ANTI-INFLAMMATORY EFFECT OF ETHANOL AND WATER CRUDE EXTRACTS FROM FRUITING BODIES OF *PHALLUS* SP.

NAPAPORN MANGMEE

A Thesis Submitted in Partial Fulfillment of the Requirements for the
Degree of Master of Science in Environmental Biology
Suranaree University of Technology
Academic Year 2022

ฤทธิ์ต้านการอักเสบของสารสกัดหยาบเอทานอลและน้ำจากฟรุตติ้งบอดี้ของ *Phallus* sp.

นางสาวนภาพร มั่งมี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาชีววิทยาสิ่งแวดล้อม
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2565

ANTI-INFLAMMATORY EFFECT OF ETHANOL AND WATER CRUDE EXTRACTS FROM FRUITING BODIES OF *PHALLUS* SP.

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

Thesis Examining Committee

(Assoc.Prof. Dr. Pongsakorn Martviset)
Chairperson

(Dr. Sirilak Chumkiew)
Member (Thesis Advisor)

(Asst. Prof. Dr. Pathanin Chantree)
Member (Thesis Co-Advisor)

(Dr. Mantana Jamklang)
Member (Thesis Co-Advisor)

(Dr. Watsana Penkhrue)
Member

(Dr. Kattika Kaarj)
Member

(Dr. Chompunoot Wangboon)
Member

(Assoc. Prof. Dr. Yupaporn Ruksakulpiwat)
Vice Rector for Academic Affairs
and Quality Assurance

(Prof. Dr. Santi Maensiri)
Dean of institute of Science

นภาพร มั่งมี : ฤทธิ์ต้านการอักเสบของสารสกัดหยาบเอทานอลและน้ำจากฟรุตติ้งบอดี้ของ *Phallus* sp. (ANTI-INFLAMMATORY EFFECT OF ETHANOLIC AND WATER CRUDE EXTRACTS FROM FRUITING BODIES OF *PHALLUS* SP.) อาจารย์ที่ปรึกษา : อาจารย์ ดร. ศิริลักษณ์ ซุมเขียว, 48 หน้า

คำสำคัญ: *PHALLUS* SP., ฤทธิ์ต้านการอักเสบ, ฟรุตติ้งบอดี้, เห็ดเยื่อไผ่

ปัจจุบันการค้นหาสารชีวโมเลกุลต้านการอักเสบจากธรรมชาติที่ส่งผลข้างเคียงต่อร่างกายน้อยได้รับความนิยมทำการศึกษา โดยเฉพาะอย่างยิ่งสารสกัดหยาบจากเห็ดเยื่อไผ่ *Phallus indusiatus* (*Dictyophora indusiata*) หรือเห็ดเยื่อไผ่สายพันธุ์จีนซึ่งได้รับความนิยมนำมาเป็นวัตถุดิบในอาหารชั้นสูงของจีน ในขณะที่รายงานการทดสอบคุณสมบัติดังกล่าวพบน้อยในเห็ดเยื่อไผ่บริโภคได้สายพันธุ์ที่พบในประเทศไทย (*Phallus* sp.) เพื่อเพิ่มโอกาสในการเพาะเลี้ยงเห็ดเยื่อไผ่สายพันธุ์ที่พบในประเทศไทยในเชิงพาณิชย์ ผ่านการศึกษาคุณสมบัติของสารออกฤทธิ์ในสารสกัดหยาบจึงมีความสำคัญ การศึกษานี้จึงมีวัตถุประสงค์เพื่อประเมินคุณสมบัติฤทธิ์ต้านการอักเสบของสารสกัดหยาบเอทานอลและสารสกัดหยาบน้ำของฟรุตติ้งบอดี้ในเซลล์แมคโครฟาจ RAW 264.7 ที่ถูกกระตุ้นด้วย lipopolysaccharide (LPS) และระบุชนิดของเห็ดเยื่อไผ่แยกได้ในประเทศไทยโดยใช้เทคนิคระดับโมเลกุล วิเคราะห์สายวิวัฒนาการด้วยวิธี neighbor-joining (NJ) ประเมินระดับความเชื่อมั่นของความสัมพันธ์ดังกล่าวด้วยวิธี bootstrap วิเคราะห์จำนวน 1,000 ซ้ำ โดยใช้โปรแกรม MEGA version 11 งานวิจัยนี้ดำเนินการตรวจสอบความเป็นพิษต่อเซลล์ด้วยการทดสอบ MTT กับสารสกัดหยาบที่มีความเข้มข้น 7.81 ถึง 1000 ไมโครกรัม/มิลลิลิตร ประเมินคุณสมบัติการต้านการอักเสบจากการตรวจสอบระดับไนไตรต์จากการทดสอบ Griess และประเมินการแสดงออกของ mRNA ไซโตไคน์ที่ทำให้เกิดการอักเสบได้แก่ IL- 1β, IL-6 และ TNF-α โดยใช้ qRT-PCR ผลการศึกษาแสดงให้เห็นว่าเห็ดเยื่อไผ่สายพันธุ์ไทยและเห็ดเยื่อไผ่สายพันธุ์จีนที่แยกได้มีความแตกต่างกันทางวิวัฒนาการ โดยตัวอย่างเห็ดเยื่อไผ่กระโปรงสั้นจัดกลุ่มร่วมกับ *Phallus atrovolvatus* และ *Phallus merulinus* ด้วยค่า bootstrap เท่ากับ 100 และเห็ดเยื่อไผ่กระโปรงยาวมีความใกล้ชิดทางสายวิวัฒนาการร่วมกับ *Phallus chiangmaiensis* และ *Phallus echinovolvatus* ที่ Bootstrap เท่ากับ 98 โดยเห็ดเยื่อไผ่เหล่านี้มีการรายงานพบในประเทศไทย และผลการทดสอบความเป็นพิษต่อเซลล์พบว่าทั้งสารสกัดหยาบเอทานอลและน้ำร้อนไม่มีความเป็นพิษต่อเซลล์ RAW 264.7 อีกทั้งไม่สามารถลดการแสดงออกในการผลิตไนไตรต์ รวมถึงการแสดงออกของ mRNA ประกอบด้วย IL-1β, IL-6 และ TNF-α แม้ทดสอบด้วยความเข้มข้นสูง การศึกษานี้ให้ผลที่แตกต่างกับการศึกษาก่อนหน้าซึ่งอธิบายถึงการลดการแสดงออกของ mRNA ไซโตไคน์ที่ทำให้เกิดการอักเสบโดยสารสกัดหยาบจาก

P. indusiatus ข้อสังเกตที่พบคือการศึกษาก่อนหน้าเลือกใช้ความเข้มข้นของสารสกัดหยาบจากเอทานอลของระยะไข่ *P. indusiatus* ที่สูงถึง 200 และ 2000 ไมโครกรัม/มิลลิลิตร ซึ่งอาจส่งผลให้ผลการศึกษาแตกต่างกัน ดังนั้นในอนาคตการศึกษาฤทธิ์ต้านการอักเสบของสารสกัดหยาบจากเห็ดเยื่อไผ่ควรใช้ความเข้มข้นสูงขึ้นและทำการทดลองเพื่อติดตามปริมาณสารชีวโมเลกุลในสารสกัดหยาบ และศึกษาเหตุการณ์ทางเคมีชีวภาพตามวิถีการส่งสัญญาณ (signaling pathway)

สาขาวิชาชีววิทยา
ปีการศึกษา 2565

ลายมือชื่อนักศึกษา นทพร ชั่งฉี
ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

NAPAPORN MANGMEE : ANTI-INFLAMMATORY EFFECT OF ETHANOLIC AND WATER CRUDE EXTRACTS FROM FRUITING BODIES OF *PHALLUS* SP. THESIS ADVISOR : SIRILAK CHUMKIEW, Ph.D. 48 PP.

Keyword: *PHALLUS* SPECIES/ ANTI-INFLAMMATORY/ FRUITING BODIES/ BAMBOO MUSHROOM

Currently, the search for natural anti-inflammatory biomolecules with minimal side effects is popular. Especially, the crude extract from the bamboo mushroom *Phallus indusiatus* (*Dictyophora indusiata*), or the Chinese bamboo mushroom, which has been popular to use as an ingredient in Chinese haute cuisine. While there are lacked scientific reports of any medicinal properties in edible bamboo mushroom species found in Thailand (*Phallus* sp.). To increase the opportunity for cultivating the Thai bamboo mushroom species as commercially, therefore the study of the properties on active ingredients in crude extracts is required. This study aimed to evaluate the anti-inflammatory properties of ethanol and hot water crude extracts from fruiting body in RAW 264.7 macrophage cells that were stimulated with lipopolysaccharide (LPS) and identify bamboo fungi isolated in Thailand using molecular techniques. The phylogenetic analysis by using neighbor-joining (NJ) and the confidence level of associations was assessed by bootstrap method, 1000 replicates using MEGA version 11. The cytotoxicity of crude extracts was investigated by MTT assay with 7.81 to 1000 µg/ml concentrations. The anti-inflammatory properties were assessed by evaluating the nitrite levels using Griess assay and the mRNA expression of the pro-inflammatory cytokines, IL-1β, IL-6 and TNF-α, using qRT-PCR. The results show that Thai bamboo mushroom and Chinese bamboo mushroom isolates are evolutionarily different, the short skirt bamboo mushroom samples grouping together with *Phallus atrovolvatus* and *Phallus merulinus* with 100 bootstrap values and long skirt bamboo mushrooms have a phylogenetic relationship closely with *Phallus chiangmaiensis* and *Phallus echinovolvatus* with 98 bootstrap values. The cytotoxicity assay showed that both of ethanol crude extract and hot water were non cytotoxic to RAW 264.7 cells and they could not decrease the expression in nitrite production including the expression of mRNA containing IL-1β, IL-6 and TNF-α even tested at high

concentrations. The results from this study showed the different finding from previous studies describing the downregulation of pro-inflammatory cytokine mRNA expression by crude extracts from *P. indusiatus*. It was noted that previous studies used concentrations of crude ethanol extract of *P. indusiatus* egg stage as high as 200 and 2000 µg/ml, which may result in different study results. Therefore, in the future, to study the anti-inflammatory activity of bamboo mushroom crude extracts should be considered at higher concentrations and the experiments should be conducted to monitor the biomolecule content and studying biochemical events along the signaling pathway.

School of Biology
Academic Year 2022

Student's Signature Napaporn
Advisor's Signature
Co-advisor's Signature
Co-advisor's Signature

ACKNOWLEDGEMENTS

The author would like to acknowledge the funding support from the Development and Promotion of Science and Technology Talent project (DPST) scholarship.

I would like to express my deepest and sincere gratitude to my advisor Dr. Sirilak Chumkiew for giving me the opportunity to do research and providing invaluable guidance and suggestions in all the time of study and writing of this thesis. Also, she always supports and encourages me to overcome all struggles in my research and my life and steer me in the right direction.

I would like to express my deepest gratitude to my co-adviser Asst. Prof. Dr. Pathanin Chantree for his invaluable guidance, support, and technical assistance throughout my research journey. His expertise and willingness to help have been instrumental in the success of this project. Moreover, his encouragement and positive attitude have motivated me to persevere through the most challenging times. I am truly fortunate to have had Asst. Prof. Dr. Pathanin Chantree and I am grateful for their unwavering commitment to my academic and personal growth. Thank you for everything.

I would like to express my sincere gratitude and appreciation to all teachers for enlightening and inspiring me in science. I also thank all staff of the department for their valuable assistance.

I thank my fellow lab members and friends in the first batch of honor program, especially in biology major, for giving me words of encouragement and sharing all the knowledge and experiences during study.

Finally, I owe my special gratitude to my family – my father, mother, and sister for being the biggest source of my strength, constantly caring, and supporting me.

Napaporn Mangmee

CONTENTS

CONTENTS (Continued)

LIST OF TABLES

Table **Page**

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal bovine serum
IL-1β	Interleukin 1 beta
IL-6	Interleukin 6
ITS-1	Internal transcribed spacer 1
ITS-2	Internal transcribed spacer 2
LPS	Lipopolysaccharide
mRNA	Messenger RNA
MTT assay	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide assay
NO	Nitric oxide
NSAIDs	Non-steroidal anti-inflammatory medicines
TNF-α	Tumor necrosis factor alpha
qRT-PCR	Real-Time Quantitative Reverse Transcription PCR

CHAPTER I

INTRODUCTION

1.1 Background

Noncommunicable diseases (NCDs) caused by inflammation have emerged as the leading cause of mortality in Thailand. These NCDs, namely cancer, cardiovascular diseases, diabetes, and chronic obstructive pulmonary disease (COPD), account for a staggering 400,000 annual fatalities, equivalent to over 1,000 lives lost per day. Remarkably, these conditions contribute to approximately 74% of all deaths in the country. The economic impact of NCDs, including premature deaths and reduced productivity due to absenteeism and presenteeism (when employees cannot fully function at work), imposes a significant burden. While the precise figures detailing this economic burden remain elusive, it is well-recognized that NCDs have substantial implications for both individual productivity and the overall economy (Organization, 2022).

Inflammation is a complex physiological response to injury, infection, or tissue damage. Inflammatory responses involve in the activation of immune cells, i.e. macrophages, which play a crucial role in the initiation and resolution of inflammation (Murray and Wynn, 2011). During the inflammatory response, macrophages release pro-inflammatory cytokines, including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α). These cytokines play a key role in the inflammatory process by attracting and activating other immune cells, such as neutrophils, and promoting the production of nitric oxide (NO) (Nathan and Xie, 1994). Nitric oxide (NO) is a small gaseous molecule that plays a crucial role in the immune response. In macrophages, NO is produced by the enzyme inducible nitric oxide synthase (iNOS) and is involved in the destruction of invading pathogens. However, overproduction of NO has been associated with various inflammatory diseases, such as rheumatoid arthritis, asthma, and atherosclerosis.

