BIOLOGICAL ACTIVITIES OF PHENOLIC COMPOUNDS EXTRACTED FROM SOME PLANT SPECIES FROM EAST KALIMANTAN PROVINCE, INDONESIA

Mrs. Franxisca Mariani

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy in Biological Sciences
Common Course
Faculty of Science
Chulalongkorn University
Academic Year 2018

แอกทิวิตีทางชีวภาพของสารประกอบฟีนอลิกที่สกัดจากพืชบางชนิดจากจังหวัดกาลิมันตันตะวันออก ประเทศอินโดนีเซีย

นางฟรานซิสก้า มาเรียนี่

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาวิทยาศาสตร์ชีวภาพ ไม่สังกัดภาควิชา/เทียบเท่า
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2561

Thesis Title	BIOLOGICAL ACTIVITIES OF PHENOLIC COMPOUNDS EXTRACTED FROM SOME PLANT SPECIES FROM EAST KALIMANTAN PROVINCE, INDONESIA
By	Mrs. Franxisca Mariani
Field of Study	Biological Sciences
Thesis Advisor	Associate Professor SEHANAT PRASONGSUK, Ph.D.
Thesis Co Advisor	Assistant Professor RACHANEEKORN TAMMACHOTE, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirement for the Doctor of Philosophy

..... Dean of the Faculty of Science
(Professor POLKIT SANGVANICH, Ph.D.)

DISSERTATION COMMITTEE

..... Chairman
(Assistant Professor KANOGWAN SERAYPHEAP, Ph.D.)

..... Thesis Advisor
(Associate Professor SEHANAT PRASONGSUK, Ph.D.)

..... Thesis Co-Advisor
(Assistant Professor RACHANEEKORN TAMMACHOTE, Ph.D.)

..... Examiner
(Assistant Professor WARINTHORN CHAVASIRI, Ph.D.)

..... Examiner
(Professor ALISA VANGNAI, Ph.D.)

..... Examiner
(Assistant Professor PONGCHAI HARNYUTTANAKORN, Ph.D.)

..... External Examiner
(Irawan Wijaya Kusuma, Ph.D.)

ฟรานซิสก้า มาเรียนี่ : แอกทิวิตีทางชีวภาพของสารประกอบฟีนอลิกที่สกัดจากพืชบางชนิดจากจังหวัดกาลิมันตันตะวันออก ประเทศอินโดนีเซีย. (BIOLOGICAL ACTIVITIES OF PHENOLIC COMPOUNDS EXTRACTED FROM SOME PLANT SPECIES FROM EAST KALIMANTAN PROVINCE, INDONESIA) อ.ที่ปรึกษาหลัก : รศ. ดร.สีหนาท ประสงค์สุข, อ.ที่ปรึกษาร่วม : ผศ. ดร. รัชนีกร ธรรมโชติ

พืชสมุนไพรสิบชนิดที่ใช้ในการศึกษานี้เป็นพืชที่พบบริเวณชนเผ่า Dayak เนื่องจากข้อมูลทางวิทยาศาสตร์เกี่ยวกับพืชเหล่านี้ยังมีจำกัด ดังนั้นวัตถุประสงค์ของการศึกษาครั้งนี้คือการวิเคราะห์องค์ประกอบของสาฟีนอลและสมบัติทางชีวภาพของตัวอย่างพืชเหล่านี้ พืชที่ได้รับการคัดเลือกถูกนำมาสกัดเพื่อแยกสารประกอบ โดยองค์ประกอบของสารประกอบฟีนอลและการต้านออกซิเดชั่นได้ถูกวิเคราะห์โดยใช้สาร Folin-Ciocalteu และวิธี phosphomolybdenum ตามลำดับ วิธี DPPH และ ABTS assays ถูกนำมาใช้วิเคราะห์การต้านทานการเกิดออกซิเดชั่น ส่วนการต้านทานเชื้อแบคทีเรียได้ทดสอบกับแบคทีเรียหกสายพันธุ์ด้วยวิธี agar well diffusion และวิธี microdilution สำหรับฤทธิ์การต้านไทโรซิเนสได้ใช้ L-tyrosine เป็นสารตั้งต้น และวิธี antidiabetic assay ได้ใช้ซูโครสและมอลโตสเป็นสารตั้งต้น เซลล์มะเร็งเต้านมของมนุษย์ (MDA-MB-231) ได้ถูกนำมาทดสอบฤทธิ์ต้านการเติบโตของเซลล์มะเร็ง ในขณะที่เซลล์เคราติโนไซต์ของมนุษย์ (HaCaT) ถูกนำมาทดสอบผลความเป็นพิษของเซลล์และฤทธิ์การรักษาบาดแผล ซึ่งซิลิกาเจลคอลัมน์โครมาโตกราฟีและ ^{1}H NMR ถูกนำมาใช้แยกสารประกอบ ผลการศึกษาพบว่า *Rhodomyrtus tomentosa* มีฤทธิ์ทางชีวภาพในวงกว้าง และ *Elaeocarpus submonoceras* สามารถนำไปใช้เป็นสารต้านการเกิดออกซิเดชั่น การต้านไทโรซิเนสและสารต้านเบาหวาน ในขณะที่ *Entada phaseoloides* สามารถนำมาเป็นสารต้านการเกิดออกซิเดชั่น การต้านไทโรซิเนสและการต้านมะเร็ง สำหรับ *Goniothalamus macrophyllus* และ *Pogostemon cablin* สามารถใช้เป็นสารต้านการเกิดออกซิเดชั่น และสารต้านมะเร็ง ในส่วน *Helicia robusta* และ *Litsea elliptica* สามารถนำไปใช้เป็นสารต้านการเกิดออกซิเดชั่น สารต้านเบาหวาน และสารต้านมะเร็ง กรดกัลลิก (gallic acid) สามารถสกัดจากใบ *E. submonoceras* ได้อย่างสมบูรณ์ จากการศึกษานี้พบว่าพืชสมุนไพรเหล่านี้มีศักยภาพเป็นยารักษาจากธรรมชาติและอาหารเสริมเพื่อสุขภาพ

สาขาวิชา วิทยาศาสตร์ชีวภาพ ลายมือชื่อนิสิต .....
ปีการศึกษา 2561 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

5672835523 : MAJOR BIOLOGICAL SCIENCES

KEYWORD: antioxidant, anti-tyrosinase, antidiabetic, anticancer, cytotoxicity

Franxisca Mariani : BIOLOGICAL ACTIVITIES OF PHENOLIC COMPOUNDS EXTRACTED FROM SOME PLANT SPECIES FROM EAST KALIMANTAN PROVINCE, INDONESIA. Advisor: Assoc. Prof. SEHANAT PRASONGSUK, Ph.D. Co-advisor: Asst. Prof. RACHANEEKORN TAMMACHOTE, Ph.D.

Ten medicinal plants used in this study are based on the experience of Dayak tribes. The scientific information about these plants are still limited, therefore, the objective of the present study was to analyze their phenolic content and biological activities. A selected species was subjected to successive extraction and compound isolation. The total phenolic content and total antioxidant capacity were analyzed by using Folin-Ciocalteu reagent and the phosphomolybdenum method, respectively. DPPH and ABTS assays were used for analyzing antioxidant activities. The antibacterial activity was performed against six bacterial strains by agar well diffusion and microdilution methods. The anti-tyrosinase activity used L-tyrosine substrate, whereas the antidiabetic assay used sucrose and maltose substrates. Human breast cancer cells (MDA-MB-231) were used for the anticancer activity test, while human keratinocyte cells (HaCaT) were used for the cytotoxicity effect and wound healing activity. Silica gel column chromatography and ^{1}H NMR were performed for compound isolation. The results showed that *Rhodomyrtus tomentosa* had broad-bioactivities, *Elaeocarpus submonoceras* could be applied as antioxidant, anti-tyrosinase, and antidiabetic agents, while *Entada phaseoloides* as antioxidant, anti-tyrosinase, and anticancer agents. *Goniothalamus macrophyllus* and *Pogostemon cablin* could be used as antioxidant and anticancer agents. Lastly, *Helicia robusta* and *Litsea elliptica* is applicable as antioxidant, antidiabetic, and anticancer agents. The gallic acid was successfully isolated from *E. submonoceras* leaves extract. These findings suggest that these plants are potential as natural medicine and health supplement.

Field of Study:	Biological Sciences	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....
		Co-advisor's Signature .....

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to:

Jesus Christ for His grace and kindness in strengthening me throughout my life.

My thesis advisor Assoc. Prof. Sehanat Prasongsuk, Ph.D. and co-advisor Asst. Prof. Rachaneekorn Tamachoote, Ph.D. for all their suggestions, guidance, patience, and encouragement throughout my study.

Asst. Prof. Warinthorn Chavasiri, Ph.D. for the guidance and inputs throughout my chemistry research and as one of my thesis committees.

Irawan Wijaya Kusuma, Ph.D. for the plant material and valuable comments regarding my research and his valuable time to come to my defense as the external committee.

Prof. Alisa Vangnai, Ph.D. and Asst. Prof. Pongchai Harnyuttanakorn, Ph.D. as my thesis committees and for the thesis evaluations and insightful suggestions.

Asst. Prof. Kanogwan Seraypheap, Ph.D. as a chairperson in my thesis defense.

Seminar Class Committees of Biological Sciences Program including Asst. Prof. Nipada Ruankaew Disyatat, Ph.D. and Asst. Prof. Sittiporn Phatdadilokrat, Ph.D. for the valuable inputs on my presentation that changes me positively.

Asst. Prof. Dr. Pongtharin Lotrakul for all supports since the beginning of the collaboration between Mulawarman University and Chulalongkorn University.

Administrative officers of the Biological Sciences Program, Botany Department, and Faculty of Sciences for their help during this degree study.

Hastaniah, M.Agr. and Paulus Matius, Ph.D. from Mulawarman University for the identification of plants and valuable information regarding Temula village plants.

Assoc. Prof. Pattamawadee Yanatatsaneejit, Ph.D. and Assoc. Prof. Pithi Chanvorachote, Ph.D. for human cells contribution.

Rico Ramadhan, Ph.D. for the contribution in antidiabetic study.

Benjawan Yanwisetpakdee, Ph.D., Ponlada Permpornsakul, Ph.D., Wichanee Bankeree, Ph.D., Siraprapa Saraihom, Ph.D., Taweeporn Sooksai, Ph.D., Sorawit Na Nongkhai, M.Sc., and all members of Plant Biomass Utilization Research Unit for the

idea, advice, and scientific techniques particularly in microbiology study.

Monthira Suntiparpluacha, Ph.D., Juthamas Sitthikornpaiboon, M.Sc., and all members of Division of Genetics (306 RTC laboratory) for the supports and scientific techniques particularly in human cells study.

Asshaima Paramita Devi, M.Sc., one of my best friends and like a sister that always support and encourage throughout my Ph.D. study. Suekanya Jaruinthusophon, Ph.D., Thuc-Huy Duong, Ph.D., and all members in Center of Excellence in Natural Product Chemistry for the scientific techniques particularly in chemistry study.

Athiwat Tilapornputt, a man who is helpful to all students and works as a laboratory assistant in Botany building.

ASEAN scholarship, Chulalongkorn University from 2013 to 2016 and Kalimantan Timur Cemerlang Grant, Indonesia 2017 which was supported financially.

Special thank to Khun Yai Som and Golf who are like grandma and daddy of my second son in Bangkok, also thank Pi' Bit, Khun Yai Nut, Pi' On, Pi' Gate, Pi' Pe, and Khun Yai Pim at Rong Rian Chalunee.

Lastly, my husband Ramaon Dearnov Saragih that always support me unconditionally for everything that I need and for our adorable sons (Jojo & Efra) who are always patient in all my activities to fulfill my study. My parents, sisters, brothers, and friends that always encourage and pray for me.

Franxisca Mariani

TABLE OF CONTENTS

Page

ABSTRACT (THAI) iii

ABSTRACT (ENGLISH) iv

ACKNOWLEDGEMENTS v

TABLE OF CONTENTS vii

LIST OF TABLES xi

LIST OF FIGURES xii

LIST OF SCHEMES xiii

LIST OF ABBREVIATIONS xiv

CHAPTER I INTRODUCTION 1

1.1 Background and rationale 1

1.2 Objective 3

1.3 Expected benefit and application 3

1.4 Research framework 3

CHAPTER II LITERATURE REVIEW 5

2.1 Plant description 6

2.1.1 *Baccaurea macrocarpa* (Miq.) Müll. Arg. 7

2.1.2 *Cinnamomum parthenoxylon* (Jack) Meisn 7

2.1.3 *Elaeocarpus submonoceras* Miq. 8

2.1.4 *Entada phaseoloides* (L.) Merr 9

2.1.5 *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson 10

2.1.6 *Gynura crepidioides* Benth 11

2.1.7 *Helicia robusta* (Roxb.) R. Br. ex Blume 12

2.1.8 *Litsea elipptica* Blume 13

2.1.9 *Pogostemon cablin* (Blanco) Benth. 14

2.1.10 *Rhodomyrtus tomentosa* (Aiton) Hassk. 15

2.2. Phenolic compounds 16

2.3 Biological activities 17

2.3.1 Antioxidants Agent 17

2.3.2 Antibacterial Agent 18

2.3.3 Tyrosinase Inhibitor 19

2.3.4 α-glucosidase Inhibitor 19

2.3.5 Anticancer Agent 21

2.3.6 Cytotoxicity Effect and Wound Healing Properties 21

CHAPTER III MATERIALS AND METHODS 23

3.1 Plant materials 23

3.2 Ethanol extracts preparation 23

3.3 Total phenolic contents (TPC) assay 24

3.4 Total antioxidant capacity (TAC) assay 25

3.5 Antioxidant activities assay 25

3.5.1 DPPH (2.2-diphenyl-1-picrylhydrazyl) inhibition assay 25

3.5.2 ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) inhibition assay 26

3.6 Antibacterial activity assay 27

3.7 Anti-tyrosinase activity assay 28

3.8 Antidiabetic activity assay 28

3.9 Anticancer activity assay 28
3.10 Cytotoxicity effect assay 29
3.11 Isolation of compound from *E. submonoceras* Miq. 29
3.11.1 Successive extraction and column chromatography 29
3.11.2 Structural identification 31
3.11.3 Wound healing assay 31
3.12 Statistical analysis 32
CHAPTER IV RESULTS AND DISCUSSION 33
4.1 Percent yield of ethanol extract 33
4.2 TPC, TAC, DPPH, and ABTS of 10 ethanol extracts 34
4.3 Antibacterial activity 39
4.4 Anti-tyrosinase activity 43
4.5 Antidiabetic activity 46
4.6 Anticancer activity 48
4.7 Cytotoxicity effect 50
4.8 Isolation of compound from *E. submonoceras* Miq. and the bioactivities 51
4.8.1 Structural identification of isolated compound 53
4.8.2 Yield, TPC, TAC, DPPH, and ABTS 54
4.8.3 Antibacterial activity 57
4.8.4 Anti-tyrosinase activities 60
4.8.5 Antidiabetic activity 61
4.8.6 Cytotoxicity effect 63
4.8.7 Wound healing activity 64
CHAPTER V CONCLUSION 68

Proposal for future research 69

REFERENCES 70

APPENDIX 83

VITA 84

LIST OF TABLES

Page

Table 4.1 Yield of leaf ethanol extracts from 10 medicinal plants from East Kalimantan province, Indonesia. 34

Table 4.2 TPC, TAC, DPPH and ABTS inhibitions of ethanol extracts 37

Table 4.3 Percentage of inhibition, MIC, and MBC of ethanol extracts against Gram-positive bacteria 40

Table 4.4 Percentage of inhibition, MIC, and MBC of ethanol extracts against Gram-negative bacteria 41

Table 4.5 Anti-tyrosinase activity of 10 ethanol extracts 44

Table 4.6 Percentage of antidiabetic activity of 10 ethanol extracts. 47

Table 4.7 Yield, TPC, TAC, DPPH, and ABTS of *E. submonoceras* Miq. leaves. 56

Table 4.8 Percentage of inhibition, MIC, and MBC of *E. submonoceras* Miq. successive extracts against Gram-positive bacteria 58

Table 4.9 Percentage of inhibition, MIC, and MBC of *E. submonoceras* Miq. successive extracts against Gram-negative bacteria 59

