靜宜大學食品營養學系

碩士論文

Department of Food and Nutrition, Providence University Department of Agricultural Technology, Soegijapranata Catholic University Master Thesis

Jaboticaba 果實萃取物對於 LPS 誘導之斑馬魚胚胎的抗發炎活性

SITAS K

Anti-Inflammatory Activity of Jaboticaba Fruit Extract in Lipopolysaccharides (LPS)-induced Inflammation of Zebrafish Embryo



研究生:布蒂曼撰

Graduate student: Josephine Claretta Budiman

指導教授: 鍾雲琴博士

Advisor: Yun-Chin Chung, Ph.D.

Dr. B. Soedarini, MP

中華民國一百一十年七月 July, 2021

Anti-Inflammatory Activity of Jaboticaba Fruit Extract in Lipopolysaccharides (LPS)induced Inflammation of Zebrafish Embryo

Jaboticaba 果實萃取物對於 LPS 誘導之斑馬魚胚胎的抗發炎活性

MASTER THESIS

Submitted in partial fulfillment of the requirements for a Food Technology Master's degree in Faculty of Agricultural Technology

Semarang, July 20th. Semarang,

Dr. B. Soedarini, MP.

靜宜大學碩士論文審定書 Providence University

Master's Thesis Oral Defense Approval Form

食品營養學系碩士班

Graduate Program Department of Food and Nutrition

學生姓名: 布蒂曼 610860181

口試日期: 2021/7/16

(STUDENT)

(DATE)

論 文 題 目 : Jaboticaba 果實萃取物對於 LPS 誘導之斑馬魚胚胎的抗發炎活性 (THESIS TITLE) Anti-Inflammatory Activity of Jaboticaba Fruit Extract in LPS-

induced Inflammation of Zebrafish Embryo

審查委員簽名 Committee Members

經本委員會評審認可,本案符合碩士資格

召集人: 黃承舜

(COMMITTEE CONVENER)

口試委員: Christiana Retnaningsih

(EXAM COMMITTEE MEMBER)

指導教授: 鍾雲琴 (THESIS ADVISOR)

,

指導教授(共同指導): Soedarini

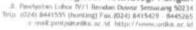
(THESIS ADVISOR)

系主任:

(CHAIRPERSON)

y. Chi chy 转款







THESIS DEFENSE REPORT MASTER PROGRAM OF FOOD TECHNOLOGY SOEGIJAPRANATA CATHOLIC UNIVERSITY

Date

: July 16, 2021

Time

: 13:00 am

Student's Name

Josephine Claretta Budiman

Student ID

19.13.0005

Thesis title

Anti-Inflammatory Activity of Jaboticaba Fruit Extract

In LPS-induced Inflammation of Zebrafish Embryo

Chair's Name (Advisor from SCU)

Advisor from RU

Dr. 14 Yun-Chin Chung

Commission Mombers

Dr. 阿米輝 Chen-Hatei Hwan

Dr. Christiana Retnamingsil

Please circle the exam result

Pass Pass with revision Fail

Committee Members Signature,

Chair Signature,

by Elin cg

HALAMAN PERNYATAAN PUBLIKASI KARYA ILMIAH UNTUK KEPENTINGAN AKADEMIS

Yang bertanda tangan dibawah ini:

Nama : Josephine Claretta Budiman

Program Studi : Teknologi Pangan

Fakultas : Teknologi Pertanian

Jenis Karya : Tesis

Menyetujui untuk memberikan kepada Universitas Katolik Soegijapranata Semarang Hak Bebas Royalti Nonekslusif atas karya ilmiah yang berjudul "Anti-Inflammatory Activity of Jaboticaba Fruit Extract in Lipopolysaccharides (LPS)-induced Inflammation of Zebrafish Embryo" beserta perangkat yang ada (jika diperlukan). Dengan Hak Bebas Royalti Nonekslusif ini Universitas Katolik Soegijapranata berhak menyimpan, mengalihkan media/formatkan, mengelola dalam bentuk pangkalan data (database), merawat, dan mempublikasikan tugas akhir ini selama tetap mencantumkan nama saya sebagai penulis / pencipta dan sebagai pemilik Hak Cipta.