Currently, non-steroidal anti-inflammatory drugs (NSAIDs) for example ibuprofen, Diclofenac, Naproxen and Aspirin are commonly prescribed for the treatment of inflammation and pain by inhibiting the activity of cyclooxygenase (COX) enzymes, which are involved in the production of prostaglandins, a group of inflammatory mediators. However, prolonged NSAIDs usage has several side effects, including gastrointestinal bleeding, renal dysfunction, and cardiovascular events. These side effects are attributed to the inhibition of COX enzymes, which are also involved in the production of protective prostaglandins that maintain the integrity of the gastrointestinal tract, regulate renal blood flow, and protect against cardiovascular disease (Johnson and Day, 1991; Wong, 2019). Consequently, researchers are exploring alternative approaches involving natural metabolites that exhibit comparable anti-inflammatory properties without the undesirable side effects of NSAIDs.

Natural products, such as medicinal plants and mushrooms, have been used for centuries to treat various diseases, including inflammation. Examples of mushrooms known for their potential anti-inflammatory properties include shiitake mushrooms, Reishi mushrooms, Maitake mushrooms, and bamboo mushrooms. For the bamboo mushroom species have been widely studied and have their origins in China. In particular, the bamboo mushroom, *Dictyophora indusiata*, has garnered significant attention in research due to its impressive effects. In Thailand, there have been reports of long white skirt and short white skirt bamboo mushrooms (*Phallus* sp.) some of which are cultivated commercially. However, there is a scarcity of studies investigating the active ingredients and anti-inflammatory effects of these mushroom species found in Thailand. Thai isolated bamboo mushrooms (*Phallus* sp.) are species of mushroom that have not been extensively studied for their anti-inflammatory potential. Thai isolate bamboo mushrooms are unique species that found in Thailand and known as their have cylindrical shape and long stalks. They are commonly used in traditional Thai medicine to treat various ailments, including inflammation. These mushrooms contain a diverse array of bioactive compounds that can modulate immune responses and alleviate inflammation.

The present study aimed to identification of bamboo mushroom samples (short and long skirt) and evaluate the anti-inflammatory potential of Thai isolate bamboo

mushrooms (short skirt) on macrophage cells, specifically RAW 264.7 cells. Understanding the phylogenetic relationship of these mushrooms will provide information of their genetic diversity and evolutionary history of edible bamboo mushroom in Thailand. Additionally, we aimed to explore the cytotoxicity of the bamboo mushrooms on RAW 264.7 cells and investigate the concentration response of NO and mRNA expression of IL-1β, IL-6, and TNF-α.

The results of this study could provide fundamental information for the development of novel anti-inflammatory agents from natural sources, such as Thai isolate bamboo mushrooms, that could alleviate inflammation and pain with small of side effects associated with NSAIDs. The findings of this study could have significant implications for the development of new therapies for inflammatory diseases.

1.2 Research objectives

1.2.1 To Identify bamboo mushroom specimens up to species level.

1.2.2 To extract the fruiting body using ethanol and water to obtain crude extracts.

1.2.3 To evaluate the anti-inflammatory activities of these crude extracts on LPS-induced inflammation in RAW 264.7 macrophage cell lines.

1.3 Scope of the study

The scope of this study is to investigate the anti-inflammatory activities of crude extracts from bamboo mushrooms using *in vitro* assays. The study involved identifying different species of bamboo mushrooms from the data base sequence, extracting the fruiting body using ethanol and water to obtain crude extracts, and evaluating the anti-inflammatory properties of these extracts on LPS-induced inflammation in RAW 264.7 macrophage cell lines as shown in figure 1.

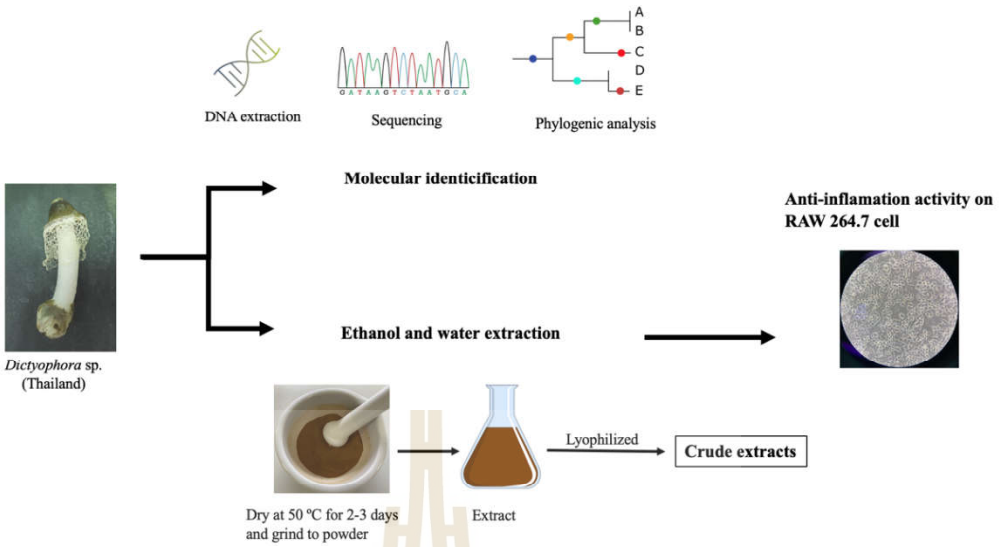


Figure 1 Schematic diagram of scope of the study.

CHAPTER II

LITERATURE REVIEWS

The potential use of bamboo mushrooms as a source of anti-inflammatory agents. Bamboo mushrooms have been traditionally used in Chinese medicine for their medicinal properties, and several studies have shown that they contain various bioactive compounds and also included the anti-inflammatory effects (Mazumder, Roy and Sarkar, 2022). Inflammation is a natural response of the body to infection or injury, however chronic inflammation can lead to various noncommunicable diseases (NCDs) such as cancer, diabetes, and cardiovascular diseases (Eid et al., 2001). Therefore, finding natural sources of anti-inflammatory agents are the importance for developing the efficient alternative therapeutic drugs.

2.1 The Inflammations

Inflammation is a biological response of the body to harmful stimuli such as injury, infection, or irritation. It is a complex process involving various cells, chemicals, and immune responses. The main goal of inflammation is to protect the body and promote healing by eliminating the harmful stimuli and initiating tissue repair. It is characterized by symptoms such as redness, swelling, heat, pain, and loss of function in the affected area. Inflammation can be classified into two types: acute and chronic. Acute inflammation is a short-term response that occurs immediately after injury, while chronic inflammation is a long-lasting response that can persist for weeks, months, or even years. Chronic inflammation is often associated with various diseases such as rheumatoid arthritis, asthma, and cardiovascular diseases (Coleman, King and Andrade, 1974).

Acute inflammation is the initial and short-term phase of the body's response to harmful stimuli, such as infections, injuries, or tissue damage. It is a vital protective mechanism aimed at eliminating the source of the injury or infection and initiating the

healing process. During acute inflammation, various proinflammatory cytokines and chemokines are released from immune cells, particularly neutrophils, which are one of the first responders to the site of inflammation. Neutrophils are a type of white blood cell and play a critical role in the early stages of acute inflammation. They migrate to the site of injury or infection, where they release inflammatory mediators and enzymes to neutralize pathogens, remove damaged tissue, and attract other immune cells to the area (Margraf, Lowell and Zarbock, 2022). In many cases, the body successfully resolves acute inflammation, leading to the restoration of normal tissue structure and function. However, if the inflammatory response persists or becomes dysregulated, it can lead to chronic inflammation (Sanada et al., 2018). Chronic inflammation is a complex biological process that involves multiple factors, including both plasma-driven and cell-driven mediators (Galvão, Sugimoto, Vago, Machado and Sousa, 2018). As shown in the table 2.1 below the plasma-driven mediators include proteins and molecules found in the blood, such as cytokines, acute-phase proteins, and complement factors, which play a role in the inflammatory response. On the other hand, cell-driven mediators are produced by various immune cells, such as macrophages, lymphocytes, and neutrophils, which infiltrate tissues and release pro-inflammatory substances (Yao and Narumiya, 2019).

Table 2.1 Plasma and cell-driven mediators.

List	Functions
Plasma-driven mediators	
Bradykinin	A vasoactive protein that causes vasodilation, higher permeability of the vascular system, smooth muscle contraction, and ache.
Plasmin	Capable of dissolving fibrin clots, activating Factor XII, and cleaving complement protein C3.
Thrombin	Releases insoluble fibrin, which combines to form a blood clot, from the soluble plasma protein fibrinogen.

Table 2.1 Plasma and cell-driven mediators (Continued).

List	Functions
Thrombin	In addition to causing nitric oxide and chemokine synthesis, thrombin may attach to cells via the PAR1 receptor to cause additional inflammatory reactions.
Cell-driven mediators	
Tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1)	Both cause comparable inflammatory responses in a wide range of cell types, including cytokine production, endothelial gene regulation, chemotaxis, leukocyte adherence, and fibroblast activation. cause of the systemic consequences of inflammation, including decreased appetite and accelerated heartbeat. Osteoblast differentiation is impeded by TNF-α.
Interleukin-6 (IL-6)	The pro-inflammatory cytokine that promotes inflammation released by macrophages in response to PAMPs, or pathogen-associated molecular patterns.
Nitric oxide	Moderate vasodilator; relaxes smooth muscle; decreases platelet aggregation; promotes leukocyte recruitment; and, at high quantities, has a direct antibacterial effect.

To fully understand the mechanisms underlying chronic inflammation, a comprehensive investigation of both plasma-driven and cell-driven mediators would be necessary. It's possible that these mediators interact with each other, and their

contributions may vary depending on the specific inflammatory condition or disease. In this study, we focus on evaluated the cell-driven mediators including IL-1β, IL-6, and TNF-α.

Inflammation mechanisms inside the body involve in multiple pathways including the activation of immune cells, production of inflammatory mediators, and recruitment of additional immune cells to the site of injury or infection follow as figure 2.1. These mechanisms are triggered by a variety of stimuli, including pathogens, toxins, and physical injury (Ahmed, 2011). The initial phase of inflammation is characterized by the release of cytokines and chemokines, such as IL-1β, IL-6, and TNF-α, which are produced by immune cells, including macrophages, dendritic cells, and mast cells. These cytokines and chemokines act as signaling molecules that attract other immune cells, such as neutrophils and monocytes, to the site of injury or infection. Once at the site of inflammation, these cells work to remove damaged tissue, debris, and invading pathogens (Lentsch, Kato, Yoshidome, McMasters and Edwards, 2000). The second phase of inflammation is characterized by the activation of immune cells, such as macrophages and dendritic cells, which phagocytose and destroy invading pathogens and other foreign particles. During this process, these cells also release additional cytokines and chemokines that recruit more immune cells to the site of inflammation. This leads to the formation of an inflammatory infiltrate, which is a complex mixture of immune cells, including neutrophils, monocytes, and lymphocytes (Pankuweit, Ruppert and Maisch, 2004). The third phase of inflammation is characterized by the resolution of the inflammatory response. This involves the removal of damaged tissue and the restoration of normal tissue function. The resolution of inflammation is controlled by several mechanisms, including the production of anti-inflammatory cytokines, such as IL-10 and TGF-β, which suppress the production of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α (Medzhitov, 2008).

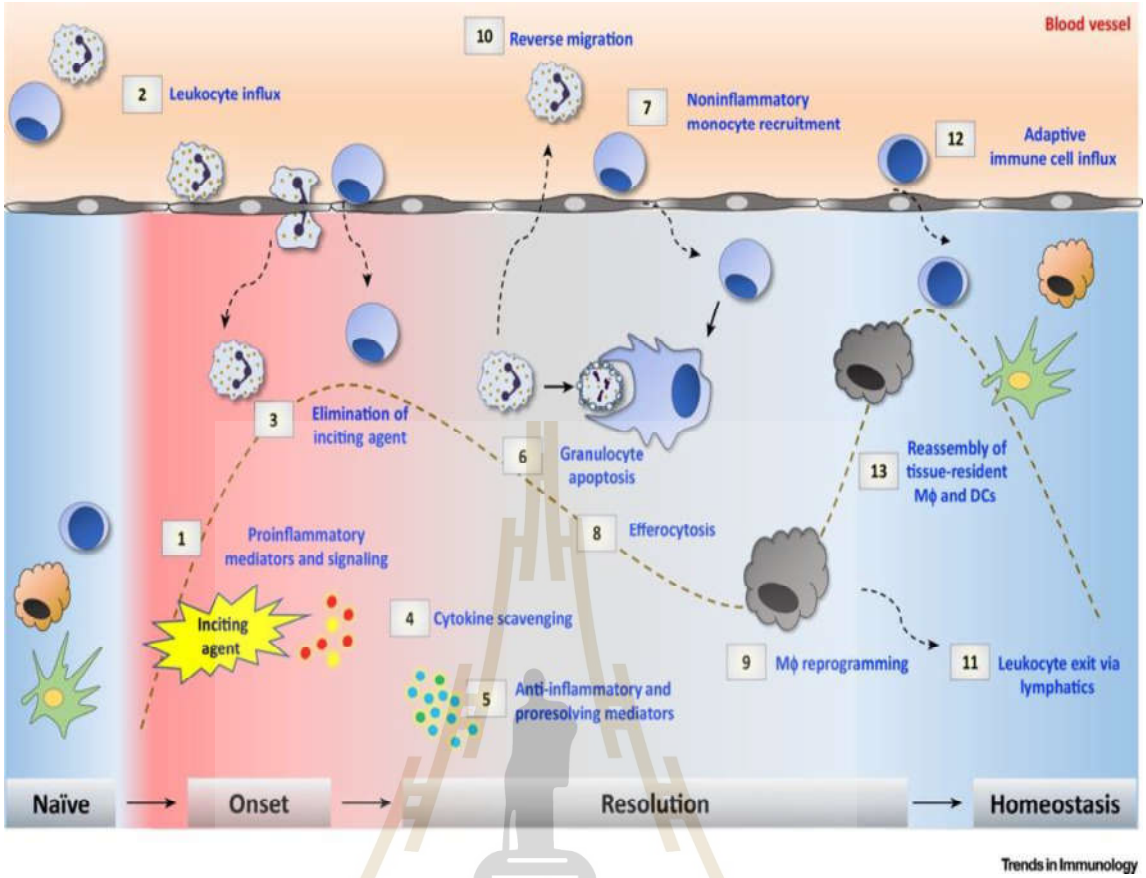


Figure 2.1 Substances that promote the resolution of the inflammatory processes (Sugimoto, Vago, Perretti and Teixeira, 2019).