Table 4.10 Tyrosinase inhibition of *E. submonoceras* Miq. 61

Table 4.11 Antidiabetic activity of *E. submonoceras* leaves 62

LIST OF FIGURES

Page

Figure 2.1 Map of Temula village (source: Google map) 6
Figure 2.2 *Baccaurea macrocarpa* (Miq.) Müll. Arg. 7
Figure 2.3 *Cinnamomum parthenoxylon* 8
Figure 2.4 *Elaeocarpus submonoceras* Miq. 9
Figure 2.5 *Entada phaseoloides* (L.) Merr. 10
Figure 2.6 *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson 11
Figure 2.7 *Gynura crepidioides* Benth. 12
Figure 2.8 *Helicia robusta* (Roxb.) R. Br. var. *robusta* 13
Figure 2.9 *Litsea elipptica* Blume 14
Figure 2.10 *Pogostemon cablin* (Blanco) Benth. 15
Figure 2.11 *Rhodomyrtus tomentosa* (Aiton) Hassk. 16
Figure 2.12 Bacteria cell structure 18
Figure 2.13 The brush-border location in intestine (www.britannica.com) 20
Figure 2.14 MDA-MB-231 cells 21
Figure 2.15 HaCaT, the human keratinocyte cells 22
Figure 4.1 IC_{50} of anticancer activity against MDA-MB-231 49
Figure 4.2 IC_{50} of cytotoxicity effect against HaCaT. 51
Figure 4.3 ^{1}H NMR of purified compound (gallic acid) 54
Figure 4.4 Cytotoxicity effect of *E. submonoceras* Miq against HaCaT 63
Figure 4.5 Wound healing activity of *E. submonoceras* Miq. 65
Figure 4.6 HaCaT migration 66
Figure A.1 Standard curve of gallic acid 83
Figure A.2 Standard curve of ascorbic acid 83

LIST OF SCHEMES

Page

Scheme 1.1 Flowchart of research..... 4

Scheme 3.2 *E. submonoceras* Miq. successive extraction..... 31

Scheme 4.1 The process of isolation of gallic acid by antioxidant-assay-guided from *E. submonoceras* Miq..... 52

LIST OF ABBREVIATIONS

α	alpha
AAE	ascorbic acid equivalent
ABTS	2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) diammonium salt
ANOVA	one-way analysis of variance
APS	autoimmune polyglandular syndrome
ATCC	American Type Culture Collection
β	beta
°C	degree Celsius
<C10	short (less than ten) alkyl chain esters
$(CD_3)_2CO$	deuterated acetone
CO2	carbon dioxide
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	dimethyl sulfoxide
DOX	doxorubicin
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW	dried weight
ECM	extracellular matrix
FBS	fetal bovine serum
g	gram
GAE	gallic acid equivalent
^{1}H NMR	proton nuclear magnetic resonance
h	hour
HaCaT	human keratinocyte cells
HepG2	liver hepatocellular carcinoma cells
HIV	human immunodeficiency virus
%I	percentage of inhibition

IC_{50}	half-maximum inhibition concentration
KCCM	Korean Culture Center of Microorganisms
m	meter
M	molar
MBC	minimum bactericidal concentration
MCF-7	human breast cancer cells with positive oestrogen receptor (ER)
MDA-MB-231	human breast cancer cells with triple-negative (ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2))
µg	microgram
mg	milligram
MHz	megahertz
MIC	minimum inhibitory concentration
min	minute
µL	microliter
mL	milliliter
mm	millimeter
µM	micromolar
mM	millimolar
N	normality
n	number of replications
NA	nutrient agar
ND	not detectable
nm	nanometer
nt	not tested
NTC	non-treatment control
p	probability value
pNPG	*p*-nitrophenyl-α-D-glucopyranoside
R^2	regression
ROS	reactive oxygen species
rpm	revolution per minute
SD	standard deviation

TAC	total antioxidant capacity
TISTR	Thailand Institute of Scientific and Technological Research
TLC	thin-layer chromatography
TPC	total phenolic contents
UV	ultraviolet
v/v	volume per volume
w/w	weight per weight
%Yield	percentage of yield

CHAPTER I

INTRODUCTION

1.1 Background and rationale

Indonesia especially Borneo Island has many potential traditional medicine sources; however, it is limited to the folkloric information and utilization. Therefore, it is necessary to establish that information and utilization scientifically (Arung, Kusuma, Christy, Shimizu, & Kondo, 2009). East Kalimantan as one part in Borneo Island is one of the areas with evergreen forests with many medicinal plants. Indigenous people of this province is the Dayak tribe that use plants to treat several diseases such as wounds, fever, scabies, sore eyes, broken bones, arthritis, pregnant, postpartum treatment, diabetes, and others. This tribe has four ways to use the medicinal herb to treat a disease like shredding, crushing, boiling or soaking in hot water, or used directly without processing (Yusro, Mariani, Diba, & Ohtani, 2014). Leaves are commonly used by people because they are easier to collect than other plant parts (Kustiawan, 2007) (Setyowati, 2010), among others *Baccaurea macrocarpa* (Miq.) Müll. Arg., *Cinnamomum parthenoxylon* (Jack) Meisn, *Elaeocarpus submonoceras* Miq., *Entada phaseoloides* (L.) Merr., *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson, *Gynura crepidioides* Benth., *Helicia robusta* (Roxb.) R. Br. ex Blume, *Litsea elliptica* Blume, *Pogostemon cablin* (Blanco) Benth, and *Rhodomyrtus*

tomentosa (Aiton) Hassk. There are several chemical constituents in leaves such amino acids, carboxylic acids, alkaloids, sterols, pigments, carbohydrates, volatiles and the most widely distributed in the plant is phenolic compounds (Leffingwell, 2001).

Phenolic compounds are one group of secondary metabolites and produced from the shikimate/phenylpropanoid pathway, which can gain simple phenolic or polyphenolic structures (Lei, Yang, Yang, Zhang, & Yu, 2015). This group closely related to antioxidant activities due to the hydroxyl groups of compounds could scavenge free radicals by donating the hydrogen atoms so that become stable or not reactive (Prihantini, Tachibana, & Itoh, 2015). Antioxidants play an important role for prevention and cure many diseases (i.e., skin and wound problem, diabetes, and cancer), and is needed to intake when the antioxidant insufficient produced in the human body (Pillai, Oresajo, & Hayward, 2005; Wilson et al., 2017). Further, phenolic is also related to antibacterial by destructing the morphology, function, or structure of the cell membrane of bacteria (Wu et al., 2016).

Base on the broad spectrum of activity of phenolic compounds, therefore, this study aims to investigate the biological activities (antioxidant, antibacterial, anti-tyrosinase, antidiabetic, anticancer, cytotoxicity effect, and wound healing activities) of the plant(s) from Temula village. Its activities can expand the applications of

plants about the beneficial and side effects of the plants and the isolated compound.

1.2 Objective

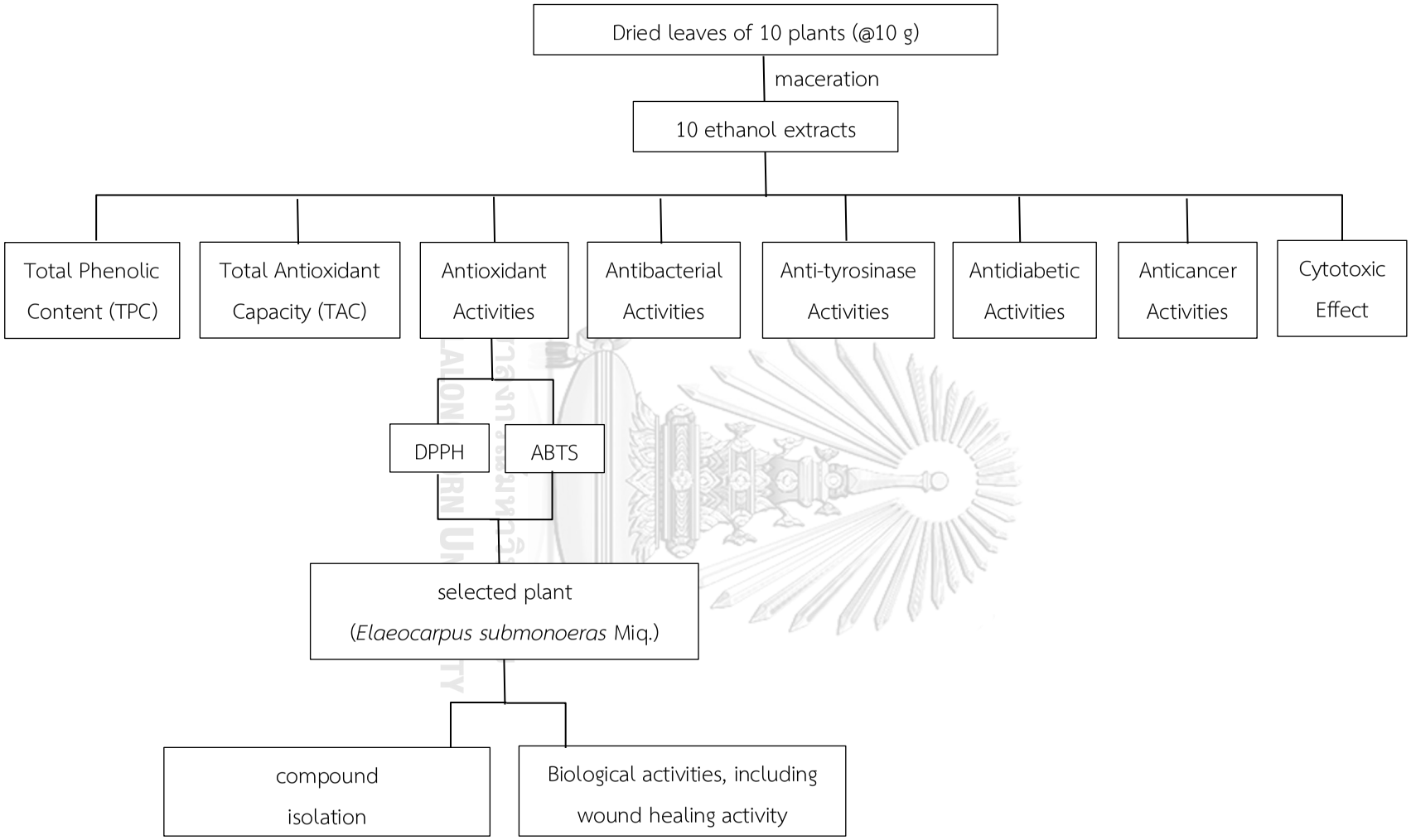
To study biological activities of phenolic compounds extracted from some plant species from East Kalimantan, Indonesia.

1.3 Expected benefit and application

Obtain the extracts and phenolic compound of local plant species from Temula village, West Kutai, East Kalimantan which exhibited biological activities and can be utilized as medicinal properties in the future.

1.4 Research framework

The framework of this study is summarized in the following scheme.



Scheme 1.1 Flowchart of research

CHAPTER II

LITERATURE REVIEW

Medicinal plants are an important source for human ailments therapeutic. The plants can be as an important source of drugs and for novel drug discovery and development process to treat or to prevent diseases (Geetha, Rajeswari, & Jayashree, 2013). Rapidly changing of botanical medicine because of some reasons such drug-resistant microorganisms, side effects of commercial drugs, and to find out new medicines for some emerging new diseases (Patwardhan, Vaidya, & Chorghade, 2004).

Indonesia is one of the countries that has many cultural ethnics for about 400 (ethnics and sub-ethnics) with their extensive knowledge about traditional medicines and medications inherited from generation to generation. This country is known as the mega-center of herbal medicines in the world, however, the effectiveness and safety have not been supported by comprehensive research (WHO, 2009). Tropical forests area of Indonesia covers about 143 million hectares and is home to about 80 % of the world's medicinal plants. It is estimated that the Indonesian tropical forests contain 28,000 plant species and more than 1300 species as medicinal plants. The abundance of natural resources makes most of the Indonesian people especially in rural areas use traditional herbal medicines to treat disease (Elfahmi, Woerdenbag, & Kayser, 2014).

Dayak tribes are indigenous people in Kalimantan Island (well known as Borneo) that use herbal medicines for many years. Every family has a garden called *simpukng* that contain medicinal plants, fruit, and many others. Their experiences of using part(s) of plants as herbal are base information to find new medicines by the scientific way (Mulyoutami, Rismawan, & Joshi, 2009).

2.1 Plant description

Temula village was the area to collect the plant material in this study located in West Kutai district, East Kalimantan province, Indonesia. It has tropical rain forest with the rainfall is 2,000 mm/year, temperature around 25 ºC – 27.1 ºC with relative humidity in the range of 83–87 %. The elevation of the village is 50–100 m above sea level (Voss, 1982). There were totally 10 plant species collected from this village based on the information of their traditional use.

Figure 2.1 Map of Temula village (source: Google map)

2.1.1 *Baccaurea macrocarpa* (Miq.) Müll. Arg.

Family Phyllanthaceae

Vernacular names Pasi Rosang (at Temula village), Tampoi (Kalimantan, Indonesia and Malaysia), Greater Tampoi (English), and ตันลังแข Lang-khae (Thai).

This plant is one of the most popular trees among Dayak tribe that used for wound treatment in Temula village, while in Mencimai village, the Dayak Benuaq and Tunjung cultivate it in their traditional home gardens and consume its sweet-tasting-fruits (Matius et al., 2018).

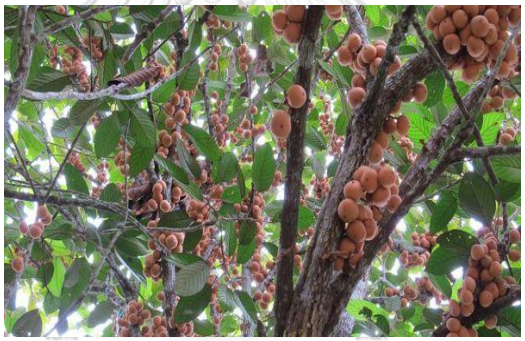


Figure 2.2 *Baccaurea macrocarpa (*Miq.*)* Müll. Arg.

2.1.2 *Cinnamomum parthenoxylon* (Jack) Meisn

Family Lauraceae

Vernacular names Pereu (at Temula village), Kayu (Sabah, Malaysia), Re Huong (Vietnam), Martaban Camphor or Saffrol Laurel (English), Huang Zhang (Chinese), and ข่าต้น ka ton (Thai).

C. parthenoxylon could be used as a hair treatment or anti-dandruff. *Cinnamomum* genus is distributed in China, Indonesia, Srilanka, India, Thailand, and Australia. This genus conceives about 250 species and the leaves are extensively used as essential oils or food spices because of their revealed a spicy odor and has a hot taste when crushed (Prasad et al., 2009).

Figure 2.3 *Cinnamomum parthenoxylon*

2.1.3 *Elaeocarpus submonoceras* Miq.

Family Elaeocarpacae

Vernacular names Nkodoi (at Temula village), Katulampa (West Java, Indonesia).

E. submonoceras Miq. is a tree with edible fruits and found at the dipterocarp forest, swamp, primary or secondary forest (Coode, 2001; Uji, 2004). This plant is native from Kalimantan, Indonesia with the seed color is dark brown, and the shape is rough, oval, and cone at the end (Lailati & Ekasari, 2015). Dayak tribes used the leaves for face washing, skin disease treatment (i.e., dark spot removing), and wound healing. There are around 200 species of genus *Elaeocarpus* all over the world and

some of them had examined for their chemical compounds. Myricetin and gallic acid, the phenolic compounds were also originated in this genus (Chand, Dasgupta, Chattopadhyay, & Ray, 1977).

Figure 2.4 *Elaeocarpus submonoceras* Miq.

2.1.4 *Entada phaseoloides* (L.) Merr.

Family Leguminosae

Vernacular names Beruruk (at Temula village), St. Thomas Bean (English), สะบ้า Saba (Thai), and Modama (Japan).

People in Temula village use *E. phaseoloides* for hair treatment or shampoo. This plant has a very large woody climber and widely distributed in Indonesia and India. The chemical constituents that present in leaves are among others entadamide A and entadamide C. This species is used traditionally for anthelminthic, antiperiodic, tonic, and emetic (Ramakrishna, Pavan, Mukkanti, & Abedulla, 2008). Seeds of this plant in Thailand is used as a soap and for skin diseases treatment. Vanillic acid,

(-) epicatechin, quercetin, luteolin, rutin, apigenin, and naringenin are some phenolic compounds contained in this plant (Sugimoto, Matsunami, & Otsuka, 2018).

Figure 2.5 *Entada phaseoloides (*L.*)* Merr.

2.1.5 *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson

Family Annonaceae

Vernacular names Somputn Planuq (at Temula village), ราชครู Rajchakru or ชิงดอกเดียว Ching Dok Diao (Thai), Limpanas Putih (Brunei), and Penawar Hitam (Peninsular Malaysia).

G. macrophyllus is used by Temula people for skin disease treatment. The genus of this plant can be found in Thailand, Indonesia, and Malaysia and possesses to have anticancer activity without inflammatory effect. Goniothalamin is one of the compounds that contains in various species of this genus and revealed the ability to kill the cancer cells including MDA-MB-231 cells but without affecting the normal liver cells compared to doxorubicin (Alabsi et al., 2013). Local people (Sakai tribe) in

Trang Province, Thailand use the decoction of this plant for blood and body nourishment (Wattanapiromsakul, Wangsintaweekul, Sangprapan, Itharat, & Keawpradub, 2005).