Demikian pernyataan ini saya buat dengan sebenarnya.

Semarang, 20 Juli 2021

Yang menyatakan

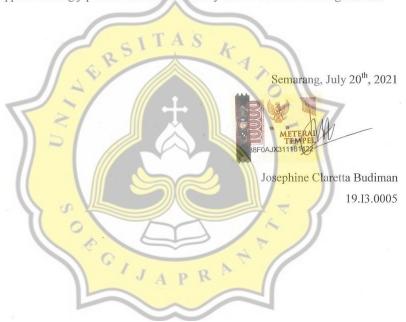
laretta Rudima

Josephine Claretta Budiman

STATEMENT OF THESIS AUTHENTICITY

I hereby declare that the thesis entitled "Anti-Inflammatory Activity of Jaboticaba Fruit Extract in Lipopolysaccharides (LPS)-induced Inflammation of Zebrafish Embryo" contains no work that ever proposed to acquire a bachelorship title in a University, and along to my knowledge, there is no work ever written or published by others, except the ones used as references in this thesis and mentioned in the list of references.

If it is proven in the future that partially or whole thesis is the result of plagiarism, therefore I am willing to be revoked with all the consequences in accordance with the law and regulations applied at Soegijapranata Catholic University and/or valid law and regulations.



SUMMARY

Inflammation can be defined as the immune system's response to harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation. Inflammation can be dangerous to human body because it may lead to various diseases. To help reduce inflammation, many people have been looking for natural sources of bioactive compounds. One of the examples is Myrciaria cauliflora (Jaboticaba), an edible fruit commonly found in Brazil. It contains considerable amount of polyphenols and other bioactive compounds which are beneficial for human health. Our previous studies showed the anti-inflammatory effect of Jaboticaba both in vitro and in vivo against COPD (Chronic Obstructive Pulmonary Disease) and IBD (Inflammatory Bowel Disease). To our knowledge, no publication on the anti-inflammatory effect of jaboticaba especially on the nerve protection, is available. Therefore, the purpose of this study is to examine the anti-inflammatory activity of jaboticaba fruit extract in LPSinduced zebrafish embryo. Jaboticaba peel and seed extracts were prepared by maceration in several concentrations of Ethanol and water. Zebrafish embryo is was used as model due to its ability to absorb the diluted small molecules in the surrounding water. Therefore, the bioactive compounds can be straightforward delivered to the targeted organs. Further, the effect of bioactive compounds can be easily observed due to the transparency of the embryos. Zebrafish embyros were divided into 6 groups, which are, LPS (Lipopolysaccharide)-induced group, LPS+Jaboticaba Peel Extract (JPE), LPS + Jaboticaba Seed Extract (JSE). The concentration of NO in zebrafish embryo is determined using Griess method, and the endocytosis of macrophage is observed using neutral red staining. While the proliferation of neutrophils is observed using Sudan Black staining. The result showed that jaboticaba fruit extract (especially the peel) may attenuate inflammation in a dose-dependent manner, indicated by reduction of both nitric oxide production and neutrophils proliferation. Extraction of jaboticaba peel is best done with 95% ethanol, followed by 50% ethanol and water. Although more work is needed to fully understand the critical role of jaboticaba on the inhibition of inflammation, our findings clearly demonstrate that jaboticaba may be a potential therapeutic intervention for the treatment of inflammatory disorders

ACKNOWLEDGEMENT

Praise the Lord because of His grace and blessing that the author able to complete the thesis

entitled: "Anti-Inflammatory Activity of Jaboticaba Fruit Extract in LPS-induced

Inflammation of Zebrafish Embryo". This thesis was written to fulfil the requirement to acquire

Master Degree of Food Technology in Soegijapranata Catholic University, Semarang,

Indonesia and Providence University, Taichung, Taiwan. The author would like to express

sincere gratitude to all people who have given support and guidance. They are:

1. Dr. R. Probo Y. Nugrahedi, STP., MSc. and Dr. Wang, Chiun-Chuang as the Dean of

Faculty of Agricultural Technology for giving the opportunity to finish the master

thesis.