In addition to cytokines and chemokines, other inflammatory mediators, such as nitric oxide (NO) and prostaglandins, also play a role in the inflammatory response. NO is produced by immune cells, such as macrophages and endothelial cells, and acts as a potent vasodilator, promoting blood flow to the site of inflammation. Prostaglandins are produced by various cells, including immune cells and damaged tissue, and contribute to the development of pain, fever, and swelling during inflammation.

Pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), and nitric oxide (NO), play a key role in the inflammatory response. These cytokines act as signaling molecules that attract immune cells to the site of inflammation, stimulate the production of other cytokines and chemokines, and regulate the function of immune cells (Yu, Kuan, Wong, Li and Tam, 2012).

IL-1β is a pro-inflammatory cytokine that is produced by immune cells, including macrophages, dendritic cells, and mast cells. It acts as a signaling molecule to attract other immune cells, such as neutrophils and monocytes, to the site of inflammation. In addition, IL-1β stimulates the production of other cytokines and chemokines, including IL-6 and TNF-α. A study published in the Journal of Immunology found that blocking IL-1β with a specific inhibitor reduced inflammation and tissue damage in a mouse model of rheumatoid arthritis (Joosten et al., 1999).

IL-6 is another pro-inflammatory cytokine that is produced by a variety of cells, including immune cells, endothelial cells, and fibroblasts. It acts as a signaling molecule to attract immune cells to the site of inflammation and stimulates the production of acute-phase proteins, such as C-reactive protein. IL-6 has been implicated in the pathogenesis of several inflammatory conditions, including rheumatoid arthritis and systemic lupus erythematosus. A study published in the Journal of Experimental Medicine found that blocking IL-6 with a specific inhibitor reduced inflammation and joint destruction in a mouse model of rheumatoid arthritis (Ohshima et al., 1998).

TNF-α is a pro-inflammatory cytokine that is produced by immune cells, including macrophages and T cells. It acts as a signaling molecule to attract other immune cells, such as neutrophils and monocytes, to the site of inflammation, and stimulates the production of other cytokines and chemokines, such as IL-1β and IL-6. TNF-α is involved in the pathogenesis of several inflammatory conditions, including rheumatoid arthritis and inflammatory bowel disease. A study published in the New England Journal of Medicine found that blocking TNF-α with a specific inhibitor reduced inflammation and joint destruction in patients with rheumatoid arthritis (Elliott et al., 1994).

Nitric oxide (NO) is a gas that is produced by immune cells, such as macrophages and endothelial cells, as well as neurons and other cells. It acts as a potent vasodilator, promoting blood flow to the site of inflammation. In addition, NO has been shown to play a role in the regulation of immune cell function and the production of other inflammatory mediators, such as cytokines and chemokines. (Wakabayashi, 1999).

NSAIDs (Nonsteroidal anti -inflammatory drugs) are commonly used to treat inflammation such as ibuprofen, Diclofenan, Naproxen and Aspirin (Bindu, Mazumder and Bandyopadhyay, 2020). However, they can cause severe side effects such as gastrointestinal bleeding and cardiovascular problems. As an alternative, there is growing interest in the use of natural plant-based treatments for inflammation. One such example is mushrooms, which have been found to possess anti-inflammatory properties. The extracts from various mushroom species have been shown to inhibit the production of pro-inflammatory cytokines and reduce inflammation in both animal and human studies (Assemie and Abaya, 2022) .

2.2 Medicinal mushroom

Medicinal mushrooms have been used for centuries in traditional medicine systems worldwide, particularly in East Asian cultures. In recent years, their therapeutic potential has gained scientific recognition, and several clinical studies have investigated their pharmacological properties. One of the most extensively studied medicinal mushrooms is the shiitake mushroom (*Lentinula edodes*). Shiitake mushrooms contain beta-glucans, polysaccharides that have immune-modulating effects and can enhance the body's ability to fight infections. Additionally, shiitake mushrooms contain ergothioneine, an antioxidant that may have protective effects against chronic diseases (Dai et al., 2015). Another commonly studied medicinal mushroom is the reishi mushroom (*Ganoderma lucidum*). Reishi mushrooms contain polysaccharides and triterpenoids that have been shown to have anti-inflammatory and antioxidant properties (Saylam Kurtipek, Ataseven, Kurtipek, Kucukosmanoglu and Toksoz, 2016). Clinical studies have suggested that reishi mushrooms may have a range of therapeutic effects, including immune system modulation, improved sleep quality, and reduced fatigue (Smith, Thayer, Malta and Utz, 1961). Other medicinal mushrooms that have been studied include *Cordyceps sinensis*, *Agaricus blazei*, and *Coriolus versicolor*. *Cordyceps sinensis* is known for its potential to improve athletic performance, while *Agaricus blazei* has been shown to have anti-cancer properties (Smith, Thayer, Malta and Utz, 1961). *Coriolus versicolor* contains polysaccharides that may have immune-

boosting effects and has been used as an adjuvant therapy in cancer treatment (Singdevsachan et al., 2016).

While the scientific evidence for the therapeutic potential of medicinal mushrooms is still emerging, their long history of use in traditional medicine systems and the growing body of research suggested that they may be a promising avenue for the development of novel therapies (Wasser, 2010). However, with any natural product, it is important to consult with a healthcare provider before using medicinal mushrooms as a treatment for any condition.

2.3 Mechanism of mushroom biomolecules for treating inflammation

There is growing interest in the potential of mushrooms and their bioactive compounds as natural remedies for inflammation and related disorders. Several studies have investigated the anti-inflammatory mechanisms of various bioactive compounds found in mushrooms, including beta-glucans, ergothioneine, triterpenoids, and phenolic compounds (Kozarski et al., 2023).

Beta-glucans are complex polysaccharides that are found in the cell walls of many mushrooms. They have been shown to have immunomodulatory effects, including the ability to activate immune cells such as macrophages and natural killer cells, and to reduce the production of pro-inflammatory cytokines (Vetvicka and Vetvickova, 2014). The study in 2012 anti-inflammatory effects of beta-glucans from mushrooms such as shiitake, reishi, and maitake, highlighting their potential as natural therapies for inflammatory disorders (Patel and Goyal, 2012). Ergothioneine is another important bioactive compound found in mushrooms, that is a potent antioxidant that has been shown to reduce oxidative stress and inflammation in the body (Mapelli et al., 2012). A study in 2017 reported that ergothioneine treatment reduced inflammation and oxidative stress by suppressing the production of pro-inflammatory cytokines and increasing the activity of antioxidant enzymes (Silva et al., 2017). Triterpenoids are a class of compounds found in many mushrooms that have been shown the potential of anti-inflammatory effects from investigated the anti-inflammatory activity of triterpenoids from the mushroom *Ganoderma lucidum*, triterpenoids inhibited the production of pro-inflammatory cytokines and reduced inflammation in a RAW 264.7

model (Wu et al., 2019). Moreover, phenolic compound extract from mushroom *Hericium erinaceu* has been reported that reduced the production of pro-inflammatory cytokines and inhibited the activity of inflammatory enzymes in a mouse model of acute liver injury (Hui Chen, 2015).

For this reason, the study on anti-inflammatory mechanisms of bioactive compounds extracted from edible mushrooms are needed to fully understand, the knowledge from this would be beneficial to develop the novel drug in the near future.

2.4 Bamboo mushroom

Bamboo mushrooms belong to the genus *Phallus* and are a group of fungi found in tropical and subtropical regions of Asia, Africa, and South America. These mushrooms have been used in traditional medicine and cuisine for centuries. The first recorded description of bamboo mushrooms dates back to the 18th century, with the species *Phallus indusiatus* being described in 1753 by the Swedish botanist Carl Linnaeus (Shukla, Singh, Tiwari and Ahirwar, 2016). The stage development of Bamboo mushroom like most fungi *Phallus* species have a complex life cycle that involves both sexual and asexual reproduction start at spore germination: The bamboo mushroom reproduces through spores, which are produced in the gills on the underside of the cap. When the spores are released into the environment, they need to find a suitable substrate to germinate. The ideal substrate for bamboo mushrooms is a mixture of sawdust, rice bran, and wheat bran then mycelial growth: Once the spores have found a suitable substrate, they begin to grow and form a network of fine, white filaments called mycelium. The mycelium is the vegetative part of the fungus and is responsible for absorbing nutrients from the substrate. Colonization of substrate: As the mycelium grows, it begins to colonize the entire substrate. This process can take several weeks, during which the substrate needs to be kept at the right temperature and humidity to ensure optimal growth. Primordia formation: After the substrate has been fully colonized by the mycelium, the bamboo mushroom begins to form primordia, which are the small, immature fruiting bodies of the mushroom. These primordia develop into the mature fruiting bodies that we eat. Fruiting body development: The primordia continue to develop and grow into the mature fruiting

bodies of the bamboo mushroom as figure 2.2. The fruiting bodies are typically long, thin, and cylindrical, with a characteristic frilly, skirt-like structure at the base. Harvesting: Once the fruiting bodies have reached maturity, they are ready to be harvested. Harvesting is typically done by hand, and the mushrooms are cut off at the base of the stem. The mushrooms are then cleaned, sorted, and packaged for distribution. Spore production: As the fruiting bodies mature, they also begin to produce spores, which are released into the environment and start the cycle over again (Dorr, Koegler, Gabrielle and Aubry, 2021; Miles and Chang, 2004; Smith, Thayer, Malta and Utz, 1961; Sridhar and Karun, 2013).

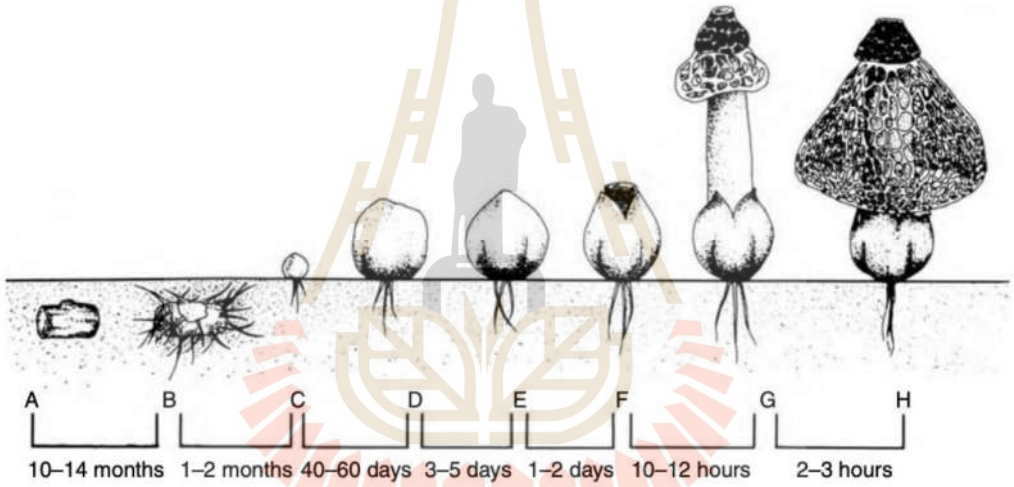


Figure 2.2 Different stages in growth and development of a fruiting body of *Dictyophora duplicata*: (A) inoculated log buried in soil, (B) development of rhizomorphs, (C) formation of primordium, (D) mushroom ball formed, (E) apical protrusion, (F) apical breakage and opening, (G) stalk elongation, and (H) mature fruiting body (Miles and Chang, 2004).

Figure 2.3 The morphology of *Phallus* sp. (Thailand) in the immature stage or egg stage (A) and fruiting body (B).

2.4.1 Utilization:

Bamboo mushrooms are highly valued for their unique flavor and texture and are commonly used in Asian cuisine. They can be used in a variety of dishes, including stir-fries, soups, stews, and curries. The delicate indusium, or veil, can also be used in various dishes, such as salads, to add a subtle flavor and texture. Several studies have been conducted to evaluate the nutritional composition of bamboo mushrooms. The studies found that bamboo mushrooms are a good source of protein, carbohydrates, dietary fiber, vitamins, and minerals. The study also found that bamboo mushrooms contain high levels of potassium, calcium, and phosphorus, which are essential for maintaining good health (Balan et al., 2022; Vivek and Venkitasamy, 2023). Moreover, in traditional Chinese medicine, bamboo mushrooms have been used to treat a variety of health conditions, including respiratory and digestive disorders, and as an immune system booster. Several studies have been conducted to evaluate the medicinal properties of bamboo mushrooms. It's have been reported of Thai strains of bamboo mushrooms extractions shown the significant of antioxidant, anti-inflammatory properties, anti-cancer properties in *invitro* experiments (Chantree,

Chumkiew, Jamklang and Martviset, 2022; Pak, Chen, Ma, Hu and Ji, 2021; Wu et al., 2020).