Figure 2.6 *Goniothalamus macrophyllus (*Blume*)* Hook. f. & Thomson

2.1.6 *Gynura crepidioides* Benth.

Family Asteraceae

Vernacular names Kemudi Patah (at Temula village), ผักกาดช้าง phak kat or หญ้าดอกคำ ya dok kham (Thai), Okinawa Spinach or Fireweed (English), Ye Tong Hao (Chinese), and Ebolo (French).

Synonym name of *G. crepidioides* is *Crassocephalum crepidioides* (Benth.) S.Moore. This species is used for the treatment of bone fracture and the leaves are widely consumed as a vegetable. This species can be found in Nigeria, Taiwan, Indonesia, and contained some phytochemicals, including alkaloids, tannins, and

monoterpenes (Adedayo et al., 2015; Aniya et al., 2005). This plant can treat some diseases like hepatitis, edema, antimalaria, antimutagenic, and fever (Aniya et al., 2005).

Figure 2.7 *Gynura crepidioides* Benth.

2.1.7 *Helicia robusta* (Roxb.) R. Br. ex Blume

Family Proteaceae

Vernacular names Tidu (at Temula village), Hang Krakok or Meat Khon (Thai), Sialhma (India), Robust Helicia (English), Medang Keladi (Peninsular Malaysia), malaantigan (Philippines).

H. robusta is endemic to Kalimantan, Sabah, Sarawak and Brunei (Chung, 2001). Its leaves are used by local people in Riau, Indonesia, to treat mothers after giving birth (Setyowati, 2010; Setyowati & Wardah, 2007). Young leaves are eaten to treat mouth sores by people in West Java, Indonesia (Susiarti, Rahayu, & Rugayah, 2018). In East Sumatera, its fruits are consumed as one of the popular local fruits,

while its leaves are soaked in bathing water for its refreshing aroma (Hasibuan, 2016). In India, its root bark is used for treating stomach ulcers and uterine problems, while its bark is used in dietary supplements (Khiangte & Lalramnghinglova, 2017).

Figure 2.8 *Helicia robusta* (Roxb.) R. Br. var. *robusta*

2.1.8 *Litsea elipptica* Blume

Family Lauraceae

Vernacular names Ayau Junuq (at Temula village), ทำมัง Thammang (Peninsular Thailand), and Medang Pepijat (Malaysia).

L. elliptica is a tree around 10 to 45 m of height and has simple leaves that consist of 4-7 pairs of secondary veins (Ngearnsaengsaruay, Middleton, & Chayamarit, 2011). Leaves of this plant have been used for sprains and bruises treatments. *Litsea* species contains about 200 plants that distributed in the tropical and subtropical area including China and Indonesia. In China, the leaves are used for stomachache, pain, traumatic injury, and arthritis treatments. The phytochemicals of these species are mainly contained of flavonoids and terpenoids. Scientific studies about the

activity of the extracts of these species revealed antibacterial, anti-HIV, anti-inflammatory, and anti-diabetic properties in both *in vitro* or *in vivo* experiments (Kong et al., 2015).

Figure 2.9 *Litsea elipptica* Blume

2.1.9 *Pogostemon cablin* (Blanco) Benth.

Family Lamiaceae

Vernacular names Nilam Koko (at Temula village), Kabling or Kadlum (Philippines), Hoắc Hương (Vietnam), Dhalum Wangi (Malaysia), and พิมเสน Phimsen (Thailand).

This plant is grown extensively in many countries, including Indonesia, Malaysia, China, and Brazil, for its essential oil, namely patchouli oil that can be used as a mosquito repellent (Bunrathep, Lockwood, Songsak, & Ruangrungsi, 2006; Gokulakrishnan, Kuppusamy, Shanmugam, Appavu, & Kaliyamoorthi, 2013). *P. cablin* leaves can cure skin allergies by being crushed (Runtunuwu, 2013).

Figure 2.10 *Pogostemon cablin (*Blanco*)* Benth.

2.1.10 *Rhodomyrtus tomentosa* (Aiton) Hassk.

Family Myrtaceae

Vernacular names Masisin Kubar (at Temula village), Kemunting or Karamunting (Indonesia and Malaysia), Downy Rose Myrtle (English), Sim (Vietnam), and โทะ Toh (Thai).

R. tomentosa is a small shrub, the height of about 1 m and has pink flowers with dark violet edible fruits (Tung et al., 2009). This plant is used for wound healing and anti-inflammatory by people in Temula village. In Vietnam, it can be used for diarrhea treatment also. Hydrolyzable tannins, triterpenes, and flavones are some of the chemical constituents contained in this plant (Tung et al., 2009). In China, Vietnam, and Thailand, the fruits are popular with the nutritious content and low of calorie, further made as wine or jam. Scientific researches revealed many bioactivities including antibacterial, anti-hepatitis, and anti-inflammation (Zhuang et al., 2017).

Figure 2.11 *Rhodomyrtus tomentosa (*Aiton*)* Hassk.

2.2. Phenolic compounds

Higher plants produce many chemical compounds that classified to primary and secondary metabolites. The plant has primary metabolites in ubiquitous and directly essential to plant itself such for basic photosynthetic or respiratory metabolism. Role of secondary metabolites to the plant is for plant survival in the environment that is synthesized through metabolic pathways as a response to stress conditions because of biotic or abiotic agents (Caretto, Linsalata, Colella, Mita, & Lattanzio, 2015; Yang et al., 2016).

Phenolic compounds as one group of secondary metabolites are the most widely distributed in plant and uncommon in bacteria, fungi, and algae. It is produced from the shikimate/phenylpropanoid pathway, which can produce simple phenolic or polyphenolic structures. Phenolics are found mostly in the vascular plants, which leaves contain amides, glycosides of hydroxycinnamic acids, esters,

glycosylated flavonoids, especially flavones and flavonols, etc (Cheynier, Comte, Davies, Lattanzio, & Martens, 2013).

The phenolic compounds can act as antioxidants in the human body to counteract reactive oxygen species (ROS). Moreover, this group is also related to other biological activities including antibacterial, anti-tyrosinase, antidiabetic, anticancer, and wound healing properties (Fawole, Makunga, & Opara, 2012; Subramanian et al., 2015; Vinayagam, Jayachandran, & Xu, 2016).

2.3 Biological activities

2.3.1 Antioxidants Agent

Antioxidants are the compounds that can be occurred in the organic material with the ability as electron donation to the oxidant or ROS and hence could inhibit the reaction of oxidative stress (Damjuti, 2013). Antioxidants amount is needed to be balanced in the body to prevent the onset of diseases (Kurutas, 2016). Therefore, natural antioxidant agents that may be contained in leaf extracts may have an important role to play in safeguarding human health. Phenolic is close related to the antioxidant activities and wildly originate in plants, for example, catechins in black tea, curcumin in turmeric, and lycopene in tomatoes (Hamid, Aiyelaagbe, Usman, Ameen, & Lawal, 2010).

2.3.2 Antibacterial Agent

Antibacterial agents are essential substances in the treatment of various infectious diseases caused by bacteria. The consumption of antibacterial agents or antibiotic without prescription and without adequate knowledge of the patients can cause negative effects such as the resistance of treated bacteria (Rezk, 2015). Bacteria are single-cell microorganism (prokaryote) that have DNA and RNA, without chlorophyll, and can be found anywhere, inside or outside of living and nonliving things. Bacteria are divided into two types base on the cell wall structure that are Gram-positive and Gram-negative. The cytoplasmic membrane of Gram-positive bacteria surrounded by hard and rigid nets, while for Gram-negative bacteria surrounded by thin cell walls and there is an outer membrane that wraps it with a large lipopolysaccharide content (Hairani, 2016).

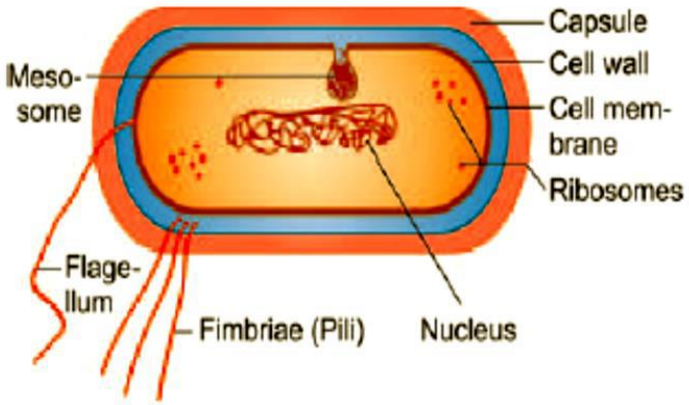


Figure 2.12 Bacteria cell structure

2.3.3 Tyrosinase Inhibitor

Tyrosinase is a copper-containing enzyme (EC 1.14.18.1) that related to melanin production with involving of the oxidative reactions of amino acid tyrosine. Melanin is a pigment produced in melanocyte cells and found in plants, animals, bacteria, and fungi. Melanin role is to be a skin protectant from ultraviolet (UV) damage by UV sunlight absorption and ROS removed, then in result make the skin darker (Wangthong, 2010). In cosmetic or food industries, the inhibitor of this enzyme is needed due to people demand to control their skin neither the skin of fruits for example. The popular tyrosinase inhibitor or whitening agent for human skin recently are among others hydroquinone and kojic acid when used in the long period, unfortunately, revealed a side effect and could be a carcinogen (Sarkar, Arora, & Garg, 2013). The natural products can be a solution, for example, *P. cablin* that showed a positive anti-tyrosinase activity (Bunrathep et al., 2006).

2.3.4 **α**-glucosidase Inhibitor

α-glucosidase is an enzyme (EC 3.2.1.20) that placed on the surface of the brush-border membrane in the small intestine as depicted in **Figure 2.12**.

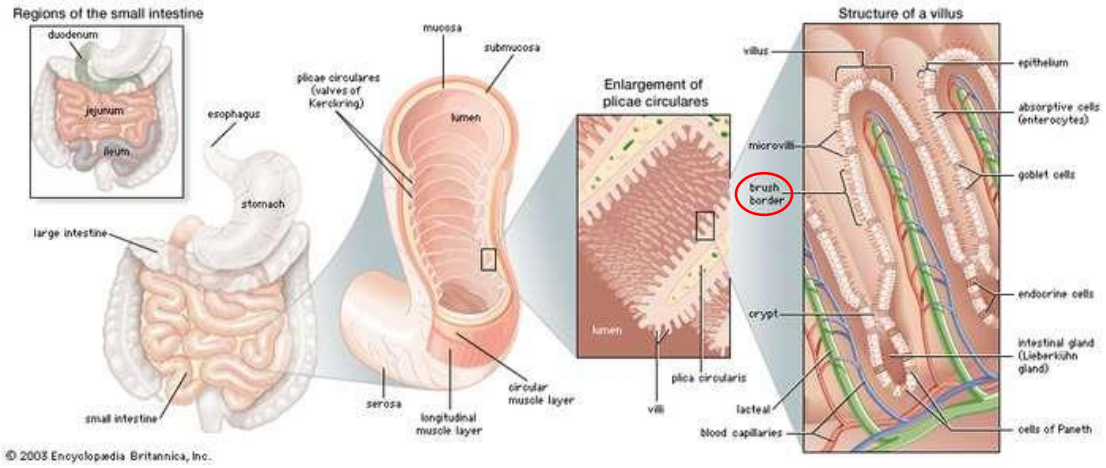


Figure 2.13 The brush-border location in intestine (www.britannica.com)

In this intestine, the process of food (carbohydrate) to be digested into monosaccharide (glucose) and further absorbed and distributed to all body cells through blood circulation. Insulin is then produced in pancreas β-cells when the glucose content is at a high level for conveying and reducing the level of glucose in the cells. Though, diabetes could happen when the pancreas cannot produce enough insulin. There are 3 types of diabetes including type I, type II, and other specific types (i.e., autoimmune polyglandular syndrome (APS) and gestational diabetes) (Ramadhan, 2015). α-glucosidase is related to type 2, the non-insulin dependent diabetes. The patients with this type are needed to be monitored and consumed oral medicine including an α-glucosidase inhibitor. The process of carbohydrates to be digested and absorbed can be slowed down by the inhibitor (Nyemb et al., 2018).

2.3.5 Anticancer Agent

An anticancer agent has a function to reduce or kill the abundance of cancer cells in the human body. Etoposide or doxorubicin among two chemotherapy medicines that commonly used. Another alternative of the agent can be produced from natural sources, which have fewer side effects and have no toxicity to the normal cells. Cancer is a disease caused by uncontrol of abnormal cells proliferation, then can spread speedily to other organs or tissues (Alam, Najum Us Saqib, & Waheed, 2017). Among many types of cancer, breast cancer (such as MDA-MB-231) is one of the leading causes of mortality in women globally (Hooshmand et al., 2014).

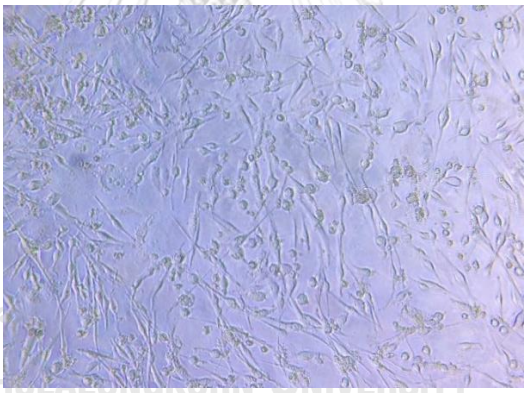


Figure 2.14 MDA-MB-231 cells

2.3.6 Cytotoxicity Effect and Wound Healing Properties

People nowadays more interest to maintain and beautify their healthy skin. This affects the increase in cosmetic and skin care products demand. Moreover, people also concern the ingredients of the products, and leads to the use of natural ingredients, mainly from plants (Fonseca-Santos, Corrêa, & Chorilli, 2015). The natural

products are effective to scavenge free radicals which cause many skin problems such as rhytides, actinic damage, photoaging, and dyschromia (Bowe & Pugliese, 2014). Thus, among the effectiveness of natural products in dermatologic care purposes, the test of cytotoxicity effect against normal skin cells such as HaCaT, the human keratinocyte cells (**Figure 2.13**), is important regarding the production of safety cosmetic or medicinal properties before releasing to the public. Keratinocytes are the major cells located in the epidermis and able to produce proteins of cellular and extracellular matrix (ECM) structural i.e. keratins. Remodeling of the keratin cytoskeleton is an important process for cells motility during wound healing (Kobiela et al., 2018).

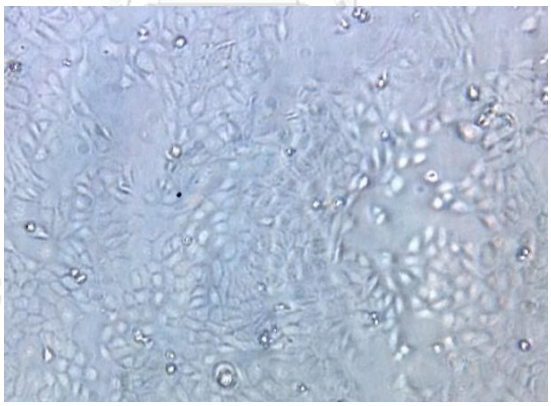


Figure 2.15 HaCaT, the human keratinocyte cells

The epidermis is the outer layer of skin and its function is to protect the human body against external attacks, therefore any wound or injury need to be healed quickly by the system in the body and can be accelerated by consuming wound healing agents (Muniandy et al., 2018).

CHAPTER III

MATERIALS AND METHODS

3.1 Plant materials

Fresh leaves of 10 medicinal plants with the voucher number in parentheses: *Baccaurea macrocarpa* (Miq.) Müll. Arg. (KK-1204-BAM001), *Cinnamomum parthenoxylon* (KK-1204-CPA001), *Elaeocarpus submonoceras* Miq. (KK-1204-ESM001), *Entada phaseoloides* (L.) Merr. (KK-1204-ENP001), *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson (KK-1204-GMA001), *Gynura crepidioides* Benth. (KK-1204-GCR001), *Helicia robusta* (Roxb.) R. Br. ex Blume (KK-1204-HRO001), *Litsea elliptica* Blume (KK-1204-LEL001), *Pogostemon cablin* (Blanco) Benth. (KK-1204-PCA001), and *Rhodomyrtus tomentosa* (Aiton) Hassk. (KK-1204-RTO001) were collected from Temula village, West Kutai, East Kalimantan, Indonesia by local people. The plants were between 3-6 years old and gathered in the rainy season (January to May 2012). Leaves with dark green or greenish color, mature, and no destruction signs by insects or fungi were collected. The specimens were deposited in the Wood Chemistry Laboratory, Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia.

