apoptosis by flow cytometry (A) Unstained, (B) control, (C) IC25; 700 $\mu\text{g}/\text{ml}$, (D) IC50; 1000 $\mu\text{g}/\text{ml}$, (E) IC75; 1450 $\mu\text{g}/\text{ml}$, (F) Negative control: FITC-only staining without apoptotic induction, (G) Negative control: PI-only staining without apoptotic induction (H) setting (Continued).

(G)



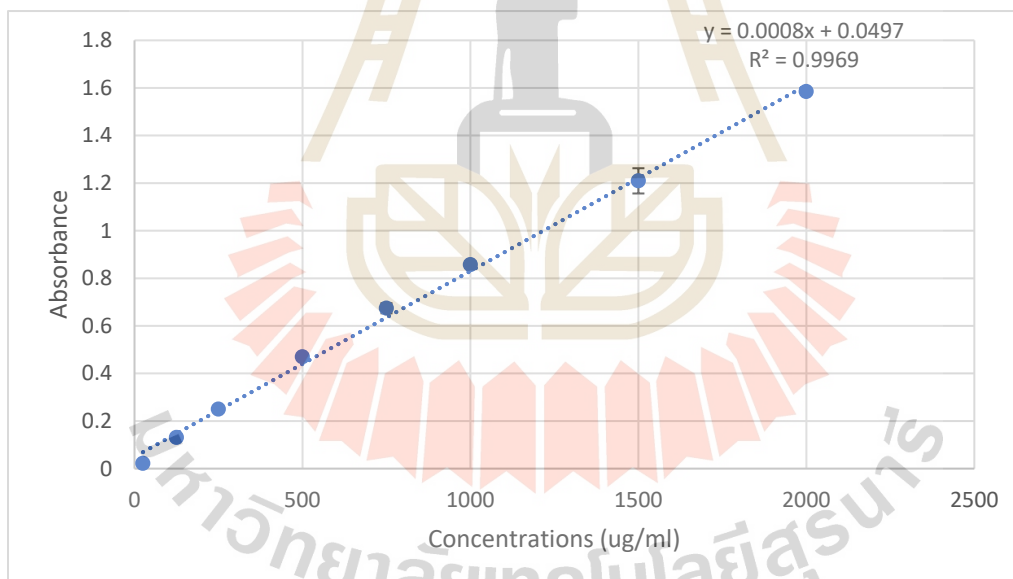
(H)

Gain	Threshold	Width
FSC	89	(1 - 3000)
SSC	230	(1 - 3000)
FITC	7	(1 - 3000)
PE	171	(1 - 3000)
ECD	302	(1 - 3000)
PC5.5	20	(1 - 3000)
PC7	582	(1 - 3000)
APC	512	(1 - 3000)
APC-A700	510	(1 - 3000)
APC-A750	481	(1 - 3000)
PB450	53	(1 - 3000)
KO525	24	(1 - 3000)
Violet610	149	(1 - 3000)

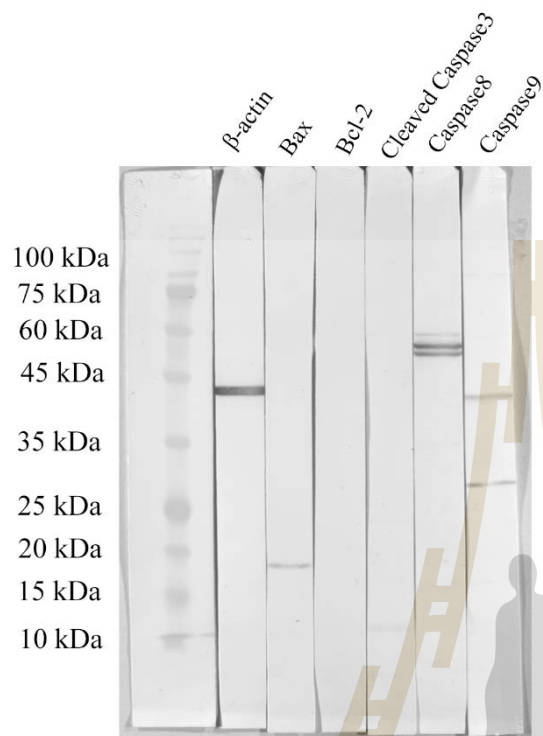
Assessment of apoptosis by flow cytometry (A) Unstained, (B) control, (C) IC25; 700 $\mu\text{g}/\text{ml}$, (D) IC50; 1000 $\mu\text{g}/\text{ml}$, (E) IC75; 1450 $\mu\text{g}/\text{ml}$, (F) Negative control: FITC-only staining without apoptotic induction, (G) Negative control: PI-only staining without apoptotic induction (H) setting (Continued).

Table showing standard protein measurement results from BCA assay

Concentration of BSA ($\mu\text{g/ml}$)	Average measured absorbance	Standard deviation
2000	1.5845	0.004949747
1500	1.2095	0.053033009
1000	0.8575	0.012020815
750	0.674	0.021213203
500	0.4695	0.010606602
250	0.25	0.007071068
125	0.131	0.002828427
25	0.022	0.002828427



Standard curve data for protein quantification using the BCA assay



Western blot analysis of apoptosis-related protein



CURRICULUM VITAE

Name: Miss Kanyapak Sakheatkarn

Date of birth: February 26, 1999

Place of birth: Bangkok, Thailand

Education:

2017 – 2021 Bachelor of Science (Biology), Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

2021 - Present Master of Science student (Environmental Biology), School of biology Sciences, Institute of Science, Suranaree University of Technology, Nakhon Ratchasima, Thailand

Scholarship:

2021 – 2023 Graduate Scholarship, Suranaree University of Technology



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