2.4.2 Species:

Bamboo mushrooms belong to the genus *Phallus*, which includes around 30 species which can be found worldwide especially, in Asia, Africa, America (Table 2.1). Interestingly, several species of bamboo mushrooms have been reported in Thailand, such as *Phallus indusiatus*, *Phallus merulinus, Phallus atrovolvatus*, and *Phallus ravenelii*. The finding of new mushroom species which related to the study of their bioactive compounds and their properties are required (Ainsworth, 2008; Chaiyama, Mau and Keawsompong, 2020; Hawkeswood and Sommung, 2019; Li, MORTIMER, Karunarathna, Xu and Hyde, 2014; Moreno, Khalid and Alvarado, 2009; Reid, 1975; Zhou et al., 2023).

Table 2.2 *Phallus* spp. and their potential medicinal properties.

Species	Activities	Founded regions	References
Phallus atrovolvatus	Antioxidant, immune modulating activities	Costa Rica, Thailand, Brazil, Australia, Myanmar	(Calonge, Kreisel and Mata, 2005; Chaiyama et al., 2020; Chaiyama, Mau and Keawsompong, 2020)
Phallus chiangmaiensis	-	Thailand	(Sommai, Khamsuntorn, Somrithipol, Luangsa-Ard and Pinruan, 2021)
Phallus cinnabarinus	-	Thailand	(Hawkeswood and Sommung, 2019)

Table 2.2 *Phallus* spp. and their potential medicinal properties (Continued).

Species	Activities	Founded regions	References
Phallus duplicatus		Thailand, Japan, North America, China	(Hosaka, 2012)
Phallus impudicus	-	Thailand, Japan, China, Taiwan, Myanmar, Europe	(Hosaka, 2012)
Phallus indusiatus	Antioxidant, Anti-inflammatory, Wound healing	Thailand, Japan, China, North America, Australia, Myanmar South America, India	(Hosaka, 2012; Lai et al., 2023; Nazir et al., 2021)
Phallus merulinus	-	India, Brazil, Thailand	(Braga-Neto and Baseia, 2014; Sridhar and Karun, 2013)
Phallus multicolor	-	Thailand, Australia, China, Indonesia, Sri Lanka, Malaysia, Papua New Guinea, Taiwan, Zaire	(Hosaka, 2012)
Phallus rubicundus	-	Thailand, Japan, Taiwan, Australia, China, India, West Indies, North America, Myanmar, Hawaii, Africa	(Hosaka, 2012)
Phallus rugulosus	-	Japan, Taiwan	(Hosaka, 2012)

2.4.3 Cultivation:

Bamboo mushrooms have traditionally been wild harvested, but there is growing interest in cultivating them due to their popularity as a delicacy and their potential medicinal properties. The cultivation of bamboo mushrooms has the potential to provide a sustainable and reliable source of mushrooms while also reducing the pressure on wild populations. Cultivation of bamboo mushrooms involves a series of steps, including substrate preparation, spawn inoculation, and fruiting body formation. The substrate used for cultivation is typically a mixture of various organic materials, such as sawdust, rice straw, and cottonseed hulls. The substrate is sterilized to kill off any competing microorganisms before being inoculated with the spawn of the desired mushroom species. After inoculation, the substrate is incubated under specific temperature and humidity conditions to promote the growth of mycelium, the vegetative part of the fungus. Once the mycelium has colonized the substrate, it is ready for fruiting body formation. Fruiting bodies are formed by exposing the colonized substrate to specific environmental conditions, including a drop in temperature and an increase in humidity. The cultivation of bamboo mushrooms requires careful management of environmental factors, such as temperature, humidity, and light, to promote optimal growth and fruiting. The timing and duration of each stage of cultivation, such as spawn inoculation and fruiting body formation, can also impact the quality and yield of the final product (Razin and Volkov, 2019).

Several studies have been conducted to evaluate the feasibility of cultivating bamboo mushrooms, and the results suggest that it is a viable option for producing high-quality mushrooms. Bunroj (2019) reported the cultivation of bamboo mushrooms was successful, and that the mushrooms had similar nutritional and medicinal properties to wild-harvested mushrooms. However, the study also noted that more research is needed to optimize cultivation practices and improve the overall efficiency of the process (Bunroj, 2019). In conclusion, the cultivation of bamboo mushrooms has the potential to provide a sustainable and reliable source of mushrooms while also reducing the pressure on wild populations. However, successful cultivation requires careful management of environmental factors and timing, and

more research is needed to optimize cultivation practices and improve the overall efficiency of the process.

2.4.4 Environmental Impact:

The wild harvesting of bamboo mushrooms has raised concerns about their impact on the environment, particularly in regions where they are heavily harvested. Several studies have been conducted to evaluate the ecological impact of bamboo mushroom harvesting, which there had a minimal impact on forest ecosystems when conducted sustainably (Langenberger, Cadisch, Martin, Min and Waibel, 2017).

Bamboo mushrooms are a diverse group of fungi with a long history of use in traditional medicine and cuisine. Several studies have been conducted to evaluate their nutritional and medicinal properties, as well as their cultivation and environmental impact. Moreover, the China strain of bamboo mushroom, especially *Phallus indusiatus* (*Dictyophora indusiata*) has been the subject of much study and research over the years, particularly in terms of its medicinal properties and potential health benefits. This strain is known for its high levels of polysaccharides, which have been shown to have immune-boosting and anti-tumor properties (Bai and Bai, 2021; Fu et al., 2015; Lai et al., 2023; Liao et al., 2015). In Thailand also found the edible bamboo mushroom strains and there is growing interested in studying and cultivating of this strain. However, Thai strains have not been studied as extensively as the China strain, there is preliminary studies have suggested that the Thai strain of bamboo mushroom may have antioxidant and anti-inflammatory properties and could potentially be used to help prevent or treat a range of health conditions, including cancer, diabetes, and cardiovascular disease (Chaiyama et al., 2020; Chaiyama, Mau and Keawsompong, 2020; Chantree, Chumkiew, Jamklang and Martviset, 2022). Promoting the cultivation of the Thai strain of bamboo mushroom is therefore seen as an important priority, both for its potential health benefits and to support local farmers and communities. Efforts are underway to study the optimal growing conditions for this strain, and to develop strategies for increasing its yield and availability.

Overall, while the China strain of bamboo mushroom has been in the focus of research attention, the recent discovery of the Thai strain highlights the importance

of continuing to explore the diversity of fungal species and the potential health benefits they provide.

2.5 Review of related studies

Bamboo mushrooms, also known as *Phallus* species, there are a type of edible fungi commonly found in Southeast Asia. A few studies have investigated the potential anti-inflammatory effects of bamboo mushrooms and their bioactive compounds on various biological systems, including RAW 264.7 cell line (Nazir et al., 2021).

Huang and faculties (2011) identified the chemical components of *Dictyophora indusiata* and evaluate their antibiotic properties, various extraction, and purification methods to isolate the compounds from the mushroom were used, and then analyzed their chemical structures using techniques such as gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy. The antibiotic activity of the compounds was also evaluated using standard methods such as the disc diffusion assay. The study found that albaflavenone was the major antibiotic compound in *Dictyophora indusiata*, and that it exhibited broad-spectrum activity against both Gram-positive and Gram-negative bacteria. The chemical analysis revealed that albaflavenone had a unique chemical structure with a fused bicyclic ring system and a long alkyl side chain, which may contribute to its antibiotic activity (Huang, Chen, Tian, Sun and Chen, 2011).

Fu and faculty (2015) evaluated the effects of the polysaccharide on the immune response of RAW 264.7 macrophages, a type of immune cell that plays a crucial role in the inflammatory response. Macrophages were treated with different concentrations of the polysaccharide and then measured various immune parameters, such as nitric oxide production, phagocytosis activity, and cytokine secretion. The structural features of the polysaccharide were evaluated using gel permeation chromatography and nuclear magnetic resonance spectroscopy. The study found that the polysaccharide from *Dictyophora indusiata* significantly increased the production of nitric oxide and cytokines in RAW 264.7 macrophages, suggesting the extraction provided an immunostimulatory effect. The polysaccharide also enhanced the phagocytic activity of the macrophages, indicating a potential role in promoting immune defense against

pathogens. The structural analysis revealed that the polysaccharide consisted of a complex arrangement of monosaccharides, which may contribute to its bioactivity (Fu et al., 2015).

Deng and faculty (2015) evaluate the effects of phosphorylation on the physiochemical and biological properties of polysaccharides from *Dictyophora indusiata*. The chemical modification was used to phosphorylate the polysaccharides, and then analyzed the resulting compounds using various techniques such as gel permeation chromatography, Fourier-transform infrared spectroscopy, and nuclear magnetic resonance spectroscopy. The biological activities of the phosphorylated polysaccharides were also evaluated, including their antioxidant and immunomodulatory properties. The study found that phosphorylation of the polysaccharides from *Dictyophora indusiata* improved their solubility and thermal stability and altered their structural features. The phosphorylated polysaccharides also exhibited enhanced antioxidant and immunomodulatory activities, as demonstrated by their ability to scavenge free radicals and stimulate the immune response of macrophages (Deng, Fu, Xu, Shang and Cheng, 2015).

Wang and faculties (2018) were isolated and characterized a bioactive polysaccharide from the fruiting bodies of *Dictyophora indusiata* and *Dictyophora echinovolvata*, two edible fungi found in China with potential health benefits. Various extraction and purification methods were used to isolate the polysaccharide, and then analyzed its structural features using techniques such as high-performance liquid chromatography and nuclear magnetic resonance spectroscopy. The bioactivities of the polysaccharide were also evaluated, including its antioxidant and immunomodulatory properties (Wang, Shi, Yin and Nie, 2018)

Nazir and faculties (2021) aimed to evaluate the effects of extracts from *Dictyophora indusiata* on in vitro wound healing models, including their ability to inhibit inflammation and stimulate collagen synthesis. The researchers used various extraction methods to prepare the extracts, and then analyzed their chemical compositions using techniques such as gas chromatography-mass spectrometry and high-performance liquid chromatography. The biological activities of the extracts were also evaluated, including their effects on cell viability, inflammation, and collagen

synthesis. The study found that extracts from *Dictyophora indusiata* exhibited high efficiency in promoting in vitro wound healing, as demonstrated by their ability to reduce inflammation and stimulate collagen synthesis. The extracts inhibited the activity of matrix metalloproteinase-2 (MMP-2), an enzyme that plays a key role in the breakdown of collagen, and thus contributed to the accumulation of collagen in the wound bed. The chemical analysis revealed that the extracts contained various bioactive compounds, including polysaccharides, fatty acids, and phenolic compounds, which may contribute to their wound healing properties (Nazir et al., 2021).

Overall, these studies suggest that bamboo mushrooms and their bioactive compounds may have potential anti-inflammatory effects on various biological systems, including the RAW 264.7 cell line. However, further research is needed to fully understand the mechanisms of action and potential therapeutic applications of these compounds.

CHAPTER III

MATERIALS AND METHODS

3.1 Bamboo mushroom samples

Two mushroom samples suspected of the China strain *(Phallus indusciatus*) which has a long indusium, and the short indusium Thai strain (*Phallus* sp.) were prepared. In this study, we used two stages of fresh bamboo mushrooms, i.e., egg stage and fruiting body for mushroom species identification and extraction, respectively. The egg stage samples were stored at -20°C until used and the fruiting body samples were dried at 50°C for 2 or 3 days to extract by ethanol and water.

3.2 Isolation of genomic DNA, PCR amplification of ITS regions, and DNA sequencing

3.2.1 Isolation of genomic DNA

A rapid, simple, and low-cost protocol for DNA extraction was followed with slight modifications from previous study that they modified protocol for extracting DNA from mushrooms and other fungal taxa that is faster, simpler, and more cost-effective than traditional methods. (Izumitsu et al., 2012). In this study we used a combination of physical disruption and chemical lysis to extract high-quality genomic DNA from a range of mushroom species, including *Phallus* sp. Thai strain *Phallus* sp. China strain. The procedure of the experiment as following this. First, 100 µl of TE buffer (1x, pH 8) was added to a 1.5 ml tube that contained the mycelium of mushroom samples that had been obtained using toothpicks during the egg phase. The tubes were subsequently microwaved twice for one minute each at 600W. The tube was then centrifuged for 5 minutes at 10,000 rpm after being kept at -20°C for at least 10 minutes. The PCR was carried out using the supernatants as templates

3.2.2 PCR amplification of ITS regions

PCR was performed using specific primers Dict_03 and Dict_04 obtained from a recent study that identified mushroom samples (*Dictyophora indusiata*) and also evaluated the wound healing activities (Nazir et al., 2021). These primers were designed from the internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), respectively, which are conservative non-coding sequences and present in high copy numbers. These primers have shown specificity for *Dictyophora indusiata* among other primers. The sequences of these primer show on the table 3.1. The PCR products were visualized on a 1.5% agarose gel and purified using a PCR purification kit.

Table 3.1 Primer sequences for sample amplification.