3.2 Ethanol extracts preparation

The leaves of 10 species were air-dried under the shade for 3 days. The dried materials were then grounded into powder at room temperature. Ten-grams of

powdered material from each plant was macerated with 95 % ethanol (3x100 mL) and constantly shaking at 120 rpm, room temperature for 48 h for each repetition. The filtration of liquid extracts was used Whatman No. 1 filter paper with a diameter of pores is 110 mm (Cat No. 1001-110, Sigma-Aldrich, USA), then concentrated by a rotary evaporator at 40 °C to acquire ethanol crude extracts. Yields of extract (w/w) were calculated and stored at -20 °C until further use.

3.3 Total phenolic contents (TPC) assay

The TPC was modified from Pientaweeratch, Panapisal, and Tansirikongkol (2016). All extracts and gallic acid standard (Merck Chemical Co., Darmstadt, Germany) were diluted in 1 % dimethyl sulfoxide (DMSO)-distilled water. Twenty microliters of samples were mixed with 100 µL of 10 % (v/v) Folin-Ciocalteu reagent 2.0 N (Loba Chemie Pvt. Ltd., India) in 96-well plate (#3599), Corning®, NY, USA), and incubated for 5 min. Afterward, 80 µL of sodium carbonate (BDH Chemicals, Toronto, Canada) (75 g/L) was added and slightly shaken, then reincubated for 120 min in room temperature and dark condition. Samples final concentrations were 50 µg/mL, while gallic acid was from 0.195 to 50 µg/mL. The reaction was measured at 760 nm of absorbance by SpectraMax M3 multi-mode microplate reader (Molecular Devices, Sunnyvale, CA, USA) with Softmax software (SoftMax, San Diego, CA, USA). Blank contained 1 % DMSO-distilled water. Phenolic content of each sample was

calculated from the equation of linear regression of gallic acid standard curve. TPCs were presented as mg gallic acid equivalent (GAE)/g of dried weight (DW) of extract.

3.4 Total antioxidant capacity (TAC) assay

The TAC method was modified from Prieto, Pineda, and Aguilar (1999). Forty microliters of sample and 400 µL of TAC reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate) were added into 1.5 ml tube, then incubated for 90 min at 95 ºC. After reached room temperature, two-hundred microliters from each tube were transferred into 96-well plate. Samples final concentrations were 50 µg/mL, while ascorbic acid was varied from 5 to 100 µg/mL. The reactions were measured at 695 nm of absorbance. Blank contained 1 % DMSO-distilled water. Antioxidant capacity of each sample was calculated from the equation of linear regression of ascorbic acid standard curve. TACs were presented as mg ascorbic acid equivalent (AAE)/g of dried weight (DW) of extract.

3.5 Antioxidant activities assay

3.5.1 DPPH (2.2-diphenyl-1-picrylhydrazyl) inhibition assay

The DPPH inhibition assay was modified from Lin et al. (2014). Fifty microliters of sample were mixed with 150 µL of 100 µM DPPH reagent in methanol, then incubated for 30 min in dark condition at room temperature. The concentrations of samples and ascorbic acid as positive control were made from zero to 1000 µg/mL in

1 % DMSO-methanol. The inhibition of DPPH was measured at 512 nm using SpectraMax M3 reader.

The percentage of DPPH inhibition was calculated by an equation that adopted from Benmehdi, Behilil, Memmou, and Amrouche (2017):

$$1 - \frac{A_{sample}}{A_{control}} \times 100\ \% \qquad (1)$$

Where A_{sample} is the absorbance from the reaction of DPPH reagent and the sample, while $A_{control}$ is the absorbance from DPPH reagent and solution only (without sample). The result of percent inhibition was calculated from 100 µg/mL of sample concentration. The results of percent inhibition from the vary of concentration were then plotted and regressed linearly to get IC_{50} value. IC_{50} means the concentration of the samples when inhibiting 50 % of DPPH.

3.5.2 ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) inhibition assay

The ABTS inhibition assay was slightly modified from Fu, Zhang, Guo, and Chen (2014). The modification was about the solution for diluting ABTS•+ and potassium persulfate reagents change to distilled water. The absorbance of reaction was measured immediately or up to 3 min. The calculation of percent inhibition and IC_{50} values were similar to DPPH inhibition assay (3.5.1). Concentrations variation of

samples and ascorbic acid were made from 0.78 to 1000 µg/mL to find the IC_{50} value, while for percent inhibition was obtained from 100 µg/mL.

3.6 Antibacterial activity assay

Agar-well diffusion method (Yadav, Trivedi, & Bhatt, 2015) and modified the microdilution method from Sarker, Nahar, and Kumarasamy (2007) were performed for antibacterial activity. There were two Gram-negative bacteria (*Pseudomonas aeruginosa* TISTR 1287 and *Salmonella typhi* ATCC 422) and four Gram-positive bacteria (*Propionibacterium acnes* KCCM 41747, *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* ATCC 25175 and *Streptococcus sobrinus* KCCM 11898) were used in this study. For the agar-well diffusion method, every plate was filled with 30 mL nutrient agar (NA) and waited until solidified, then 100 µL of bacteria inoculums was swabbed. The concentration of samples and chloramphenicol (positive control) were 1,000 µg/mL in acetone. Percentage of inhibition values were obtained from dividing the zone of inhibition of samples (mm) with the zone of inhibition of positive control (mm) and multiplied by 100 %.

Minimum inhibitory concentration (MIC) values were performed by microdilution method using a 96-well plate. Every well contained 50 µL samples or controls, 40 µL of nutrient broth, and 10 µL of bacterial suspension based on 0.5 McFarland standard. There were 8 varied concentrations of samples and positive control and the final concentrations were from 7.8125 to 1000 µg/mL (2-fold serial

dilution). After the samples and bacterial incubated for 18-24 h, MIC result was shown by added 10 µL of 0.01 % resazurin colorimetric agent and incubated for 10 min. Thereafter, minimum bactericidal concentration (MBC) value was shown from the lowest concentration that absence of bacteria growth by transfer 10 µL of the solution in the 96-well plate that revealed MIC value and other three higher concentrations onto NA plate, then incubated for next to 18-24 h.

3.7 Anti-tyrosinase activity assay

This assay was slightly modified from Mapunya, Nikolova, and Lall (2012). The samples and kojic acid (Sigma-Aldrich, MO, USA) as positive control were reacted with mushroom tyrosinase (Sigma-Aldrich, MO, USA) and 100 µL of L-tyrosine (Sigma-Aldrich, MO, USA).

3.8 Antidiabetic activity assay

The antidiabetic assay used rat intestinal α-glucosidase and two substrates: sucrose and maltose; as described in detail at Ramadhan, Worawalai, and Phuwapraisirisan (2018). The percentage inhibition was performed from three final concentrations of samples (40, 200, and 1000 µg/mL) against control (the reaction without sample).

3.9 Anticancer activity assay

The assay was performed by Banerjee et al. (2016) with modification. MDA-MB-231 human breast cancer cell lines were cultured in complete media (high-

glucose DMEM, 1 % antibiotic-antimycotic (100x), and 10 % heat-inactivated fetal bovine serum) at 37 ºC, 5 % CO_2. All reagents were gained from Gibco (NY, USA). In 96-well-plates, ten thousand cells per well were seeded and incubated for 48 h. IC_{50} was obtained by varying final concentrations of samples that dissolved in 1 % DMSO-complete media. After the treatments, then were re-incubated for next to 48 h. The media was then changed with 90 µL of complete media and 10 µl of PrestoBlue colorimetric agent (Invitrogen, Carlsbad, CA, USA) and incubated for 2 h. Fluorescence units were measured according to the manufacturer's recommendation at 570 nm as excitation and 600 nm as emission wavelengths. The logarithmic regression in Excel was used to obtain the IC_{50} value.

3.10 Cytotoxicity effect assay

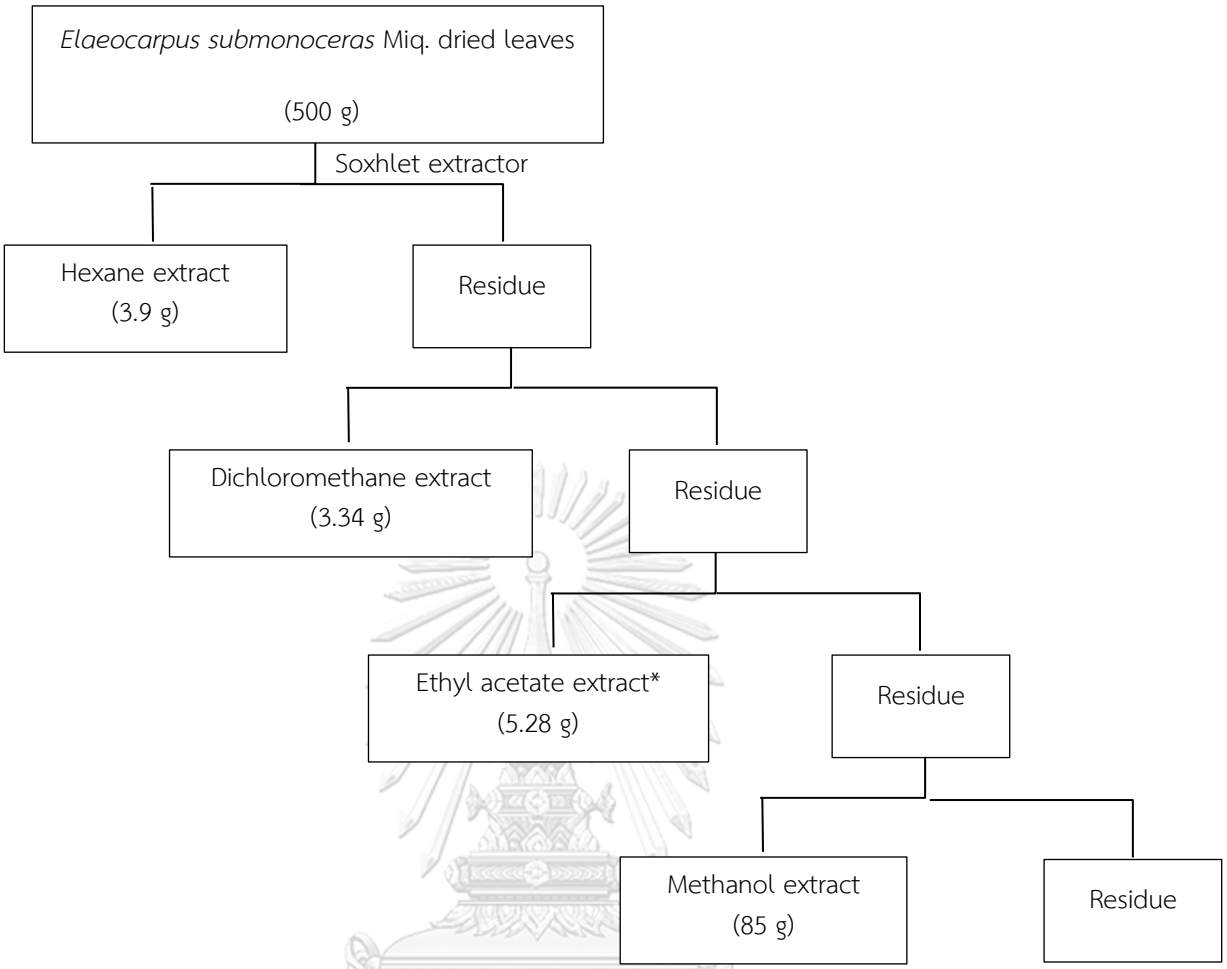
Cytotoxicity effect was performed by Ritto et al. (2017) with modification. HaCaT human keratinocyte cells were cultured in complete media (same with **3.9**) with additional of 1 % 200 mM glutamine (PAA, Pasching, Austria) at 37 ºC, 5 % CO_2. The amount of the cells in every well, incubation times, measurement by fluorescence units and calculation were similar with section **3.9**.

3.11 Isolation of compound from *E. submonoceras* Miq.

3.11.1 Successive extraction and column chromatography

Compound isolation process was begun by Soxhlet successive extraction of 500 g of *E. submonoceras* Miq. dried leaves (collected from April to June 2016) with

four different polarity solvents (**Scheme 3.1**). This process yielded hexane, dichloromethane, ethyl acetate, and methanol extracts. All the process of extraction and purification was guided by antioxidant assays (section **3.5.1** and **3.5.2**) and thin-layer chromatography (TLC; Merck $60F^{254}$, Darmstadt, Germany). The ethyl acetate extract was then fractionated using silica gel 60 column chromatography with hexane-ethyl acetate and ethyl acetate-methanol solvents gradients and elicited 13 fractions (E.1 – E.13). The E.10 active fraction was then washed with dichloromethane and the precipitated was purified by silica gel 60 column chromatography. A mixture of dichloromethane, methanol, water, and acetic acid was used as the solvents system. From 41 collection tubes, the pure compound was found in tube 11 (code as E.10.11). The yields of extracts and compound were weighed and calculated as a percent (w/w).



Scheme 3.2 *E. submonoceras* Miq. successive extraction

3.11.2 Structural identification

The isolated compound (E10.11) was confirmed by TLC and structurally identified by using BRUKER proton nuclear magnetic resonance spectroscopy (^{1}H NMR) 400 MHz in acetone ($(CD_3)_2CO$).

3.11.3 Wound healing assay

The extracts were tested with the biological activities including wound healing assay. This assay was performed in HaCaT cell lines according to Juneja et al. (2019).

The cells were seeded in a 12-well plate (3 x 10^5 cells/well). Each well contained one mL of 10 % FBS, 1 % glutamine, 1 % antibiotic, and 90 % DMEM. After 24 h and reaching confluency, three vertical scratch lines were made in each well by using a p200 tip. Afterward, the wells were washed with low serum-media (1 % FBS, 1 % antibiotic, and 99 % DMEM) for two times and consecutively gave the treatment. The concentration of the samples was 10 µg/mL in low serum-media. The treatments were done in every two wells of replication and incubated at 37 ℃ in 5% CO_2. Three representative images from each well of the scratched areas were photographed under 4x magnifications of an inverted microscope to estimate the relative proliferation or migration of cells. The images were taken at 0 h, 24 h, 48 h, and 72 h after scratching and continued to analyze using ImageJ software, then presented as percentage of the wound closure compared to the initial scratch area at 0 h.

3.12 Statistical analysis

The results were shown as mean and standard deviation (mean±SD) from three replications**.** One-way analysis of variance (ANOVA) was used for statistical analyses with significant differences ($p \leq 0.05$) using Microsoft Excel Office 365 and IBM SPSS statistics 22 with Duncan's post hoc test.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Percent yield of ethanol extract

Ten plant species from nine different families were macerated with ethanol. **Table 4.1** showed the percentage yields of those ethanol extracts. *E. submonoceras* gave the highest yield (16.19 %), followed by *L. elliptica* (10.42 %), while *B. macrocarpa* had the lowest yield (1.42 %).

Ethanol solvent has been widely used for polyphenols recovery and antioxidant studies in plants (Alam, Bristi, & Rafiquzzaman, 2013; Do et al., 2014). The 70 % ethanol extract yield (3.57 %) from *E. serratus* was lower than the present study (Biswas et al., 2012). The yield of *L. elliptica* ethanol extracts was in the range of 8.96 % – 13.62 % (Ahmmad et al., 2012; Pradeepa et al., 2011). The difference of percentage of extract yields may be due to the amount of compound dissolved in ethanol solvent.

Table 4.1 Yield of leaf ethanol extracts from 10 medicinal plants from East Kalimantan province, Indonesia.

Scientific name	Local name	Family name	Yield (%, w/w)*
Baccaurea macrocarpa (Miq.) Müll. Arg.	Pasi Rosang	Phyllanthaceae	1.42
Cinnamomum parthenoxylon (Jack) Meisn	Pereu	Lauraceae	5.83
Elaeocarpus submonoceras Miq.	Nkodoi	Elaeocarpacae	16.19
Entada phaseoloides (L.) Merr.	Beruruk	Leguminosae	3.65
Goniothalamus macrophyllus (Blume) Hook. f. & Thomson	Somputn Planuq	Annonaceae	2.67
Gynura crepidioides Benth.	Kemudi Patah	Asteraceae	1.51
Helicia robusta (Roxb.) R. Br ex Blume	Tidu	Proteaceae	4.73
Litsea elliptica Blume	Ayau Junuq	Lauraceae	10.42
Pogostemon cablin (Blanco) Benth.	Nilam Koko	Lamiaceae	4.36
Rhodomyrtus tomentosa (Aiton) Hassk.	Masisin Kubar	Myrtaceae	5.68

* Percentage of yield was calculated by dividing the weight of the ethanol extract with the weight of the dried leaves multiplied by 100 %.