2. Dr. B. Soedarini, MP and Dr. Chung, Yun-Chin (鍾雲琴) as supervisor for giving the

great guidance during the process.

3. All lecturers in Food Technology Department of Soegijapranata Catholic University

and Providence University for giving all great lessons during the learning activities

4. All laboratory and administration staffs for providing great services and information

during the learning activities.

5. Katharina Ardanareswari, STP, MSC as both laboratory partner and friend who always

support and accompany author during research period at Providence University.

6. Author's family and friends for always supporting and cheering for author.

The author realizes that there were unintended errors in writing this report. The author

really allows all readers to give suggestions to improve its content. However, the author

hopes that this report can be an inspiration and provide useful information for others.

Semarang, July 20th 2021

Author,

Josephine Claretta Budiman (布蒂曼)

ii

TABLE OF CONTENT

SUMMARY	1
ACKNOWLEDGEMENT	ii
TABLE OF CONTENT	iii
LIST OF FIGURES	v
LIST OF TABLES	vi
1. Introduction	1
1.1. Background	1
2. Literature Review	4
2.1. Inflammation	
2.1.1. Mechanism	4
2.2. Jaboticaba and Bioactives	5
2.2.1. Anti-inflammation activity of Jaboticaba	6
2.3. Zebrafish.	11
2.3.1. Advantages of Zebrafish Models	11
2.3.2. Cultivation and Breeding	11
2.3.3. Zebrafish Biology and Immunology	11
3. Material and Methods	
3.1. Preparation of Jaboticaba Seed and Peel Extract	13
3.2. Zebrafish Breeding	13
3.3. Zebrafish Grouping	14
3.4. LPS Zebrafish Treatment.	14
3.5. Nitric Oxide Determination (Griess Method)	15
3.6. Neutral Red Staining (NR)	15
3.7. Sudan Black Staining (SB)	15
4. Results	17
4.1. Nitric Oxide Determination	17
4.2. Macrophage Determination	20
4.3. Neutrophils Determination	24
4.3.1. Individual Size	24
4.3.2. Total Number of Neutrophil	24
4.3.3. Total Size of Neutrophil	24
	30

5.	Discussion	
6.	Conclusion	38
7	References	39



LIST OF FIGURES

Figure 1. Jaboticaba Fruit	6
Figure 2. Flowchart of Jaboticaba Extraction	
Figure 3. Breeding Box of Zebrafish	14
Figure 4. Determination of nitric oxide content in zebrafish	18
Figure 5. Representative pictures of neutral red stained zebrafish larvaes	
Figure 6. Determination of macrophages in zebrafish	22
Figure 7. Representative pictures of Sudan Black stained zebrafish larvaes	26
Figure 8. Evaluation of neutrophils individual size in zebrafish	27
Figure 9. Evaluation of neutrophils total number in zebrafish	30
Figure 10. Evaluation of neutrophils total size in zebrafish	



LIST OF TABLES

Table 1. Jaboticaba Bioactive Compounds	.8
Table 2. Nitric Oxide Determination in Zebrafish with Various Treatments of Jaboticaba	19
Table 3. Effect of Jaboticaba on Macrophage Staining of Zebrafish	. 23
Table 4. Effects of Jaboticaba on Individual Size of Zebrafish Neutrophils	. 28
Table 5. Percentage Inhibition of Jaboticaba Extract in Individual Size of Neutrophils	. 29
Table 6. Effect of Jaboticaba on Total Number of Zebrafish Neutrophils	. 31
Table 7. Percentage Inhibition of Jaboticaba Extract in Total Number of Neutrophils	. 32
Table 8. Effect of Jaboticaba on Total Size of Zebrafish Neutrophils	. 34
Table 9. Percentage Inhibition of Jaboticaba Extract in Total Size of Neutrophils	. 35