Sequence primer		Size
Dict 03 Forward	TGCCTGTTTGAGTGTCGTGA	20
Dict 03 Reverse	ACGGACGACGCAAGACTTAT	20
Dict 04 Forward	GGAAGTAAAAGTCGTAACAAGG	22
Dict 04 Reverse	TCCTCCGCTTATTGATATGC	20

To minimize pipetting errors and ensure consistency across multiple PCR reactions, a PCR master mix was prepared by combining water, buffer, dNTPs, primers, and Taq DNA polymerase in a single tube, plus an additional reaction's worth of the master mix to account for any loss during pipetting. The master mix was then distributed evenly among individual PCR tubes, and template DNA was added to each tube.

Table 3.2 Polymerized Chain Reaction condition for amplify isolated DNA.

Components	Volume (µl)
DNA template	1
Forward primer (10 µM)	0.5
Reverse primer (10 µM)	0.5
Mixed dNTPs (2.5 mM each)	0.5

Table 3.2 Polymerized Chain Reaction condition for amplify isolated DNA (Continued).

Components	**Volume (µl)**
10X $((NH_4))_2SO_4$ buffer	2.5
$MgCl_2$ (25 mM)	1.5
Taq DNA polymerase (5 U/µl)	0.25
Ultrapure water	18.25
Total volume	**25.00**

The PCR reactions were carried out in several cycles, beginning with an initial denaturation step at 94°C for 5 minutes. This was followed by 35 cycles of amplification, consisting of a 1-minute denaturation step at 94°C, 1-minute annealing step at temperatures between 50°C to 59°C (using a gradient temperature approach), and 1-minute extension step at 72°C. After completion of the cycles, a final extension step was carried out at 72°C for 10 minutes. The amplified DNA products were visualized by subjecting them to 0.2% (w/v) agarose gel electrophoresis.

3.2.3 DNA sequencing by using pGEM-T easy vector

Since the size of the PCR product is small, it is necessary to clone it into a vector. In this experiment, the PCR product was inserted into the pGEM-T easy vector (Promega, USA) and transformed into XL-1 blue competent cells. The resulting DNA products was sent for sequencing by Solgent Co. Ltd. (South Korea). The obtained nucleotide sequences were aligned using the Clustal Omega program and compared to sequences in the GenBank databases using BLAST analysis. Phylogenetic trees were constructed using the Molecular Evolutionary Genetics Analysis (MEGA) XI software.

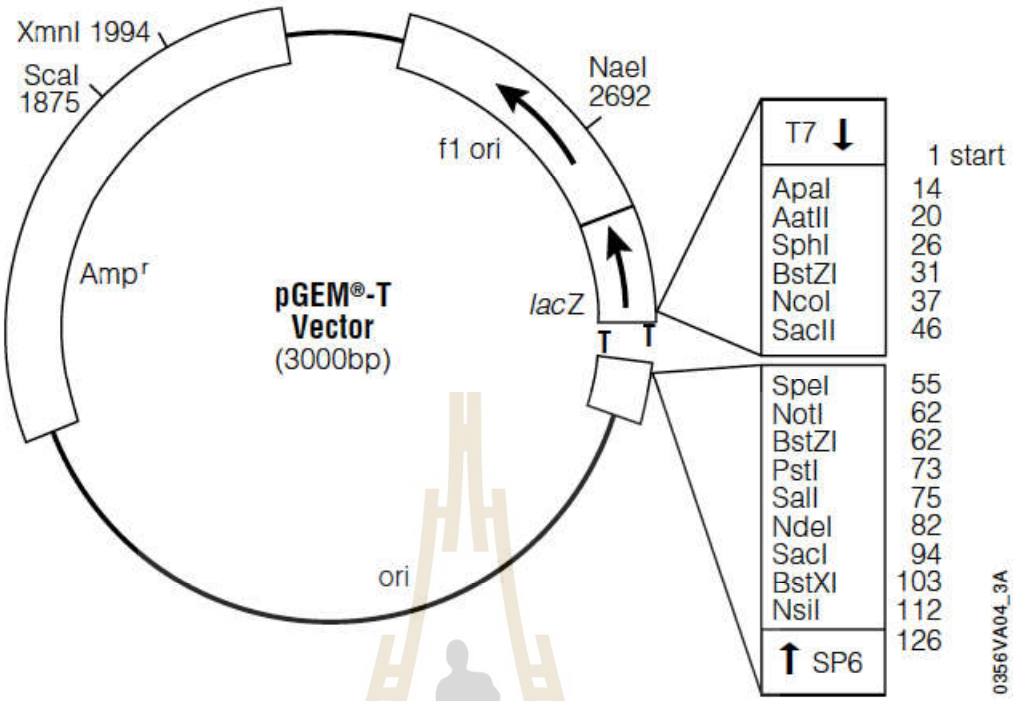


Figure 3.1 Convenient systems for the routine subcloning of PCR products. pGEM®-T Vector sequence reference points.

3.3 Phylogenetic analysis

The ITS sequences of *Phallus* sp. (CH0306, CH0407, TH0306, and TH0406) which were the samples strain amplified by primer Dict 03 and Dict 04 from selected colonies number 06, 07 were aligned with selected *Phallus* sp. and *Mutinus albotruncatus* using muscle alignments. The Neighbor-Joining method was used to generate the evolutionary history. The branches indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Each analysis involved 29 nucleotide sequences, and all ambiguous positions were removed for each sequence pair using the pairwise deletion option. The evolutionary analyses were conducted in MEGA11 (https://www.megasoftware.net).

Table 3.3 List of species, collection code, and GenBank accession number.

Species	Collection code (Voucher/Strain)	ITS GenBank acc. no
Mutinus albotruncatus	UFRN Fungos 2025	MF447826
Phallus atrovolvatus	INPA 240016	MG678531
Phallus aureolatus	ICN 176962^{T}	MF372135
Phallus campanulatus	ICN 176970	MF372138
Phallus chiangmaiensis	BCC 92055	MT452883
Phallus cinnabarinus	NPA 255835	KJ764821
Phallus coronatus	LE 295238	MG678522
Phallus denigricans	UFRN Fungos 2805	MG678485
Phallus dongsun	GDGM 29086	MN307394
Phallus echinovolvatus	GDGM 79020	MN523216
Phallus flavocostatus	RE 2004	MG67852
Phallus hadriani	AH 39161	KF481956
Phallus haitangensis	HKAS 88197^{T}	KU705383
Phallus impudicus	GDGM 77656	MN307393
Phallus indusiatus	INPA 264931	MG678500
Phallus hadriani	AH 39161	KF481956
Phallus haitangensis	HKAS 88197^{T}	KU705383
Phallus impudicus	GDGM 77656	MN307393
Phallus lutescens	GDGM 72218^{T}	MN131079
Phallus luteus	TNS Kasuya B 218	KP222543
Phallus mengsongensis	HKAS 78343^{T}	KF052624
Phallus merulinus	INPA 240010	MG678530
Phallus multicolor	MEL 2382891	KP012762
Phallus purpurascens	UFRN Fungos 2808 ^{T}	MG678487
Phallus rubrovolvatus	YZS 040	KF939503

Table 3.3 Species, Collection Code and GenBank Accession Number (Continued).

Species	Collection code (Voucher/Strain)	ITS GenBank acc. no
Phallus rugulosus	TNS F 46049	MF372142
Phallus serratus	HKAS 78340^{T}	KF052622
Phallus squamulosus	UFRN Fungos 2806^{T}	MG678497
Phallus ultraduplicatus	HMAS 253050^{T}	KJ591584

In this study, the ex-holotype strains are denoted by the letter "T". An outgroup, *Mutinus albotruncatus*, was also included.

3.4 Crude extracts preparation

To extract compounds from *Phallus* sp. fruiting bodies, the fruiting bodies were first dried at 50°C for 2-3 days and then ground into a powder at a concentration of 33g/L. The powder is then soaked in 95% ethanol for 7 days in an amber glass bottle. Additionally, a hot water extraction method was used in which the same powder mixed with distilled water at a ratio of 10:1 and heated to 95°C for 2 hours. The resulting solution is then centrifuged at 25°C, 3214 rcf for 10 minutes, and the supernatant was collected. Both the ethanol (ThE) and water (ThW) extracted samples were then filtered through filter paper and the extracts were freeze-dried and stored at -20°C until further used.

3.5 Cell culture and cell viability analysis

3.5.1 Cell culture

The RAW 264.7 cell line was used in this study. Cultured in DMEM containing 10% fetal bovine serum (FBS) and 1% anti-anti (a mixture of 100 IU/ml penicillin and 100 ug/ml streptomycin) to make the completed medium, at a temperature of 37°C and with 5% CO_2. When the cells reach 80% confluence in the flask, they were sub-cultured into new flasks by removing the old medium, rinsing with PBS (pH 7.4), and adding trypsin (0.025%,1X) to detach the cells for 5 min at 37°C in an environment containing 5% CO_2. The reaction was then stopped with completed medium (at twice

the volume of trypsin used). The cells were then centrifuged at 2,000 rpm for 5 min, the supernatant was discarded, and the cells were resuspended in completed medium and transferred to a new flask for further growth.

3.5.2 Cell viability analysis

The MTT assay was a method used to determine the percentage of viable cells in a culture treated with various concentrations of crude extracts from *Phallus* sp. To begin the process, 10,000 cells were seeded into each well of a 96-well plate and cultured at 37°C under 5% CO_2 for 24 hours. The cell quality was checked using an inverted light microscope before treating the cells with various concentrations of ethanol, ranging from 5% to 1% with a control group that concentration has no cytotoxicity was chosen for further experiment. The *Phallus* sp. ethanol crude extract was then dissolved in ethanol and used to treat the cells, while the water crude extracts are dissolved in Milli-Q water (18 MΩ-cm). The cells were then treated with different concentrations of the crude extracts for 24 or 48 hours include 10 conditions which 1000 µg/ml was highest concentration then 2-fold dilution and control.

After treatment, 20 µl of MTT solution (5 mg/ml filtered) was added to each well without mixing and incubated for 3 hours. The supernatant was aspirated, and 100 µl of DMSO is added to each well. The plate was then placed in a plate-reader for 10 minutes, and the absorbance at 562 nm was read. The cell viability was presented as a ratio following the equation, and the experiment is performed in triplicate. The IC50 value is calculated and three concentrations, including upper, equal, and lower than the IC50 value, are chosen for further experiments.

Equation:

$$\%\text{Cell viability} = \frac{(A_{sample} - A_{blamk})}{(A_{control} - A_{blank})} \times 100$$

Which A was the absorbance at 562 nm.

3.6 Anti-inflammatory effects determination

The purpose of this study was to investigate anti-inflammatory effect of *Phallus* sp. Thai strain by determining the levels of proinflammatory mediators such as TNF-α, IL-1β, IL-6 and nitric oxide (NO). To ensure proper comparison, four control groups were included in the experiment: a negative control group (not treated), a group treated with the highest concentration of crude extract without LPS, a group treated with LPS (0.5 μg/ml) only, and a group treated with standard medicine; dexamethasone (5 μg/ml) and LPS (0.5 μg/ml). Additionally, there were three experimental groups which were treated with three different concentrations of crude extract.

3.6.1 Nitric oxide assay

To determine nitric oxide (NO) concentration which produced by LPS-induce inflammation on RAW 264.7 macrophage cells. The Griess test was the technique chosen for evaluated NO concentration in this experiment. The protocol follow as the culture supernatant was first collected from the treated cells. Then, 50 μL of culture supernatant was mixed with 50 μL of Griess reagent (equal volume of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in water) in a 96-well plate. The mixture was incubated for 10 min at room temperature in the dark. The absorbance of the samples is measured at 540 nm using a microplate reader. The concentration of NO was determined using a sodium nitrite standard curve.

Note that the Griess test measures the concentration of nitrite, which was a stable oxidation product of nitric oxide. Therefore, the nitrite concentration was used as an indirect measurement of nitric oxide concentration.

3.6.2 Semiquantitative real-time RT-PCR

Semiquantitative real-time RT-PCR (reverse transcription polymerase chain reaction) was used to measure the mRNA expression of proinflammatory cytokines in the RAW 264.7 macrophage cells. Total RNA was extracted from the cells using the TRIzol reagent following the manufacturer's instructions. The concentration and purity of the RNA were determined by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer (nanodrop). The RNA was then reverse transcribed into

complementary DNA (cDNA) using a reverse transcription kit. The kit used oligo(dT) primers and random hexamers to initiate the reverse transcription reaction. The oligo(dT) primers selectively anneal to the poly(A) tail of mRNA molecules, while the random hexamers anneal to random regions throughout the RNA sample. This approach ensures that cDNA synthesis is not limited to specific transcripts and allows for a more comprehensive analysis of gene expression. The resulting cDNA was used as a template for the PCR amplification of the target genes and GAPDH.

The expression of target genes, including TNF-α, IL-1β, and IL-6, was measured by real-time PCR using the SYBR Green PCR master mix consist of specific primers for the target genes and GAPDH. The thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s. The fluorescence signal was monitored during the annealing step, and the cycle threshold (Ct) value was determined for each gene.

In this study, we used GAPDH as a reference gene for the semiquantitative real-time RT-PCR analysis. The GAPDH gene was commonly used as a reference gene due to its stable expression in various cell types and under different experimental conditions. The relative expression levels of the target genes were calculated using the comparative CT ($2^{-\Delta\Delta CT}$) method, with GAPDH as the reference gene. The PCR amplification efficiency was determined by the slope of the standard curve generated from a dilution series of cDNA samples. The specificity of the PCR amplification was confirmed by melting curve analysis and agarose gel electrophoresis of the PCR products. The results were presented as the mean ± standard deviation.