4.2 TPC, TAC, DPPH, and ABTS of 10 ethanol extracts

Phenolics have several unique characteristics, including their flavor, color, bitterness, odor, and biological activities, which have led to their use in many medicinal and food applications (Naczk & Shahidi, 2006). One of the biological activities that highly related to phenolic is antioxidant activity. This condition due to

the donation of a hydrogen cation of the phenolics to free radicals, and generate a relatively stable free radical (Prihantini et al., 2015). Studies about antioxidant activities are widely used by phosphomolybdenum, DPPH, and ABTS methods in plant and food research (Basumatary, Das, Nanjian, & Sharma, 2015; Pientaweeratch et al., 2016). **Table 4.2** presented the TPC, TAC, DPPH, and ABTS inhibition of ethanol extracts.

TPC analysis used gallic acid as a standard. The TPCs from the 10 plants ranged from 55.20 to 578.45 mg GAE/g DW. The highest TPC values were obtained from *E. submonoceras* (578.45 ± 31.44 mg GAE/g DW) and *H. robusta* (576.04 ± 9.15 mg GAE/g DW), while the lowest value was obtained from *C. parthenoxylon* (55.20 ± 0.78 mg GAE/g DW).

TAC was performed by phosphomolybdenum method and used ascorbic acid as a standard. The highest TAC value was obtained from *C. parthenoxylon* (642.85 ± 5.27 mg AAE/g DW), followed by *E. submonoceras* (419.73 ± 3.95 mg AAE/g DW) and *H. robusta* (363.28 ± 1.88 mg AAE/g DW), while the lowest value was obtained from *L. elliptica* (91.61 ± 5.84 mg AAE/g DW).

DPPH and ABTS assays results are presented as the percentage of inhibition and as IC_{50} values. The percentage of DPPH inhibition ranged from 25.27 ± 0.45 to 99.06 ± 1.17 %, while the IC_{50} values ranged from 3.81 ± 0.06 to 346.90 ± 22.34 µg/mL. The percentage of ABTS inhibition ranged from 19.69 ± 0.29 % to

98.13 ± 0.55 %, whereas the IC_{50} values ranged from 13.51 ± 0.67 to 600.74 ± 2.86 µg/mL. *E. submonoceras* extract gave the highest antioxidant levels (with IC_{50} values of 3.81 and 13.51 µg/mL), then followed by *H. robusta* (IC_{50} values of 6.86 and 35.93 µg/mL for DPPH and ABTS inhibition, respectively).

The trend of the four methods presented in this section was relatively similar. The high content of phenolic revealed the high of antioxidant activities, exclude for one plant (*C. parthenoxylon*). This plant contained low of TPC but high of TAC. This condition could be happened because of the antioxidant in this plant not only from phenolic compounds. Ascorbic acid is one example of a compound contained in leaves but is not included in the phenolic group and is well-known as an antioxidant (Nowak, Goslinski, Wojtowicz, & Przygonski, 2018; Rivelli, Caruso, Maria, & Galgano, 2017).

E. submonoceras tends to be an excellent extract among ten plants in terms of phenolic content and antioxidant activities. Its percentages of DPPH and ABTS inhibitions at 100 µg/mL of concentration were similar to the positive control (ascorbic acid) in the range from 94.98 ± 0.29 to 100 ± 0.10 %. The IC_{50} of DPPH of this extract was even better than ascorbic acid and comparable when against ABTS radical. This plant is interesting to study further regarding its biological activities because there have been no previous information about this species.

Table 4.2 TPC, TAC, DPPH and ABTS inhibitions of ethanol extracts

Sample	TPC (mg GAE/g DW)	TAC (mg AAE/g DW)	DPPH		ABTS	
			%I	IC_{50} (µg/mL)	%I	IC_{50} (µg/mL)
B. macrocarpa	97.06 ± 2.90^{d}	145.97 ± 7.78^{g}	25.27 ± 0.45^{i}	143.51 ± 8.13^{f}	29.78 ± 0.32^{g}	380.28 ± 10.94^{i}
C. parthenoxylon	55.20 ± 0.78^{e}	642.85 ± 5.27^{a}	54.33 ± 1.73^{g}	88.43 ± 2.63^{e}	52.14 ± 2.15^{e}	124.10 ± 2.38^{g}
E. submonoceras	578.45 ± 31.44^{a}	419.73 ± 3.95^{b}	94.98 ± 0.29^{bc}	3.81 ± 0.06^{a}	96.85 ± 0.11^{a}	13.51 ± 0.67^{b}
E. phaseoloides	150.21 ± 12.46^{c}	105.77 ± 2.02^{h}	88.74 ± 1.64^{e}	10.14 ± 0.54^{ab}	64.51 ± 2.87^{c}	71.00 ± 4.80^{e}
G. macrophyllus	170.08 ± 9.31^{c}	200.07 ± 15.80^{e}	94.72 ± 1.47^{bc}	23.33 ± 1.15^{bc}	54.22 ± 1.64^{e}	89.73 ± 4.82^{f}
G. crepidioides	61.79 ± 6.14^{e}	209.78 ± 13.14^{de}	33.09 ± 0.05^{h}	346.90 ± 22.34^{g}	19.69 ± 0.29^{h}	600.74 ± 2.86^{j}
H. robusta	576.04 ± 9.15^{a}	363.28 ± 1.88^{c}	93.10 ± 0.53^{cd}	6.86 ± 0.29^{a}	92.62 ± 0.13^{b}	35.93 ± 0.29^{c}
L. elliptica	115.10 ± 6.71^{d}	91.61 ± 5.84^{h}	81.46 ± 3.19^{f}	13.71 ± 1.13^{ab}	60.83 ± 4.39^{d}	84.69 ± 4.20^{f}
P. cablin	169.73 ± 12.18^{c}	167.48 ± 6.27^{f}	99.06 ± 1.17^{a}	36.30 ± 1.26^{d}	42.28 ± 0.48^{f}	139.43 ± 1.42^{h}
R. tomentosa	307.50 ± 28.49^{b}	218.37 ± 14.34^{d}	91.11 ± 0.39^{d}	27.11 ± 1.16^{cd}	98.13 ± 0.55^{a}	45.81 ± 0.41^{d}
ascorbic acid	-	-	96.60 ± 1.05^{b}	12.72 ± 0.23^{ab}	100.00 ± 0.10^{a}	3.07 ± 0.04^{a}

The concentration of percentage inhibition of DPPH and ABTS was at 100 µg/mL. All results are presented by the mean ± SD (n = 3) with significantly different ($p \leq 0.05$) shown by different superscript letters.

In various *Elaeocarpus* species, there are some phenolic compounds that are also effective as antioxidants. *E. lanceofolius* leaf ethanol extract contained 4'-methylmyricetin and myricetin-3-O-rhamnoside (Ray, Dutta, & Dasgupta, 1976). *E. serratus, E. oblongus, and E. floribundus* contained mearnsetin. *E. tuberculatus* contained quercetin, kaempferol, and ethyl gallate. *E. serratus* and *E. ganitrus* Roxb. contained ellagic acid (Chand et al., 1977). Gallic acid was also found in leaf ethanol extract of *E. sphaericus* (Garg, Goswami, & Khurana, 2012). *E. submonoceras* ethanol extract is pledging as an antioxidant agent and even could scavenge ABTS better than *E. ganitrus* leaf ethanol extract. Percentage of ABTS inhibition of *E. ganitrus* at a concentration of 500 µg/mL showed 55.77 % and revealed higher of IC_{50} value (297.12 µg/mL), also contained lower TPC (56.79 mg GAE/g DW) (Kumar, Shanmugam, Palvannan, & Bharathi Kumar, 2008). Ethanol extract of *E. ganitrus* from bark also showed a higher IC_{50} value (81.85 µg/mL) against DPPH free radicals and contained phenol compounds by a phytochemicals test (Talukdar, Dutta, Chakraborty, & Das, 2017).

Previous studies regarding the bioactivities of *H. robusta* and its compounds are limited. This is the first information about the phenolic content and antioxidant activities of *H. robusta* leaves. Bark methanolic extract of this plant contained flavonoids and tannins when detected by phytochemical screening. This part also contained ferulic acid and gallic acid (phenolic group) with an IC_{50} value of DPPH

inhibition was relatively lower (49.4 µg/mL) compared to this study result (Lallawmawma, 2016). The stem bark of other species from the same genus (*Helicia nilagirica*) revealed relatively lower of phenolic content (1078 mg GAE/100 g of ethanol extract) and also lower of ABTS inhibition (IC_{50} = 154.79 µg/mL) (Zoremsiami, 2017) compared to the present study.

4.3 Antibacterial activity

The antibacterial activities of the extracts were displayed in the form of a percentage of inhibition, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) values. **Table 4.3** presented the data of the extracts against four Gram-positive bacteria (*P. acnes*, *S. aureus*, *S. mutans*, and *S. sobrinus*), while **Table 4.4** presented the data of the extracts against two Gram-negative bacteria (*P. aeruginosa* and *S. typhi*). All the extracts showed antibacterial activity against six strains compared to chloramphenicol. The extracts could inhibit *P. acnes* in the range from 24.1 % to 33.9 %, *S. aureus* from 26.2 % to 44.3 %, *S. mutans* from 39.4 % to 56.2 %, and *S. sobrinus* from 29.7 % to 41.9 % (**Table 4.3**). The inhibition of the extracts against *P. aeruginosa* in the range of 48.8 % – 73.9 %, while against *S. typhi* in the range of 36 % – 43.1 % (**Table 4.4**).

Table 4.3 Percentage of inhibition, MIC, and MBC of ethanol extracts against Gram-positive bacteria

Sample	*P. acnes* KCCM 41747			*S. aureus* ATCC 25923			*S. mutans* ATCC 25175			*S. sobrinus* KCCM 11898		
	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC
B. macrocarpa	29 ± 1.8^{cd}	>1000	>1000	28.1 ± 0.8^{fg}	1000	>1000	47.1 ± 2.0^{cd}	>1000	>1000	35.1 ± 1.8^{de}	1000	>1000
C. parthenoxylon	32.2 ± 0.7^{b}	1000	>1000	26.6 ± 1.9^{g}	1000	>1000	48.2 ± 3.6^{c}	1000	1000	39.7 ± 2.5^{bc}	1000	>1000
E. submonoceras	28.6 ± 0.9^{cd}	>1000	>1000	44.3 ± 1.0^{b}	>1000	>1000	44.7 ± 1.7^{cde}	>1000	>1000	37.6 ± 3.4^{cd}	1000	>1000
E. phaseoloides	24.1 ± 1.9^{e}	>1000	>1000	31.4 ± 1.1^{cd}	>1000	>1000	43.9 ± 0.8^{de}	1000	>1000	39.6 ± 0.1^{bc}	1000	>1000
G. macrophyllus	27.9 ± 2.7^{d}	>1000	>1000	30.8 ± 1.7^{cde}	1000	>1000	39.4 ± 1.9^{f}	1000	>1000	29.7 ± 2.5^{g}	>1000	>1000
G. crepidioides	33.9 ± 0.9^{b}	>1000	>1000	28.5 ± 1.6^{efg}	1000	>1000	42.3 ± 1.1^{ef}	1000	>1000	34.5 ± 1.1^{def}	1000	>1000
H. robusta	28.6 ± 2.2^{cd}	>1000	>1000	29.7 ± 0.8^{def}	1000	>1000	41.8 ± 4.0^{ef}	>1000	>1000	33.6 ± 0.5^{ef}	1000	>1000
L. elliptica	32.6 ± 1.0^{b}	>1000	>1000	32.8 ± 1.9^{c}	1000	>1000	55.3 ± 1.8^{b}	1000	>1000	39.0 ± 1.8^{bc}	>1000	>1000
P. cablin	28.3 ± 0.5^{cd}	>1000	>1000	26.2 ± 1.6^{g}	>1000	>1000	42.1 ± 1.1^{ef}	>1000	>1000	31.5 ± 1.8^{fg}	>1000	>1000
R. tomentosa	31.2 ± 2.6^{bc}	31.25	>1000	32.0 ± 1.9^{cd}	62.5	250	56.2 ± 1.0^{b}	31.25	125	41.9 ± 2.1^{b}	15.625	500
Chloramphenicol	100.0 ± 0^{a}	3.90625	15.625	100.0 ± 0^{a}	15.625	31.25	100.0 ± 0^{a}	3.90625	7.8125	100.0 ± 0^{a}	3.90625	3.90625

Percentage of inhibition (%inhibition) was performed at 1000 µg/mL of sample concentration. The results of %inhibition are presented by the mean ± SD (n = 3) with significantly different ($p \leq 0.05$) shown by different superscript letters.

Table 4.4 Percentage of inhibition, MIC, and MBC of ethanol extracts against Gram-negative bacteria

Sample	*P. aeruginosa* TISTR 1287			*S. typhi* ATCC 422		
	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC
B. macrocarpa	73.9 ± 6.8^{b}	1000	>1000	38.6 ± 2.4^{cde}	1000	>1000
C. parthenoxylon	50.1 ± 1.2^{d}	1000	>1000	43.1 ± 1.5^{b}	1000	>1000
E. submonoceras	53.8 ± 2.6^{cd}	1000	>1000	42.1 ± 1.9^{bc}	1000	>1000
E. phaseoloides	60.0 ± 4.1^{cd}	1000	>1000	41.0 ± 1.0^{bcd}	1000	>1000
G. macrophyllus	64.7 ± 1.9^{bc}	1000	>1000	40.5 ± 3.2^{bcd}	1000	>1000
G. crepidioides	57.8 ± 4.9^{cd}	1000	>1000	37.8 ± 3.5^{de}	1000	>1000
H. robusta	57.4 ± 1.6^{cd}	1000	>1000	37.1 ± 2.0^{de}	1000	>1000
L. elliptica	48.8 ± 0.1^{d}	>1000	>1000	42.0 ± 3.2^{bc}	>1000	>1000
P. cablin	55.1 ± 3.9^{cd}	1000	>1000	36.0 ± 1.2^{e}	>1000	>1000
R. tomentosa	53.8 ± 2.6^{cd}	1000	>1000	37.4 ± 1.2^{de}	31.25	>1000
Chloramphenicol	100.0 ± 0^{a}	62.5	250	100.0 ± 0^{a}	15.625	31.25

Percentage of inhibition (%inhibition) was performed at 1000 µg/mL of sample concentration. The results of %inhibition are presented by the mean ± SD (n = 3) with significantly different ($p \leq 0.05$) shown by different superscript letters.

R. tomentosa revealed the highest activities of antibacterial especially when referred to its MIC and MBC values. Other extracts had MIC and MBC values at 1000 µg/mL or above. MIC values of *R. tomentosa* were 15.625 µg/mL (against *S. sobrinus*), 31.25 µg/mL (against *P. acnes*, *S. mutans*, and *S. typhi*), 62.5 µg/mL (against *S. aureus*), and 1000 µg/mL (against *P. aeruginosa*). At below 1000 µg/mL, MBC values of *R. tomentosa* were 125 µg/mL (against *S. mutans*), 250 µg/mL (against *S. aureus*), and 500 µg/mL (against *S. sobrinus*).

There were some previous studies about *R. tomentosa* in term of the antioxidant and antibacterial activities. However, studies about leaf ethanol extract of this plant were scarce and no information about TPC, TAC, ABTS, and antibacterial activities against *S. sobrinus*. The ethanol extract of this plant was reported from Songkhla, Thailand, to have antibacterial activity against *S. aureus* with MIC and MBC values lower than to those in the present study (Saising, Ongsakul, & Voravuthikunchai, 2011). *R. tomentosa* extract at a higher concentration (100 µg/well) also exhibited antibacterial activity against *P. acnes* and *S. typhi* (Kusuma, Ainiyati, & Suwinarti, 2016). The leaf ethanol extract had a lower of MIC value but the MBC value was the same as this study when against *S. mutans* NPRCM 2010 (Limsuwan, Subhadhirasakul, & Voravuthikunchai, 2009).

There were 14 compounds isolated from the ethanol extract of *R. tomentosa* leaves among others gallic acid, myricetin, myricetin-3,7,3′-trimethyl ether, myricetin-3,7,3′-trimethyl ether 5′-O-β-glucopyranoside, quercetin, rhodomyrtone, rhodomyrtosone C, and tomentosone C that performed antibacterial activity against *S. aureus* ATCC 6538 (Liu, Tan, & Qiu, 2016). Rhodomyrtosone C was also found in ethyl acetate extract of *R. tomentosa* leaves and revealed the activity against *Salmonella typhimurium* ATCC 14028 (Krisyanella, Dachriyanus, & Marlina, 2011). Gallic acid was reviewed by Subramanian et al. (2015) and informed that synthetic derivatives of this compound could inhibit *S. aureus*. Another review about myricetin

informed that this compound was less active against *S. mutans*, but had good activity against *S. aureus*, methicillin-resistant *S. aureus*, *P. aeruginosa,* and *S. typhi* (Semwal, Semwal, Combrinck, & Viljoen, 2016). Myricetin-3,7,3′-trimethyl ether and quercetin that found from a mixture of dichloromethane and ethyl acetate fractions of *Premna resinosa* aerial parts performed a strong activity against *S. aureus* ATCC 25923 and *S. typhimurium* ATCC 14028 (Albadawi et al., 2017). This research recommended that *R. tomentosa* could be used as an antibacterial agent in a broad spectrum. The higher concentration of the extract was needed to inhibit Gram-negative bacteria. This condition may be caused by a complex cell wall structure of Gram-negative bacteria, including the presence of a lipid outer membrane, which makes antibacterial compounds difficult to penetrate to lyse the cells (Beveridge, 1999). *P. aeruginosa* is one of the bacteria known to be resistant to many antibiotics (Duletić-Laušević et al., 2018).

4.4 Anti-tyrosinase activity

The activity of tyrosinase inhibition of all the extracts was presented in **Table 4.5** and until 1000 µg/mL, the inhibition was shown from five plants in the range from 13 % for *B. macrocarpa* to 83.02 % for *E. submonoceras*. Further, the IC_{50} values were only shown from three extracts, with the best from *E. submonoceras* at 103.65 µg/mL, then *E. phaseoloides* at 543.83 µg/mL, and the lowest one was *P.*

cablin at 944.4 µg/mL. Those extracts were significantly different from the positive control (kojic acid) that performed the IC_{50} value at 1.18 µg/mL.

Table 4.5 Anti-tyrosinase activity of 10 ethanol extracts

Sample	%inhibition	IC_{50} (µg/mL)
B. macrocarpa	13.00 ± 1.20^{f}	>1000
C. parthenoxylon	ND	>1000
E. submonoceras	83.02 ± 8.30^{b}	103.65 ± 5.74^{b}
E. phaseoloides	76.44 ± 0.96^{c}	543.83 ± 51.06^{c}
G. macrophyllus	ND	>1000
G. crepidioides	ND	>1000
H. robusta	ND	ND
L. elliptica	45.93 ± 4.03^{e}	>1000
P. cablin	68.63 ± 1.03^{d}	944.40 ± 58.94^{d}
R. tomentosa	ND	ND
Kojic acid	99.96 ± 0.32^{a}	1.18 ± 0.02^{a}

ND: not detectable. %inhibition was done at 1000 µg/mL. All results are displayed as mean ± SD (n = 3) and followed by different letters to show statistically significant differences ($p \leq 0.05$).

To the best of our knowledge, this is the first study into the enzyme inhibition of *E. submonoceras and E. phaseoloides* and could elaborate a possible function of those species for use in phytocosmetics. The activity against this enzyme was reported before for *Elaeocarpus* species. At 500 µg/mL of concentration, ethanol extract of *E. serratus* leaves could inhibit 20.29 % (Liyanaarachchi, Samarasekera, Mahanama, & Hemalal, 2018) and 80 % ethanol extract of *E. sylvestris* (Lour.) Poir.

leaves performed a similar percentage of inhibition at 27.8 ± 2.4 % (Moon, Yim, Song, Lee, & Hyun, 2010).

The information regarding tyrosinase inhibition of *Entada* species was very scarce. The bark of *E. africana* showed good activity against this enzyme and comparable with kojic acid (Baurin, Arnoult, Scior, Do, & Bernard, 2002). Findings isolated compounds from *E. phaseoloides* were also limited. A previous study revealed that from 75 % ethanol extract of this species was isolated entadamides A and C, and a sulfur-containing amide (Ikegami, Sekine, Duangteraprecha, et al., 1989). These compounds may effective as an anti-inflammatory agent and also could inhibit 5-lipoxygenase activity (Ikegami, Sekine, Aburada, et al., 1989). Therefore, those compounds could be responsible for tyrosinase inhibition, as for example entadamide A also performed melanin inhibition with the same activity to a common cosmetic ingredient arbutin (Sugimoto et al., 2018).

P. cablin is well-known for its essential oil, namely patchouli oil. Patchouli alcohol, a tricyclic sesquiterpenoid is one of the major constituents of this essential oil and important in the fragrance industry (Bunrathep et al., 2006). Patchouli alcohol possesses some of the bioactivities, including antioxidative, antibacterial, and whitening properties and is more abundant in the leaf than in the stem or root (Hu, Peng, Xie, Zhang, & Cao, 2017). Leaf extract and patchouli alcohol in term of the

whitening property could reduce melanin contents and intracellular tyrosinase activity in B16 melanoma cells (Bae, Lee, Son, & Lee, 2009).

4.5 Antidiabetic activity

Study about **α**-glucosidase inhibition was done with eight plants and for sucrose substrate, the activity was revealed from only three plants in a dose-dependent manner (**Table 4.6**). The best activity came from *E. submonoceras*, then *H. robusta* and the lowest was *R. tomentosa* at the lower concentration (40 or 200 µg/mL), while at 1000 µg/mL revealed no significantly different for the percentage of inhibition in the range from 91.48 % to 94.58 % and even better than the control (acarbose) for all concentrations. These results were different when using maltose substrate which showed the activity for all the eight extracts. The percentage of inhibition of those plants and the control exhibited dose-dependent manner. At concentration of 40 µg/mL performed the inhibition ranged from 24.48 % to 70.39 %, at 200 µg/mL in the range from 29.66 % to 80.58 %, whereas at 1000 µg/mL in the range from 33.84 % to 94.85 %. The highest activities were presented by *E. submonoceras* and *H. robusta*, while the lowest activities by *C. parthenoxylon* and *P. cablin*.

Table 4.6 Percentage of antidiabetic activity of 10 ethanol extracts.

sample name	sucrose substrate			maltose substrate		
	%I at 40 µg/mL	%I at 200 µg/mL	%I at 1000 µg/mL	%I at 40 µg/mL	%I at 200 µg/mL	%I at 1000 µg/mL
B. macrocarpa	nt	nt	nt	nt	nt	nt
C. parthenoxylon	ND	ND	ND	24.48 ± 0.97^{c}	29.66 ± 0.48^{d}	36.55 ± 2.71^{ef}
E. submonoceras	58.90 ± 2.61^{a}	76.26 ± 0.59^{a}	91.48 ± 8.13^{a}	63.94 ± 1.13^{a}	79.51 ± 0.82^{a}	93.64 ± 1.08^{a}
E. phaseoloides	ND	ND	ND	29.08 ± 0.12^{c}	30.73 ± 2.46^{d}	38.94 ± 2.05^{e}
G. macrophyllus	ND	ND	ND	25.83 ± 1.85^{c}	31.47 ± 0.82^{d}	43.08 ± 0.51^{d}
G. crepidioides	nt	nt	nt	nt	nt	nt
H. robusta	50.25 ± 1.86^{b}	70.44 ± 2.75^{b}	94.58 ± 2.21^{a}	70.39 ± 1.17^{a}	80.58 ± 0.91^{a}	94.85 ± 0.93^{a}
L. elliptica	ND	ND	ND	48.91 ± 3.64^{b}	53.58 ± 1.01^{c}	57.64 ± 1.58^{c}
P. cablin	ND	ND	ND	27.62 ± 0.24^{c}	31.20 ± 0.81^{d}	33.84 ± 1.04^{f}
R. tomentosa	54.35 ± 3.77^{b}	64.21 ± 3.47^{c}	91.66 ± 0.26^{a}	67.50 ± 1.26^{a}	73.34 ± 2.29^{a}	89.29 ± 1.92^{b}
acarbose	28.44 ± 0.94^{c}	53.11 ± 0.92^{d}	71.89 ± 1.85^{b}	29.89 ± 2.50^{c}	62.29 ± 2.11^{b}	86.88 ± 1.91^{b}

nt: not tested; ND: not detectable; %I: percentage of inhibition. All results are displayed as mean ± SD (n = 3) and followed by different letters to show statistically significant differences ($p \leq 0.05$).

Elaeocarpus species was reported to be effective as an **α**-glucosidase inhibitor. Ethanol extract of *E. serratus* fruits when was mixed with *p*-nitrophenyl-**α**-D-glucopyranoside (pNPG) substrate at 20 to 100 µg/mL of concentration exhibited moderate activity in the range from 6.68 % to 25.93 % (Geetha, Jayashree, & Rajeswari, 2015).

Previous reports about the antidiabetic activity of *Helicia* species have not been found yet. Therefore, this species became more interest to study further to know what compounds that responded to the activity.

4.6 Anticancer activity

The plant extracts were tested on breast cancer cells (MDA-MB-231) for their anticancer activity. The IC_{50} values ranged from 49.74 ± 3.82 µg/mL for *R. tomentosa* to 877.71 ± 7.58 µg/mL for *L. elliptica* after 24 h of treatment incubation (**Figure 4.1**). Unfortunately, all the extracts showed significantly different compared to etoposide with IC_{50} value was only 7.55 ± 0.74 µg/mL. *P. cablin* was relatively effective against MDA-MB-231 with an IC_{50} value of 90.59 ± 3.60 µg/mL, followed by *C. parthenoxylon* and *H. robusta* with IC_{50} values of 158.32 ± 0.91 and 186.03 ± 15.01 µg/mL, respectively.

Some compounds were isolated from the ethanol extract of *R. tomentosa* leaves among others gallic acid, myricetin, myricetin-3,7,3′-trimethyl ether, naringenin, and quercetin (Liu et al., 2016). Other reports beside of the *R. tomentosa* species

revealed the effectiveness of those compounds to inhibit the growth of cancer cells. Gallic acid could inhibit the growth of MCF-7 and MDA-MB-231, without interfering normal cells (Subramanian et al., 2015). Myricetin could inhibit MCF-7 cell growth compared to the positive control, vinblastine (Semwal et al., 2016). The IC_{50} value of myricetin-3,7,3′-trimethyl ether was only 7 μg/mL when inhibited the HepG2 hepatocellular carcinoma cells (Albadawi et al., 2017). Then, naringenin could also inhibit MDA-MB-231 growth by apoptosis induction and by suppressing caspase-3 and -9 activities (Wang et al., 2019). Study about quercetin showed the potential of this compound to inhibit angiogenesis in tamoxifen-resistant MCF-7 cells (Maalik et al., 2014).

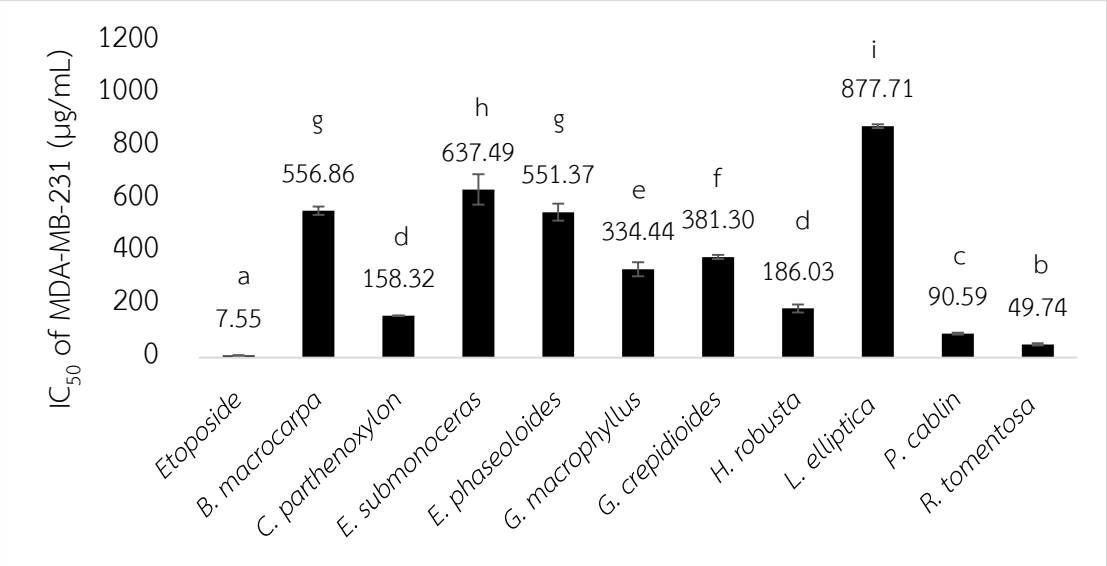


Figure 4.1 IC_{50} of anticancer activity against MDA-MB-231

All results are represented by mean + SD (n = 3). Statistically significant differences ($p \leq 0.05$) are shown by the differentiation of letters.

Tomentodione H-J and M was also isolated from the leaf ethanol extract of *R. tomentosa* and when combined with doxorubicin revealed more effective to treat doxorubicin-resistant human breast cancer cells (MCF-7/DOX) (Zhang et al., 2017).

4.7 Cytotoxicity effect

Comprehensive information about the content, including the cytotoxicity effect to normal cells, is needed for commercial applications (Fonseca-Santos et al., 2015). The present study treated HaCaT, human skin non-carcinoma cells by 10 ethanol extracts and presented the IC_{50} values ranged from 92.95 ± 3.09 µg/mL for *C. parthenoxylon* to 1000 µg/mL and above for *E. phaseoloides* and *L. elliptica* (**Figure 4.2**). The IC_{50} values of cytotoxicity effect were then compared to other biological activities results and found *R. tomentosa* is safe and the most effective for all the activities (antioxidant, antibacterial, anti-tyrosinase, antidiabetic, and anticancer). *E. submonoceras* are useful as an antioxidant, anti-tyrosinase, and antidiabetic agents, while *E. phaseoloides* can be applied as an antioxidant, anti-tyrosinase, and anticancer agents. Furthermore, *G. macrophyllus* and *P. cablin* can be used as antioxidant and anticancer candidates. Lastly, *H. robusta* and *L. elliptica* may be applied as antioxidant, antidiabetic, and anticancer mediators.

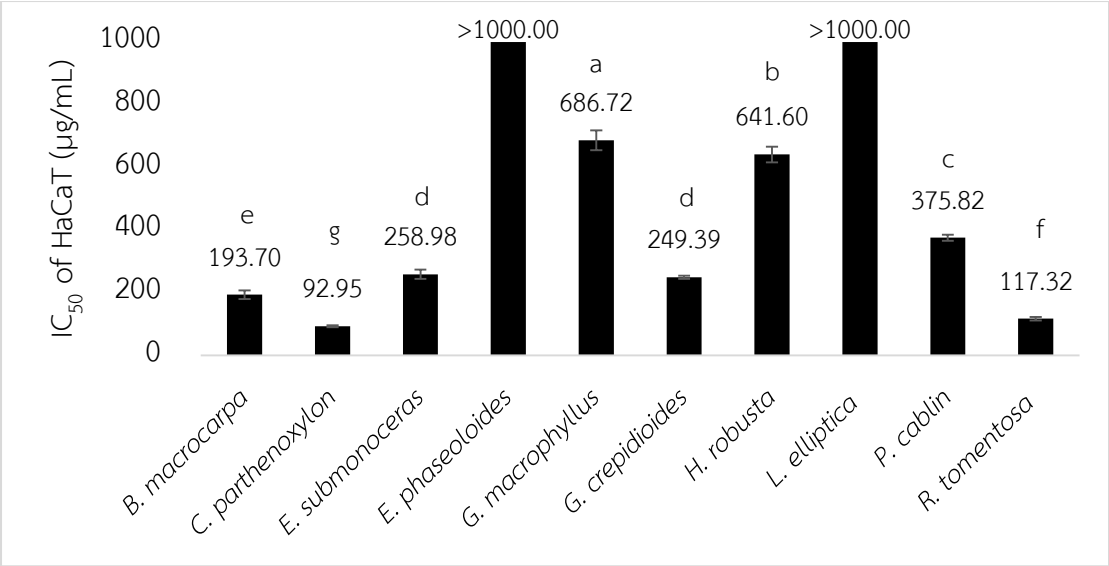
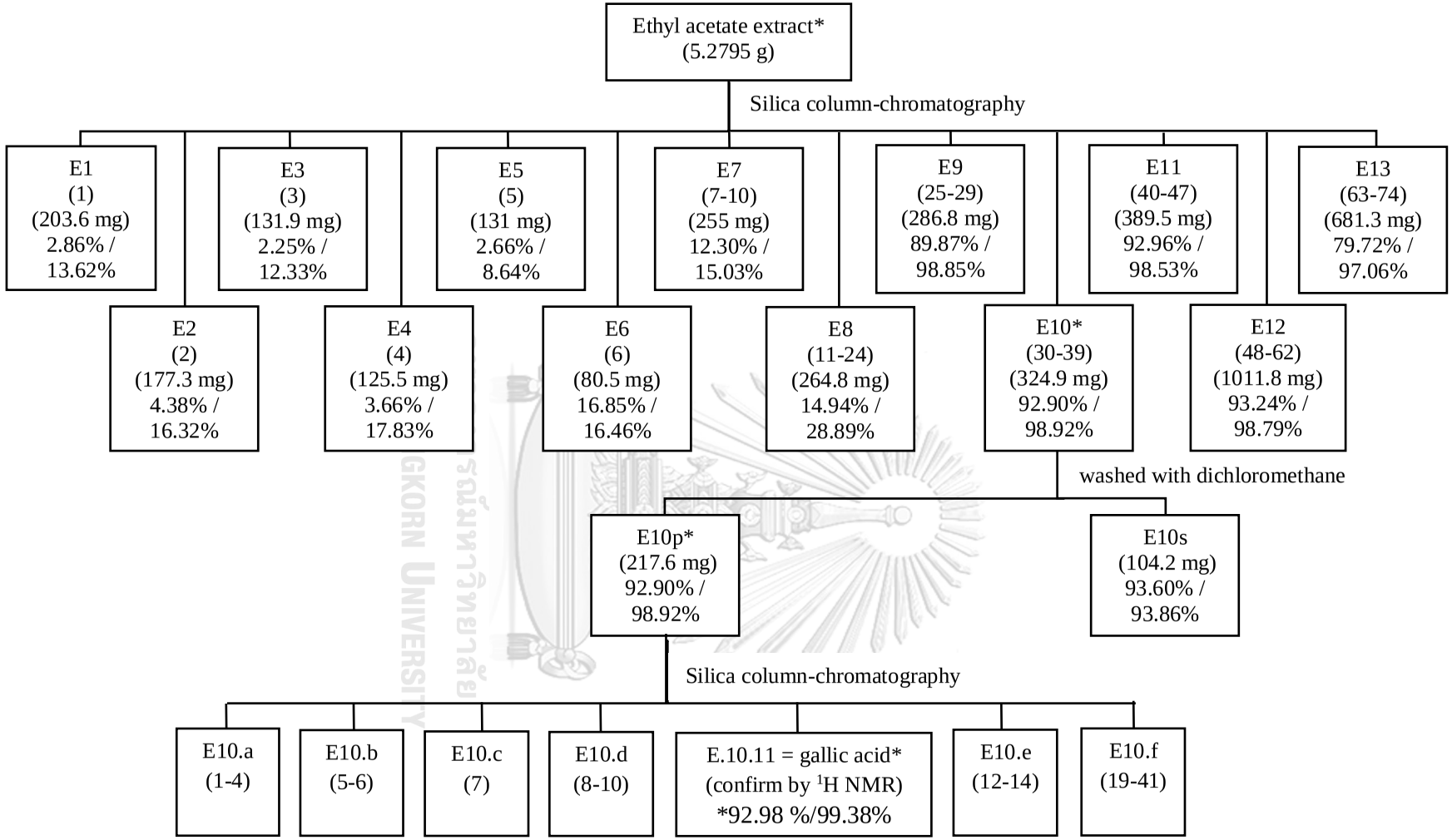