3.7 Statistic analysis

The data analysis was performed with three replicates and presented as the mean and standard deviation. To determine significant differences between the data groups, the one-way ANOVA with Dunnett's test was used with a significance level set at $p<0.05$. The SPSS Statistics Software (Chicago, IL, USA) was used for data analysis and using independent T-test to compare different value between two group condition which has one factor of interest.

CHAPTER IV
RESULTS AND DISCUSSIONS

4.1 Bamboo mushroom identification

Molecular phylogenetic analyses results based on sequences of the ITS1 and ITS2 gene regions shown in Figure 4.1, the results shown that Thai isolate bamboo mushroom species separately from other *Phallus* species indicated with high bootstrap values. The results shown that, *Phallus atrovolvatus* and *Phallus merulinus* which found in Thailand are closely related to our Thai isolation sample. Those three species had shared character as a short white indusium (Sommai et al., 2021). The results from phylogenetic analysis clearly shown the unsimilar between Thai-isolated *Phallus* (TH0306) and Chinese strain, *Phallus* sp. (CH0306), by high bootstrap values. However, the results shown the similarity between *Phallus* sp. (China strain) and *Phallus echinovalvotus* and *Phallus chiangmaiensis* which reported to find in North Thailand as Thai isolated (Nazir et al., 2021; Sommai, Khamsuntorn, Somrithipol, Luangsa-Ard and Pinruan, 2021). These suggested that our *Phallus* sp. Which we expected to be Chinese strain should be one of Thai strain. To confirm these assumption, there are more information needed such as mushroom morphology i.e., shape and surface configuration of the receptacle, the coloration of the receptacle, volva with rhizomorphs, the presence of an erect to curved sponge-like and hollow pseudo stipe, the size of the basidium, and the presence or absence of indusia (skirt-like structures) (Yan, Liu, Li and Wang, 2023).

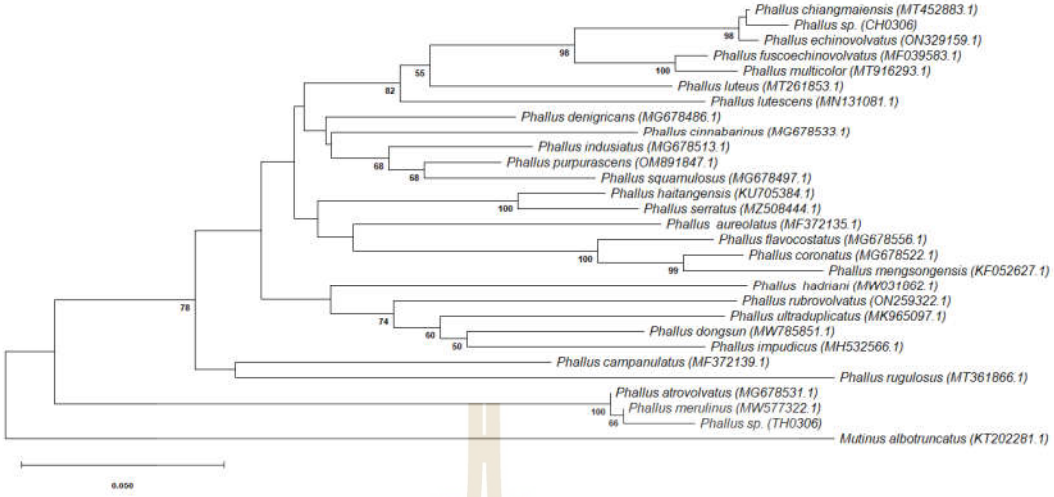


Figure 4.1 The phylogenetic relationship of Thai bamboo mushrooms (*Phallus* sp.; TH0306) was inferred from ITS-1 sequences. The number at the significant nodes represents the bootstrap value. *Mutinus albotruncatus* was used as the outgroup control.

4.2 Cytotoxicity of ethanol and water crude extract of *Phallus* sp. (Thai isolate) from fruiting body

Based on cell viability results from the MTT assay, it appears that both the ethanol and hot water crude extracts from *Phallus* sp. fruiting body were not cytotoxic to RAW 264.7 macrophage cells (Figure 4.2), even at the highest concentration (1,000 µg/ml). Additionally, the IC_{50} value was calculated to be more than 5,000 µg/ml, indicating that the extracts have no significant effect on cell viability at the tested concentrations.

These findings suggested that the extracts from *Phallus* sp. fruiting body may have potential for use in therapeutic applications, as they do not appear to have harmful effects on cells at concentrations that are higher than what would be expected to be used in such applications. However, it is important to note that further studies are needed to determine the safety and efficacy of these extracts *in vivo* and in human clinical trials. Our results show a similar trend with Nazir et al., 2021 studied, that even in high concentration of bamboo mushroom extracted (0.2 mg/ml and 2 mg/ml of bamboo mushroom extractions) would not have any toxicity to the normal cell line.

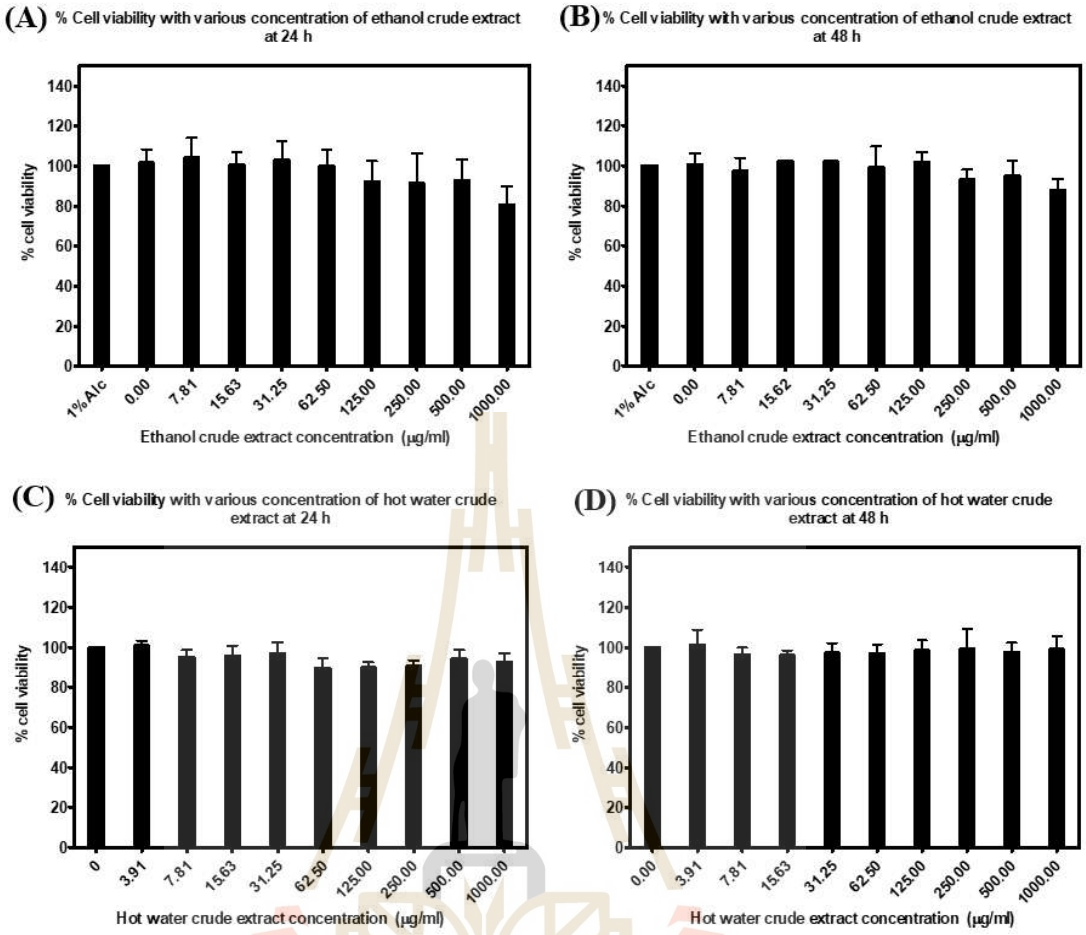


Figure 4.2 Cell viability of RAW 264.7 cells treated with various concentrations of TH0306 ethanolic crude extract at 24 hours (A) and 48 hours (B) and hot water crude extract at 24 hours (C) and 48 hours (D).

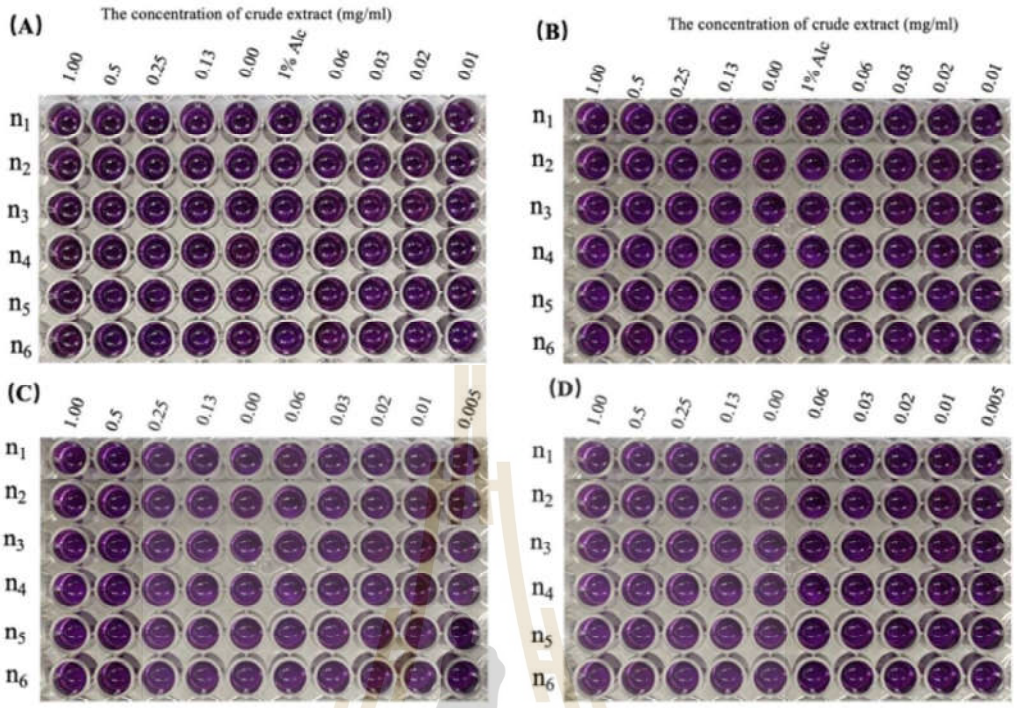


Figure 4.3 96-well plate of cell survival studies by MTT assay of various concentrations of ethanol crude extract incubated for 24 and 48 h, respectively (A, B) and various concentrations of aqueous crude extract incubated. for 24 and 48 hours respectively (C, D).

4.3 Nitric oxide analysis via Griess test

The result of the Griess test indicated that the crude extracts from *Phallus* sp. fruiting body did not induce any inflammation or reduce the production of nitric oxide (NO) in RAW 264.7 macrophage cells. This is demonstrated by the fact that the nitrite levels produced by LPS stimulation alone was significantly higher than those produced by crude extracts alone. However, when the crude extracts were combined with LPS, there was no significant difference in the nitrite levels compared to LPS stimulation alone. This suggests that the crude extracts did not have any inhibitory effect on the NO production induced by LPS, and therefore cannot be considered as a potential anti-inflammatory agent through NO cascade. It is worth noting that the positive control, dexamethasone, was able to significantly reduce the nitrite levels produced

by LPS stimulation, indicating its potent anti-inflammatory activity through the NO cascade (Figure 4.4).

Overall, the result suggested that the crude extracts from *Phallus* sp. fruiting bodies did not possess significant anti-inflammatory activity in this model system. The results of my experiment suggested that the crude extracts of *Phallus* sp. at the selected concentrations did not have a significant effect on reducing nitrite levels and, therefore, did not show anti-inflammatory properties through the NO cascade. One possible explanation for this result is that the extracts may not contain enough active compounds, or the concentrations used were not high enough to have an effect on NO production.

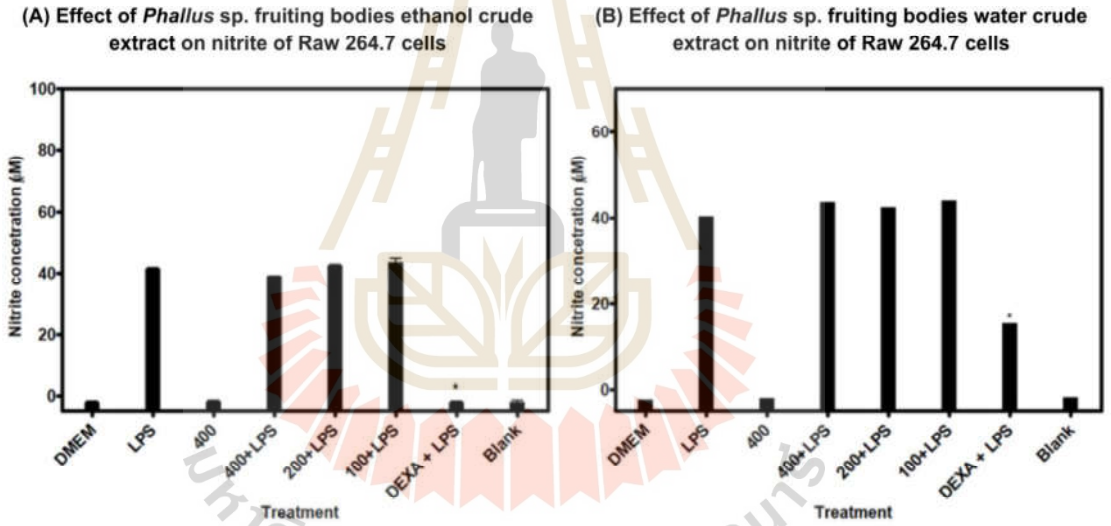


Figure 4.4 The nitrite concentration of RAW 264.7 cells was treated with various conditions. of ethanol crude extract (A, B) and hot water crude extract (C, D) *denotes a significant difference compared to the LPS-stimulated control group only at $p<0.05$.