Figure 4.2 IC_{50} of cytotoxicity effect against HaCaT.

All results are represented by mean + SD (n = 3). Statistically significant differences ($p \leq 0.05$) are shown by the differentiation of letters.

4.8 Isolation of compound from *E. submonoceras* Miq. and the bioactivities

The plant that selected for further compound isolation was *E. submonoceras* because of its ethanol extract performed excellent values by antioxidant assays. Successive extraction was carried out in the amount of more leaf material with four different polarities of solvents as depicted in **Scheme 3.1** then continued to **Scheme 4.1**. The scheme in this chapter is represented in detail from which extract and fractions that the compound carried out (marked with "*****") with the data of the percentage of DPPH and ABTS inhibition as the bio-guided assays for the isolation.



Scheme 4.1 The process of isolation of gallic acid by antioxidant-assay-guided from *E. submonoceras* Miq.

Numbering in parentheses indicate the numbers of the tube that collected in one fraction. The numbers in mg unit mean the weight of each fraction. The numbers in percent mean the percentage of DPPH / ABTS inhibition at 100 µg/mL of sample concentration, except for %DPPH inhibition value of ethyl acetate extract obtained from 25 µg/mL of sample concentration.

4.8.1 Structural identification of isolated compound

A compound was isolated from ethyl acetate successive extract and showed a blue color reaction when mixed with Folin–Ciocalteu's reagent. It was then confirmed by a TLC plate and showed only one spot even though the solvent systems were varied. This compound coded by E10.11 was obtained at about 12.2 mg of weight with white-crystallin-powder. Identification of the compound used ^{1}H NMR ($(CD_3)_2CO$, 400 MHz), δ (ppm): 7.15 (d, *J*= 1.2 Hz, 2 H (H-2 and H-6)) (**Figure 4.3**), and the results revealed that it was gallic acid.

The 1H NMR result of gallic acid was similar to another report for *Elaeocarpus* species. This compound was contained in leaf extracts of *E. sylvestris* and *E. tonkinensis* (Dao et al., 2019; Prihantini et al., 2015). This compound was discovered as one of the major compounds in *E. ganitrus* and *E. tuberculatus* (Chand et al., 1977). Gallic acid is known as 3,4,5-trihydroxybenzoic acid and is one of the phenolic group members. It has one aromatic ring, one carboxylic acid, and three hydroxyl substituents. It has a low molecular weight with a simple structure but possesses various biological activities including antioxidant properties and easy to be absorbed in the human metabolic system (Badhani, Sharma, & Kakkar, 2015). The present study confirmed that this is the first report regarding compound isolation including gallic acid from *E. submonoceras* and also the biological activities information of this plant.

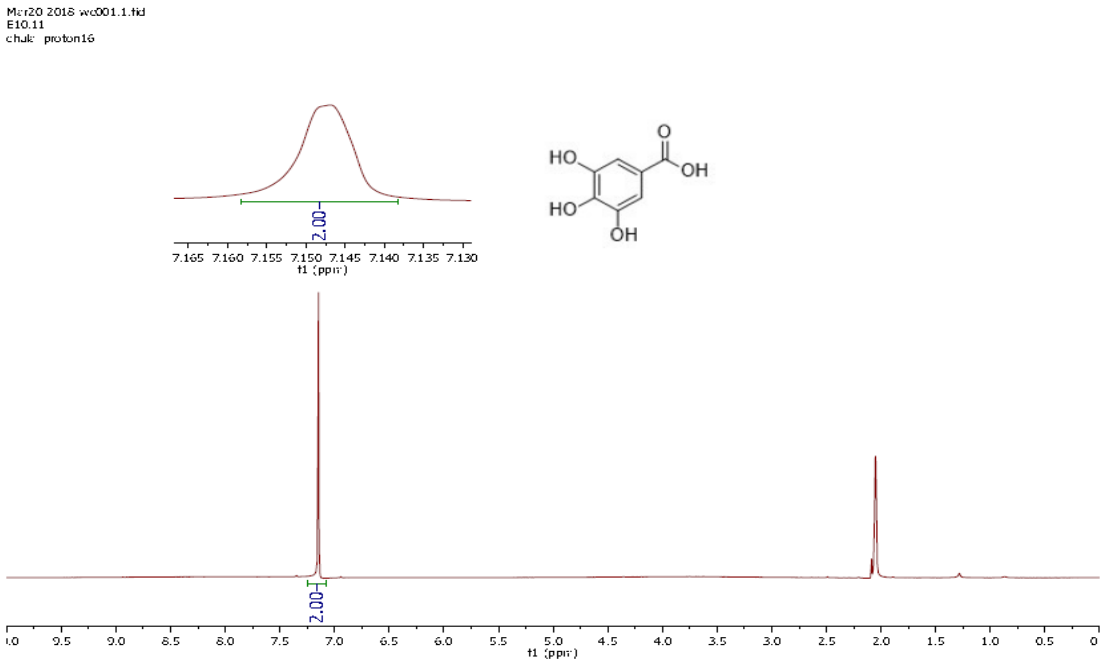


Figure 4.3 ^{1}H NMR of purified compound (gallic acid)

4.8.2 Yield, TPC, TAC, DPPH, and ABTS

The percentage yields, TPC, TAC, DPPH, and ABTS results of four extracts and the isolated compound (gallic acid) obtained from *E. submonoceras* leaves are presented in **Table 4.7**. Methanol extract has the highest yield (17 %) compare to other crude extracts, followed by ethyl acetate extract (1.06 %).

TPC and TAC analyses were carried out to get information about the effect of polarity on the content of phenolic or antioxidant. The highest TPC was obtained from methanol extract (542.81 ± 50.11 mg GAE/g DW) and not significantly different from ethyl acetate extract (504.92 ± 43.23 mg GAE/g DW). The lowest TPC value was obtained from hexane extract, followed by dichloromethane extract. TAC values

trend was the same with TPC values in the range from 53.22 to 343.45 mg AAE/g DW. The different phenolic content and antioxidant capacity of each extract could be due to polarity characteristic of the extraction solvents (Abarca-Vargas, Pena Malacara, & Petricevich, 2016). The common solvents to extract phenolics are methanol, ethanol, acetone, propanol, ethyl acetate, water, or the combination of them (Naczk & Shahidi, 2006).

Studies about antioxidant activities related to successive extracts of *Elaeocarpus* species have never been reported before. Results of DPPH percentage of inhibition were shown in **Table 4.7** from 100 µg/mL of samples concentration exhibited in the range from 35.37 % for hexane extract to 94.71 % for ethyl acetate extract. The ABTS percentage inhibition from the same concentration had ranged from 11.04 to 98.41 %. Ethyl acetate and methanol extracts exhibited to be the best antioxidant performance by both methods in the IC_{50} ranged from 7.67 to 14 µg/mL, while the last one was from the hexane extract. Based on these results, the antioxidant activities may originate from polar compounds. This condition was similar to the data of successive extraction of three species of *Mentha* (*M. spicata*, *M. pulegium*, and *M. piperita*) that performed the activity of DPPH inhibition was showed only from methanol and water (the polar extracts), rather than from hexane and dichloromethane (the non-polar extracts) (Barchan, Bakkali, Arakrak, Pagán, & Laglaoui, 2014). Barchan et al. (2014) also revealed that the activity of polar extracts related to their phenolic content.

Table 4.7 Yield, TPC, TAC, DPPH, and ABTS of *E. submonoceras* Miq. leaves.

Extract / compound	%Yield*	TPC (mg GAE/g DW)	TAC (mg AAE/g DW)	DPPH		ABTS	
				%I	IC_{50} (µg/mL)	%I	IC_{50} (µg/mL)
Hexane	0.78	7.65 ± 0.55^{b}	53.22 ± 3.08^{d}	35.37 ± 2.31^{d}	92.10 ± 4.69^{b}	11.04 ± 0.34^{d}	>1000
Dichloromethane	0.67	57.23 ± 4.08^{b}	90.11 ± 4.59^{c}	64.99 ± 2.69^{c}	307.33 ± 0.06^{c}	13.98 ± 0.76^{c}	827.82 ± 5.20^{c}
Ethyl acetate	1.06	504.92 ± 43.23^{a}	274.21 ± 6.43^{b}	94.71 ± 0.66^{a}	7.81 ± 0.19^{a}	95.94 ± 1.10^{b}	14.00 ± 0.57^{a}
Methanol	17.00	542.81 ± 50.11^{a}	343.45 ± 1.51^{a}	94.07 ± 0.14^{a}	7.67 ± 0.09^{a}	96.75 ± 0.18^{ab}	13.89 ± 0.34^{a}
Gallic acid	0.23	-	-	90.05 ± 1.13^{b}	10.04 ± 0.84^{a}	98.41 ± 0.24^{a}	25.33 ± 0.54^{b}

*: the yield was calculated by dividing the weight of obtained gallic acid with the weight of ethyl acetate extract at 100 %. The sample concentration for %I (percentage of inhibition) value of DPPH and ABTS was 100 µg/mL. All result in this table excludes %Yield denotes the mean ± SD (n = 3). Significantly different ($p \leq 0.05$) are shown by different superscript letters.

Fifteen phenolic compounds that identified by Li et al. (2017) also performed a positive correlation when tested by DPPH and ABTS assays. Results of antioxidant activities also related to TPC and TAC and it might be because of the existence of gallic acid and other phenolic compounds contained in the plant (Fawole et al., 2012).

Furthermore, the ABTS scavenging assay in all results showed lower than DPPH due to the characteristic of ABTS that more sensitive to hydrophilic and high-pigmented antioxidants, while DPPH is appropriate to hydrophobic antioxidants (Floegel, Kim, Chung, Koo, & Chun, 2011). This phenomenon also answered the low activities of hexane and dichloromethane, especially when reacting to ABTS free radical.

4.8.3 Antibacterial activity

The six strains of bacteria were used to examine the antibacterial activities of four successive extracts. Data, as shown in **Table 4.8** and **Table 4.9**, contained the percentage of inhibition compared to chloramphenicol, MIC, and MBC against Gram-positive and Gram-negative, respectively. Percentage of inhibition at 1000 µg/mL of extracts against Gram-positive: *P. acnes, S. aureus, S. mutans*, and *S. sobrinus* were 23.9 %–31.2 %, 29 %–36.6 %, 37 %–47.3 %, and 31.3 %–42.9 %, respectively, while against Gram-negative: *P. aeruginosa* and *S. typhi* were 57.5 %–73.9 % and 32.4 %–45.9 %, respectively.

Table 4.8 Percentage of inhibition, MIC, and MBC of *E. submonoceras* Miq. successive extracts against Gram-positive bacteria

Sample	*P. acnes* KCCM 41747			*S. aureus* ATCC 25923			*S. mutans* ATCC 25175			*S. sobrinus* KCCM 11898		
	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC
Hexane	23.9 ± 2.4^{d}	>1000	>1000	30.1 ± 1.1^{d}	>1000	>1000	37.0 ± 1.2^{d}	>1000	>1000	31.3 ± 1.6^{d}	>1000	>1000
Dichloromethane	27.6 ± 1.2^{c}	>1000	>1000	29.0 ± 2.3^{d}	1000	>1000	43.1 ± 5.1^{bc}	>1000	>1000	37.8 ± 0.3^{c}	>1000	>1000
Ethyl acetate	27.2 ± 0.5^{c}	1000	>1000	34.2 ± 0.8^{c}	1000	>1000	38.3 ± 2.5^{cd}	1000	>1000	38.7 ± 1.1^{c}	1000	>1000
Methanol	31.2 ± 2.9^{b}	>1000	>1000	36.6 ± 2.9^{c}	1000	>1000	47.3 ± 2.7^{b}	>1000	>1000	42.9 ± 3.9^{b}	1000	>1000
Chloramphenicol	100 ± 0^{a}	3.90625	15.625	100 ± 0^{a}	15.625	31.25	100 ± 0^{a}	3.90625	7.8125	100 ± 0^{a}	3.90625	3.90625

The concentration of extracts for %inhibition was 1000 µg/mL. The %inhibition was calculated by comparing to chloramphenicol. All the %inhibition values are denoted by the mean ± SD (n = 3). Different superscript letters show significantly different ($p \leq 0.05$). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) units are µg/mL.

Table 4.9 Percentage of inhibition, MIC, and MBC of *E. submonoceras* Miq. successive extracts against Gram-negative bacteria

Sample	*P. aeruginosa* TISTR 1287			*S. typhi* ATCC 422		
	%Inhibition	MIC	MBC	%Inhibition	MIC	MBC
Hexane	73.9 ± 6.1^{b}	1000	>1000	32.4 ± 2.4^{e}	>1000	>1000
Dichloromethane	65.0 ± 6.8^{bc}	1000	>1000	38.7 ± 3.6^{d}	>1000	>1000
Ethyl acetate	60.1 ± 6.6^{bc}	1000	>1000	43.0 ± 0.4^{bc}	500	>1000
Methanol	57.5 ± 4.0^{c}	1000	>1000	45.9 ± 1.6^{b}	1000	>1000
Chloramphenicol	100 ± 0^{a}	62.5	250	100 ± 0^{a}	15.625	31.25

The concentration of extracts for %inhibition was 1000 µg/mL. The %inhibition was calculated by comparing to chloramphenicol. All the %inhibition values are denoted by the mean ± SD (n = 3). Different superscript letters show significantly different ($p \leq 0.05$). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) units are µg/mL.

Ethyl acetate and methanol extracts showed more effective especially when against *S. typhi* with MIC values were 500 and 1000 µg/mL, respectively. The MBC value of all extracts was more than 1000 µg/mL. Even though all the extracts showed the activity against all the bacteria strains, unfortunately, the results were significantly different compared to chloramphenicol. Among the two groups, all the samples were more effective to Gram-negative than to Gram-positive. It is contrary to many research (Koohsari, Ghaemi, Sadegh Sheshpoli, Jahedi, & Zahiri, 2015; Limsuwan et al., 2009) that usually more effective against Gram-positive bacteria. The less activity of the samples up to 1000 µg/mL, even though some plants use for skin disease and wound treatment by local people, maybe related to protection effect, instead of pharmacological (Taylor, Rabe, McGaw, Jäger, & van Staden, 2001).