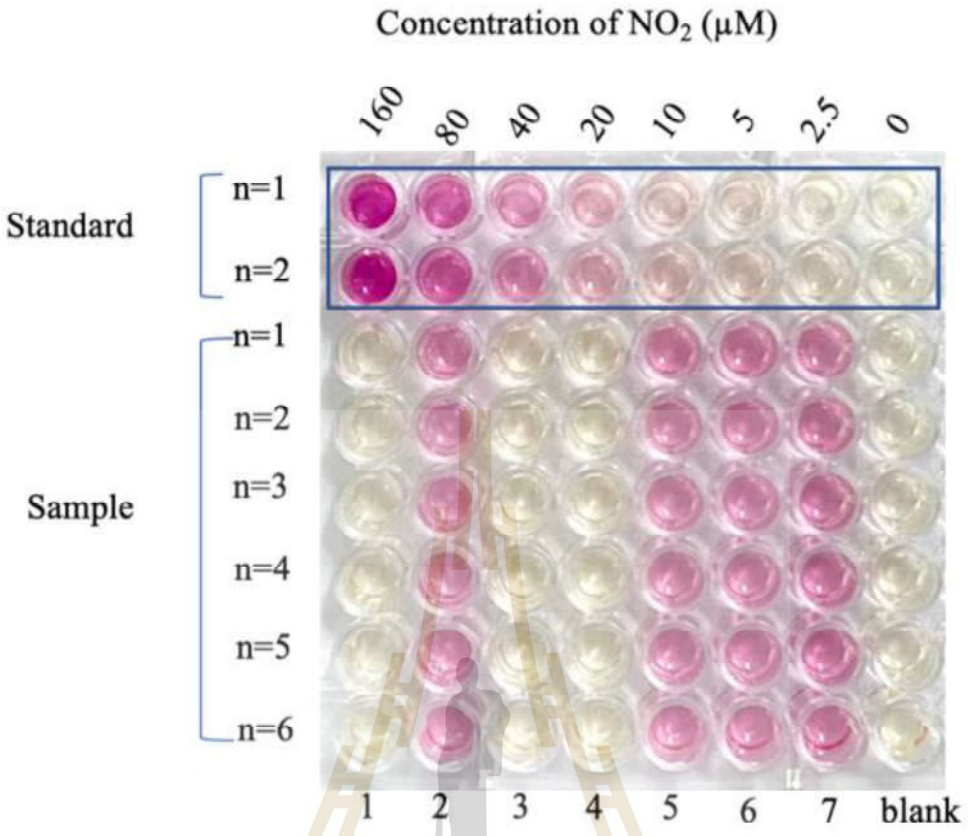


Figure 4.5 The Griess test determined the nitrite concentration from each treatment condition: (1) Control, (2) LPS (0.5 μg/ml), (3) Crude extract 400 μg/ml, (4) LPS plus dexamethasone (5 μg/ml), (5) LPS plus Crude extract 400 μg/ml, (6) LPS plus crude extract 200 μg/ml, and (7) LPS plus crude extract 100 μg/ml.

4.4 Relative mRNA level of inflammatory cytokine determined by semiquantitative real-time PCR

The pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α play a key role in the immune response and are often elevated in response to infection, injury, or other inflammatory stimuli. In this study were investigating whether the ethanolic crude extract of TH0306 could potentially reduce the expression of these pro-inflammatory cytokines in LPS-stimulated RAW 264.7 cells.

However, the results showed as in figure 4.6 no significant reduction of any of the cytokines (IL-1β, IL-6, and TNF-α) in the LPS plus ethanolic crude extract treated cells compared to LPS-treated cells alone. This suggests that at the selected concentration

(400 µg/ml), the TH0306 crude extract was not able to effectively modulate the expression of these cytokines in response to LPS stimulation.

The result is somewhat contradictory to a previous study on *P. indusiatus*, which reported that the ethanolic crude extract of mushroom eggs stage was able to significantly down-regulate pro-inflammatory cytokines (Nazir et al., 2021). However, it's important to note that the previous study used different parts of the mushroom and different concentration levels, so it's possible that the differences in results could be due to these factors.

Overall, this result suggests that further investigation is needed to determine the potential anti-inflammatory effects of *Phallus* sp. crude extract, and additional concentration levels should be explored. Additionally, other assays or methods for measuring inflammation could be considered to apply.

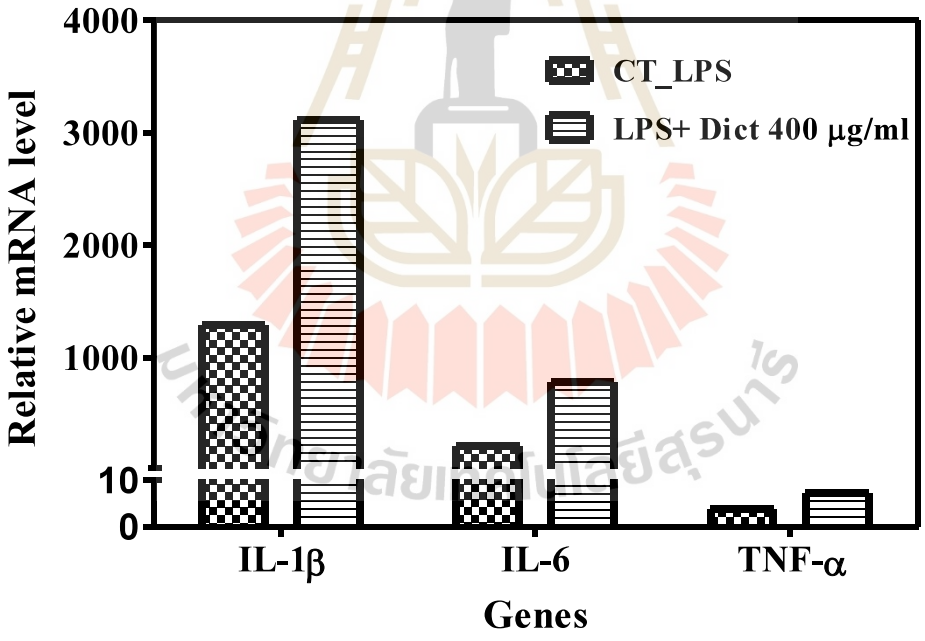


Figure 4.6 The relative mRNA expression levels of pro-inflammatory cytokines, including IL-1β, IL-6 and TNF-α in the only LPS treated condition and LPS plus 400 µg/ml of ethanolic crude extract treated RAW 264.7 cells.

CHAPTER V
CONCLUSIONS

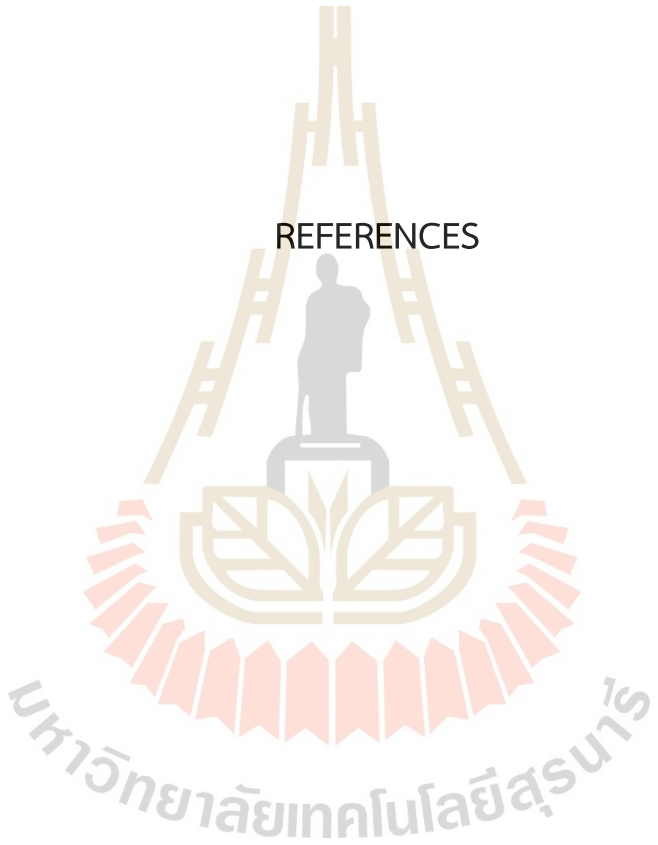
Based on the molecular phylogenetic analysis, the TH0306 was identified as different species from *Phallus indusiatus*. The MTT assay results showed that both the ethanol and hot water crude extracts from the fruiting body of *Phallus* sp. did not have cytotoxic effects on RAW 264.7 macrophage cells even at the highest concentration of 1000 µg/ml (the IC_{50} values were more than 5000 µg/ml).

The nitric oxide production assay using Griess reagent indicated that NO production was induced by LPS, but not by the crude extracts. This finding confirmed that the crude extracts were endotoxin-free without any inducible inflammatory properties. Unfortunately, the results from LPS plus crude extracts, both ethanolic and hot water crude extracts, in various concentrations existed comparative level to LPS alone. Significant levels of nitrite were detected in LPS plus dexamethasone (positive control) group only. Our result suggested that TH0306 crude extracts at these selected concentrations could not alter the nitrite level, which inferred that the reduction of inflammation through NO cascade could not be concluded.

The relative mRNA expression levels of pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α in the LPS-treated and LPS plus 400 µg/ml of ethanolic crude extract treated RAW 264.7 cells showed no significant reduction of all cytokines. Therefore, further investigations using different concentrations of TH0306 extracts are required.

The results from this study suggested that the crude extracts from both hot water and ethanolic extractions of *Phallus* sp. fruiting body did not exhibit cytotoxicity or anti-inflammatory effects on RAW 264.7 cells. Further studies are needed to explore the potential anti-inflammatory properties of different parts and concentrations of the mushroom extract.

REFERENCES



REFERENCES

Ahmed, A. U. (2011). An overview of inflammation: mechanism and consequences. *Frontiers in Biology. 6*(4), 274-281.

Assemie, A., and Abaya, G. (2022). The Effect of Edible Mushroom on Health and Their Biochemistry. *International Journal of Microbiology. 2022*, 874-882.

Bai, X.-w., and Bai, X.-f. (2021). Antioxidant activity of a polysaccharide from *Dictyophora indusiata* volva and MECC analysis of its monosaccharide composition. *Journal of the Indian Chemical Society. 98*(10), 140-146.

Balan, V., Zhu, W., Krishnamoorthy, H., Benhaddou, D., Mowrer, J., Husain, H., and Eskandari, A. (2022). Challenges and opportunities in producing high-quality edible mushrooms from lignocellulosic biomass in a small scale. *Applied Microbiology and Biotechnology. 106*(4), 1355-1374.

Bindu, S., Mazumder, S., and Bandyopadhyay, U. (2020). Non-steroidal anti-inflammatory drugs (NSAIDs) and organ damage: A current perspective. *Biochemical Pharmacology. 180*(1), 114-147.

Braga-Neto, C. R., and Baseia, I. G. (2014). A new species and new records of gasteroid fungi (Basidiomycota) from Central Amazonia, Brazil. *Phytotaxa. 183*(4), 239-253.

Bunroj, A. (2019). Bamboo fungus (*Phallus indusiatus*) cultivation using various fruit tree leaves beneath sponge gourd and bitter gourd holds. *PSRU Journal of Science and Technology. 4*(3), 48-57.

Calonge, F. D., Kreisel, H., and Mata, M. (2005). *Phallus atrovolvatus*, a new species from Costa Rica. *Journal of Agricultural and Food Chemistry. 29*, 777-778.

Chaiyama, V., Keawsompong, S., LeBlanc, J. G., de LeBlanc, A. d. M., Chatel, J.-M., and Chanput, W. (2020). Action modes of the immune modulating activities of crude mushroom polysaccharide from *Phallus atrovolvatus*. *Bioactive Carbohydrates and Dietary Fibre. 23*(5), 996-1003

Chaiyama, V., Mau, J.-L., and Keawsompong, S. (2020). Morphological characteristics, molecular identification and antioxidant activities of *Phallus atrovolvatus* (Agaricomycetes) isolated from Thailand. *International Journal of Medicinal Mushrooms. 22*(8), 743-753.

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), 33-42.

Coleman, D., King, R., and Andrade, J. (1974). The foreign body reaction: a chronic inflammatory response. *Journal of Biomedical Materials Research. 8*(5), 199-211.

Dai, X., Stanilka, J. M., Rowe, C. A., Esteves, E. A., Nieves Jr, C., Spaiser, S. J., Christman, M. C., Langkamp-Henken, B., and Percival, S. S. (2015). Consuming *Lentinulaedodes* (Shiitake) mushrooms daily improves human immunity: A randomized dietary intervention in healthy young adults. *Journal of the American College of Nutrition. 34*(6), 478-487.

Deng, C., Fu, H., Xu, J., Shang, J., and Cheng, Y. (2015). Physiochemical and biological properties of phosphorylated polysaccharides from *Dictyophora indusiata*. *International Journal of Biological Macromolecules. 72*, 894-899.

Dorr, E., Koegler, M., Gabrielle, B., and Aubry, C. (2021). Life cycle assessment of a circular, urban mushroom farm. *Journal of Cleaner Production. 288,* 655-668.

Eid, A. A., Ionescu, A. A., Nixon, L. S., Lewis-Jenkins, V., Matthews, S. B., Griffiths, T. L., and Shale, D. J. (2001). Inflammatory response and body composition in chronic obstructive pulmonary disease. *American journal of respiratory and critical care medicine. 164*(8), 1414-1418.

Elliott, M. J., Maini, R. N., Feldmann, M., Kalden, J. R., Antoni, C., Smolen, J., Leeb, B., Breedveld, F., Macfarlane, J., and Bijl, J. (1994). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *The Lancet. 344*(8930), 1105-1110.

Fu, H., Deng, C., Teng, L., Yu, L., Su, T., Xu, X., Chen, J., and Yang, C. (2015). Immunomodulatory activities on RAW 264.7 macrophages of a polysaccharide

from veiled lady mushroom, *Dictyophora indusiata* (higher basidiomycetes). *International Journal of Medicinal Mushrooms. 17*(2), 151-162.

Galvão, I., Sugimoto, M. A., Vago, J. P., Machado, M. G., and Sousa, L. P. (2018). Mediators of inflammation. *Immunopharmacology and Inflammation. 23*(2), 3-32.

Hawkeswood, T., and Sommung, B. (2019). First record of the stinkhorn fungus, *Phallus cinnabarinus* (WS Lee) Kreisel (1996)(Basidiomycota: Phallaceae) from Thailand. *Calodema. 711*, 1-3.

Hosaka, K. (2012). Preliminary list of phallales (phallomycetidae, basidiomycota) in Thailand. *Memoirs of the National Museum of Nature and Science. 48,* 81-89.

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.

Hui Chen, J. S., Yanchun Li, Yan Zhang, Yanhong Liang, Yiran Sun, and Honggao Wang. (2015). Anti-inflammatory effect of water-soluble polysaccharide from *Hericium erinaceus* against LPS-induced hepatic inflammation in mice. *Journal of Medicinal Food. 18*(3), 255-263.

Izumitsu, K., Hatoh, K., Sumita, T., Kitade, Y., Morita, A., Tanaka, C., Gafur, A., Ohta, A., Kawai, M., and Yamanaka, T. (2012). Rapid and simple preparation of mushroom DNA directly from colonies and fruiting bodies for PCR. *Mycoscience*. *53*(5), 396-401.

Johnson, A., and Day, R. (1991). The problems and pitfalls of NSAID therapy in the elderly (Part I). *Drugs & Aging. 1*, 130-143.

Joosten, L. A., Helsen, M., Saxne, T., van de Loo, F. A., Heinegård, D., and van den Berg, W. B. (1999). IL-1αβ blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-α blockade only ameliorates joint inflammation. *The Journal of Immunology. 163*(9), 5049-5055.

Kozarski, M., Klaus, A., van Griensven, L., Jakovljevic, D., Todorovic, N., Wan, W. A. A. Q. I., and Vunduk, J. (2023). Mushroom β-glucan and polyphenol formulations as natural immunity boosters and balancers: Nature of the application. *Food Science and Human Wellness. 12*(2), 378-396.

Lai, Y., Fang, Q., Guo, X., Lei, H., Zhou, Q., Wu, N., and Song, C. (2023). Effect of polysaccharides from *Dictyophora indusiata* on regulating gut microbiota and short-chain fatty acids in mice. *Journal of Food Measurement and Characterization. 17*(1), 1-11.

Langenberger, G., Cadisch, G., Martin, K., Min, S., and Waibel, H. (2017). Rubber intercropping: a viable concept for the 21st century? *Agroforestry Systems. 910*, 577-596.

Lentsch, A. B., Kato, A., Yoshidome, H., McMasters, K. M., and Edwards, M. J. (2000). Inflammatory mechanisms and therapeutic strategies for warm hepatic ischemia/reperfusion injury. *Hepatology. 32*(2), 169-173.

Li, H., MORTIMER, E. E., Karunarathna, S. C., Xu, J., and Hyde, K. D. (2014). New species of Phallus from a subtropical forest in Xishuangbanna, China. *Phytotaxa. 163*(2), 91–103.

Liao, W., Luo, Z., Liu, D., Ning, Z., Yang, J., and Ren, J. (2015). Structure characterization of a novel polysaccharide from *Dictyophora indusiata* and its macrophage immunomodulatory activities. *Journal of Agricultural and Food Chemistry. 63*(2), 535-544.

Mapelli, L., Canale, C., Pesci, D., Averaimo, S., Guizzardi, F., Fortunati, V., Falasca, L., Piacentini, M., Gliozzi, A., and Relini, A. (2012). Toxic effects of expanded ataxin-1 involve mechanical instability of the nuclear membrane. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1822*(6), 906-917.

Margraf, A., Lowell, C. A., and Zarbock, A. (2022). Neutrophils in acute inflammation: current concepts and translational implications. *Blood, The Journal of the American Society of Hematology. 139*(14), 2130-2144.

Mazumder, M., Roy, S., and Sarkar, A. K. (2022). Pharmacological and therapeutic value of bamboo mushroom Phallus indusiatus (Agaricomycetes). *Italian Journal of Mycology. 51*, 47-57.

Medzhitov, R. (2008). Origin and physiological roles of inflammation. *Nature. 454*(7203), 428-435.

Moreno, G., Khalid, A., and Alvarado, P. (2009). A new species of Phallus from Pakistan. *Mycotaxon. 108*(1), 457-462.

Murray, P. J., and Wynn, T. A. (2011). Protective and pathogenic functions of macrophage subsets. *Nature Reviews Immunology. 11*(11), 723-737.

Nathan, C., and Xie, Q.-w. (1994). Nitric oxide synthases: roles, tolls, and controls. *Cell. 78*(6), 915-918.

Nazir, Y., Linsaenkart, P., Khantham, C., Chaitep, T., Jantrawut, P., Chittasupho, C., Rachtanapun, P., Jantanasakulwong, K., Phimolsiripol, Y., and Sommano, S. R. (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), 1100-1118.

Ohshima, S., Saeki, Y., Mima, T., Sasai, M., Nishioka, K., Nomura, S., Kopf, M., Katada, Y., Tanaka, T., and Suemura, M. (1998). Interleukin 6 plays a key role in the development of antigen-induced arthritis. *Proceedings of the National Academy of Sciences. 95*(14), 8222-8226.

Pak, S., Chen, F., Ma, L., Hu, X., and Ji, J. (2021). Functional perspective of black fungi (*Auricularia auricula*): Major bioactive components, health benefits and potential mechanisms. *Trends in Food Science & Technology. 114*, 245-261.

Pankuweit, S., Ruppert, V., and Maisch, B. (2004). Inflammation in dilated cardiomyopathy. *Herz. 29*(8), 788.

Patel, S., and Goyal, A. (2012). Recent developments in mushrooms as anti-cancer therapeutics: a review. *3 Biotech. 2*, 1-15.

Razin, A., and Volkov, M. (2019). Modelling of cultivation conditions of *Phallus impudicus* to obtain mycelium with technological properties *Social Science and Humanity. 35*(9), 30-37.

Reid, D. A. (1975). A new species of Lindtneria (Basidiomycetes) from West Africa. *Kew Bulletin. 30*(4), 597-600.

Sanada, F., Taniyama, Y., Muratsu, J., Otsu, R., Shimizu, H., Rakugi, H., and Morishita, R. (2018). Source of chronic inflammation in aging. *Frontiers in Cardiovascular Medicine. 5*, 12.

Saylam Kurtipek, G., Ataseven, A., Kurtipek, E., Kucukosmanoglu, I., and Toksoz, M. R. (2016). Resolution of cutaneous sarcoidosis following topical application of *Ganoderma lucidum* (Reishi Mushroom). *Dermatology and Therapy. 6*(1), 105-109.

Shukla, A., Singh, A., Tiwari, D., and Ahirwar, B. K. (2016). Bambusicolous fungi: a reviewed documentation. *International Jounal of Pure & Applied Bioscience. 4*(1), 304-310.

Silva, F. C., Bramatti, I. C., Toledo, A. G., Salles, F. M., Itinose, A. M., and Marek, C. B. (2017). Antihyperglycemic effect of quercetin in ovariectomized rats treated with tamoxifen. *Journal of Medicinal Food. 20*(3), 235-242.

Singdevsachan, S. K., Auroshree, P., Mishra, J., Baliyarsingh, B., Tayung, K., and Thatoi, H. (2016). Mushroom polysaccharides as potential prebiotics with their antitumor and immunomodulating properties: A review. *Bioactive Carbohydrates and Dietary Fibre. 7*(1), 1-14.

Smith, L. G., Thayer, W. R., Malta, E. M., and Utz, J. P. (1961). Relationship of the Griess nitrite test to bacterial culture in the diagnosis of urinary tract infection. *Annals of Internal Medicine. 54*(1), 66-72.

Sommai, S., Khamsuntorn, P., Somrithipol, S., Luangsa-Ard, J. J., and Pinruan, U. (2021). *Phallus chiangmaiensis* sp. nov. and a Record of *P. merulinus* in Thailand. *Mycobiology. 49*(5), 439-453.

Sridhar, K., and Karun, N. (2013). On the basket stinkhorn mushroom *Phallus merulinus* (Phallaceae) in Mangalore, Karnataka, India. *Journal of Threatened Taxa. 9,* 3985-3988.

Sugimoto, M. A., Vago, J. P., Perretti, M., and Teixeira, M. M. (2019). Mediators of the resolution of the inflammatory response. *Trends in Immunology. 40*(3), 212-227.

Vetvicka, V., and Vetvickova, J. (2014). Immune-enhancing effects of Maitake (*Grifola frondosa*) and Shiitake (*Lentinula edodes*) extracts. *Annals of Translational Medicine. 2*(2), 14-20.

Vivek, K., and Venkitasamy, C. (2023). Role and applications of fungi in food and fermentation technology. In Joseph Heitman (Ed.), *Fungal Resources for Sustainable Economy: Current Status and Future Perspectives* (p.71-87). Singapore: Springer.

Wakabayashi, I. (1999). Inhibitory effects of baicalein and wogonin on lipopolysaccharide-induced nitric oxide production in macrophages. *Pharmacology & Toxicology. 84*(6), 288-291.

Wang, Y., Shi, X., Yin, J., and Nie, S. (2018). Bioactive polysaccharide from edible *Dictyophora* spp.: Extraction, purification, structural features and bioactivities. *Bioactive Carbohydrates and Dietary Fibre. 14*, 25-32.

Wasser, S. P. (2010). Medicinal mushroom science: history, current status, future trends, and unsolved problems. *International Journal of Medicinal Mushrooms. 12*(1), 1-16.

Wong, R. S. (2019). Role of nonsteroidal anti-inflammatory drugs (NSAIDs) in cancer prevention and cancer promotion. *Advances in Pharmacological and Pharmaceutical Sciences. 2019*, 1-10.

Wu, Q., Dai, T., Song, J., Liu, X., Song, S., Li, L., Liu, J., Pugazhendhi, A., and Jacob, J. A. (2020). Effects of herbal and mushroom formulations used in traditional chinese medicine on in vitro human cancer cell lines at the preclinical level: An empirical review of the cell killing mechanisms. *Process Biochemistry. 94*(1), 136-142.

Wu, Y.-L., Han, F., Luan, S.-S., Ai, R., Zhang, P., Li, H., and Chen, L.-X. (2019). Triterpenoids from *Ganoderma lucidum* and their potential anti-inflammatory effects. *Journal of Agricultural and Food Chemistry. 67*(18), 5147-5158.

Yan, Z., Liu, H., Li, J., and Wang, Y. (2023). Application of Identification and Evaluation Techniques for Edible Mushrooms: A Review. *Critical Reviews in Analytical Chemistry. 53*(3), 634-654.

Yao, C., and Narumiya, S. (2019). Prostaglandin-cytokine crosstalk in chronic inflammation. *British Journal of Pharmacology. 176*(3), 337-354.

Yu, S.-L., Kuan, W.-P., Wong, C.-K., Li, E. K., and Tam, L.-S. (2012). Immunopathological roles of cytokines, chemokines, signaling molecules, and pattern-recognition receptors in systemic lupus erythematosus. *Journal of Immunology Research. 2012*, 1-15.

Zhou, Z.-L., LI, Z., Lyu, Y., Liu, A., Gao, Y.-M., and Wang, P.-M. (2023). A new species of *Mutinus* (Phallaceae, Phallales) from Shandong Province, China. *Phytotaxa. 583*(2), 174–182.

CURRICULUM VITAE

Name Napaporn Mangmee

Date of Birth May 21, 1998

Place of Birth Nakhonsawan, Thailand

Education

2020-2022

M.Sc. in Environmental Biology

Suranaree University of Technology, Nakhon Ratchasima, Thailand

2016-2020

B.Sc. in Biology (Honor Program, First Class Degree Honor)

Suranaree University of Technology, Nakhon Ratchasima, Thailand

Publication Study on anti-inflammatory activities of ethanolic and water crude extracts (Proceeding in Second International Conference on TU Health Science (ICTUHS)