4.8.4 Anti-tyrosinase activities

Percentage of tyrosinase inhibition at 500 µg/mL of sample concentration was shown by all extracts and gallic acid except hexane extract (**Table 4.10**). The lowest value was performed by ethyl acetate extract, which was the highest value performed by gallic acid. The IC_{50} value was then only revealed by gallic acid (358.89 µg/mL) for the maximum concentration of tested was 1000 µg/mL. The activity of this compound was significantly different from kojic acid. It is an interesting result when the separation of compounds based on the polarity performed not too excellent than when the compound was isolated if refer to the IC_{50} value. Therefore, the activity of gallic acid, which contained in the polar extracts may interfere by other compounds or known as antagonism effect as confirmed by Olszowy, Dawidowicz, and Jóźwik-Dolęba (2019). Furthermore, compared to kojic acid, the activity of gallic acid was also lower in this study. It similar to a review mentioned about the activity of gallic acid was 100-fold lower than kojic acid. It could be happened because of gallic acid or other short alkyl chain esters (<C10) worked as substrates when reacted with mushroom tyrosinase (Chang, 2009).

Table 4.10 Tyrosinase inhibition of *E. submonoceras* Miq.

Sample Name	% inhibition	IC_{50} (µg/mL)
Kojic acid	100.00 ± 0.43[a]	1.18 ± 0.02[a]
Hexane	ND	>1000
Dichloromethane	36.21 ± 0.83[c]	>1000
Ethyl acetate	20.07 ± 1.99[d]	>1000
Methanol	41.33 ± 2.76[c]	>1000
Gallic acid (isolated compound)	56.62 ± 1.06[b]	358.89 ± 8.92[b]

ND: not detectable. The %inhibition was done at 500 µg/mL. Data are represented as mean ± SD (n = 3). Values in the same column are significantly different ($p \leq 0.05$) when followed by different superscript letters.

4.8.5 Antidiabetic activity

The four extracts and gallic acid when was reacted with α-glucosidase in the presence of a sucrose substrate performed no activity at 40 µg/mL of sample concentration, while at 200 µg/mL was only shown by gallic acid (57.74 % of inhibition). At the highest concentration (1000 µg/mL), gallic acid was still the best (70.07 %), followed by ethyl acetate and then methanol extracts. Gallic acid was not significantly different with ethyl acetate extract and acarbose. All the extracts in the presence of a maltose substrate performed the activity to inhibit α-glucosidase in all three variations of concentrations with a dose-dependent manner. At the lowest concentration (40 µg/mL) was expressed in the range from 32.42 % for ethyl acetate extract to 73.9 % for gallic acid, while at 200 µg/mL in the range from 51.52 % for dichloromethane extract to 88.79 % for gallic acid also.

Table 4.11 Antidiabetic activity of *E. submonoceras* leaves

sample name	sucrose substrate			maltose substrate		
	%I at 40 µg/mL	%I at 200 µg/mL	%I at 1000 µg/mL	%I at 40 µg/mL	%I at 200 µg/mL	%I at 1000 µg/mL
Hexane	ND	ND	ND	52.63 ± 0.40^{b}	52.72 ± 0.86^{de}	54.74 ± 4.12^{c}
Dichloromethane	ND	ND	ND	48.93 ± 1.18^{c}	51.52 ± 3.59^{e}	53.14 ± 0.52^{c}
Ethyl acetate	ND	ND	64.97 ± 2.15^{bc}	32.42 ± 3.40^{d}	56.71 ± 3.16^{d}	88.08 ± 1.24^{ab}
Methanol	ND	ND	62.77 ± 4.09^{c}	59.50 ± 2.81^{b}	67.77 ± 2.71^{b}	90.14 ± 2.89^{a}
Gallic acid	ND	57.74 ± 1.26	70.07 ± 6.37^{ab}	73.90 ± 4.06^{a}	88.79 ± 1.07^{a}	89.50 ± 0.13^{ab}
Acarbose	28.44 ± 0.94	53.11 ± 0.92	71.89 ± 1.85^{a}	29.89 ± 2.50^{d}	62.29 ± 2.11^{c}	86.88 ± 1.91^{b}

ND: not detectable; %I: percentage of inhibition. All results are displayed as mean ± SD (n = 3) and followed by different letters to show statistically significant differences ($p \leq 0.05$).

At the highest concentration, dichloromethane extract showed the lowest performance, while methanol was the best and even better than acarbose. There was a limited report for the antidiabetic activity of *Elaeocarpus* species. A report revealed about the activity of *E. sylvestris* leaf methanol extract with low of IC_{50} value (74.4 ± 0.9 µg/mL) in the reaction of the pNPG substrate (Prihantini, Tachibana, & Itoh, 2014).

4.8.6 Cytotoxicity effect

The cytotoxicity effect of extracts and gallic acid against HaCaT were displayed by IC_{50} value in **Figure 4.4**. Gallic acid had the lowest value at 92.28 ± 4.47 µg/mL, while ethyl acetate had the highest value at 446.25 ± 24.78 µg/mL. The values were then compared to their biological activities. Ethyl acetate and methanol extracts, also gallic acid are safe for antioxidant agents.

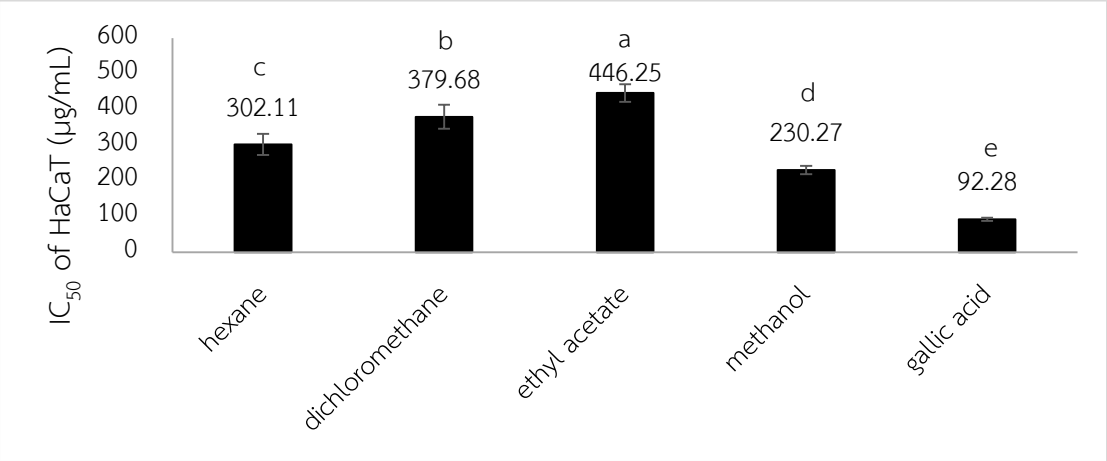


Figure 4.4 Cytotoxicity effect of *E. submonoceras* Miq against HaCaT.

All results are expressed by mean ± SD (n = 3). Statistically significant differences ($p \leq 0.05$) are shown by the differentiation of letters.

All the extracts could be applied as an antidiabetic agent in the presence of maltose substrate, while gallic acid could be applied in the presence of both substrates.

4.8.7 Wound healing activity

Local people (Dayak tribe) use the leaves of this plant since ancient times including treatment of skin diseases and wound healing. Therefore, this assay was performed to ethanol maceration extract (detail in section **3.2**), four successive extracts (section **3.11.1**), and the isolated gallic acid compound (section **3.11.2**). **Figure 4.5** presented the percentage of wound healing activity of HaCaT cells under the treatment of samples at 10 µg/mL of concentration. The successive extracts (hexane, dichloromethane, ethyl acetate, and methanol) performed lower of activity compared to the non-treatment control, while gallic acid (30.08 ± 0.33 % and 35.99 ± 1.35 %) was the best and followed by ethanol extract (21.26 ± 1.64 % and 33.45 ± 3.15 %) after incubated for 24h and 48h, respectively. The cells migration of ethanol extract and gallic acid was displayed in **Figure 4.6**.

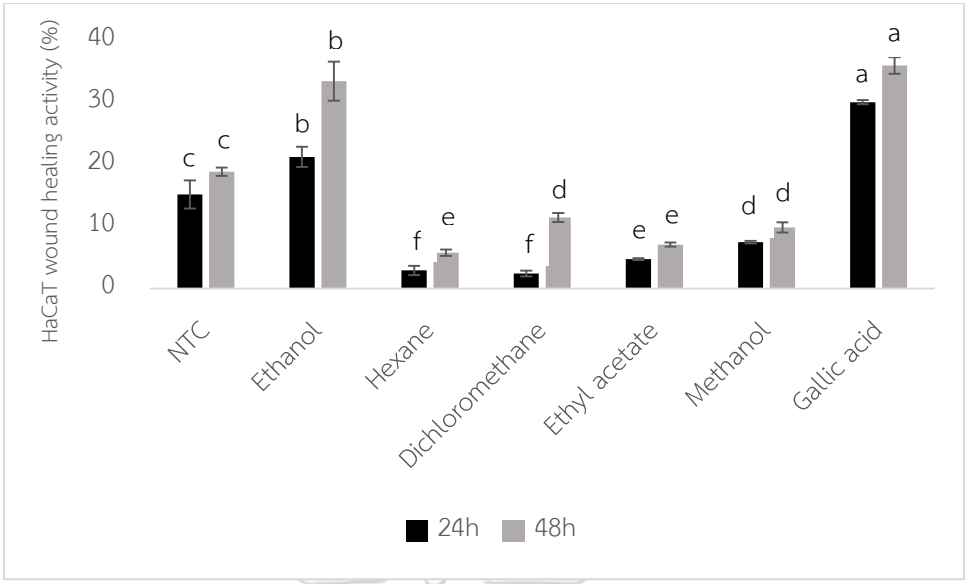


Figure 4.5 Wound healing activity of *E. submonoceras* Miq.

NTC: non-treatment control. The bars are displayed as mean ± SD with statistically significant differences ($p \leq 0.05$) shown by differentiation of letters at the same incubation time. The incubation was performed at 24 h (black bar) and 48 h (grey bar) after scratching with the concentration of sample was 10 µg/mL.

Dried fruit of *E. ganitrus* (another related species) is also used for wound healing of Ayurveda treatment (Hardainiyan, Nandy, & Saxena, 2015). Interestingly, this is the first scientific report of *Elaeocarpus* species. Gallic acid was reported for HaCaT wound healing activity with the same scratch assay in lower concentration at 10 µM (equal to 1.7012 µg/mL) (Yang et al., 2016).

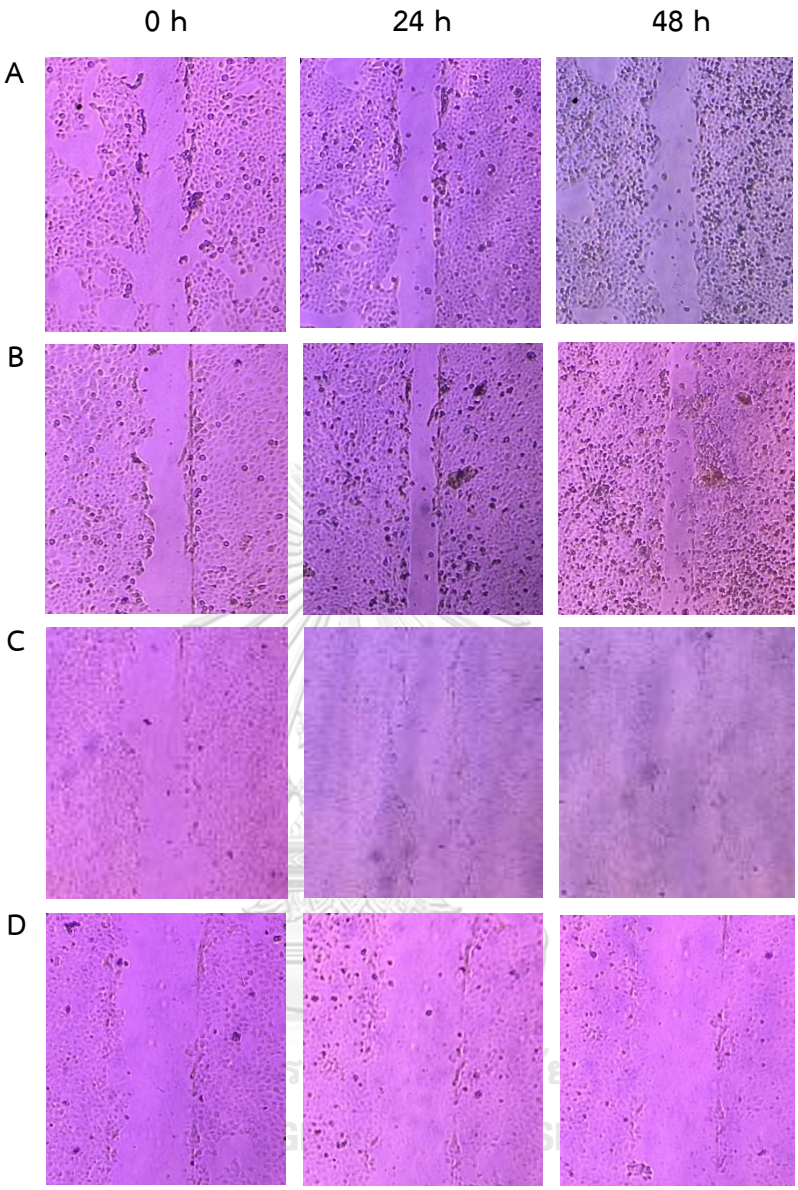


Figure 4.6 HaCaT migration of A) non-treatment control; B) ethanol extract; C) hexane extract; D) dichloromethane extract at 0 h, 24 h, and 48 h of incubation times.

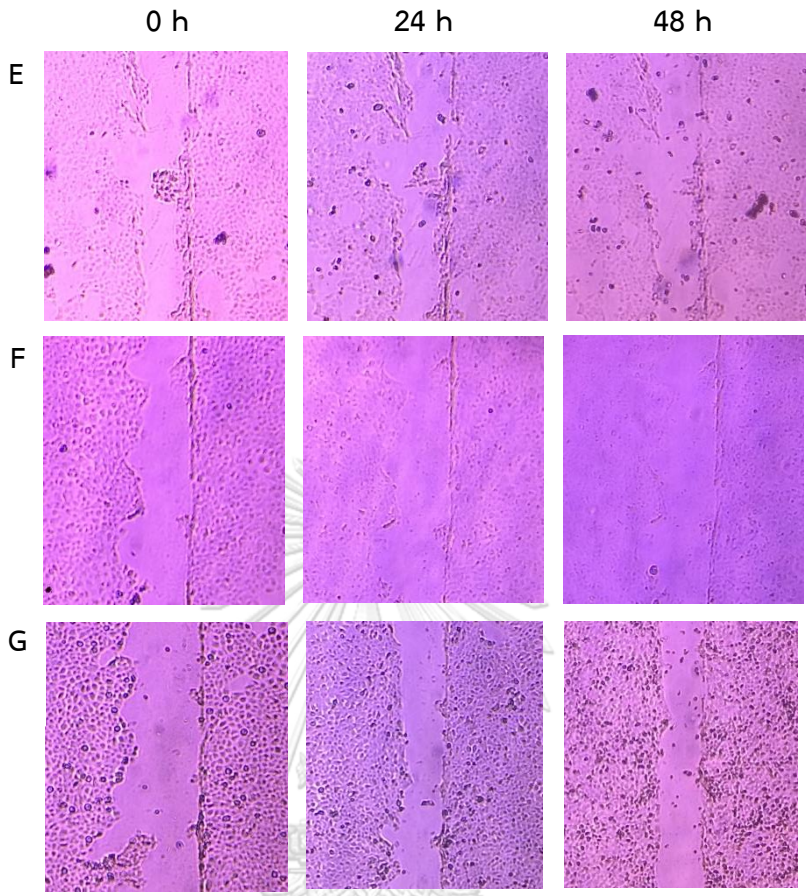


Figure 4.6 HaCaT migration of E) ethyl acetate extract; F) methanol extract; G) gallic acid at 0 h, 24 h, and 48 h of incubation times.

CHAPTER V

CONCLUSION

The ethanol extracts from leaves of ten medicinal plants used by the Dayak tribes in East Kalimantan, Indonesia exhibited some biological activities which were then compared with their cytotoxicity effect against HaCaT, the non-carcinoma cells. *R. tomentosa* is recommended for all the activities (antioxidant, antibacterial, anti-tyrosinase, antidiabetic, and anticancer). *E. submonoceras* is promising as an antioxidant, anti-tyrosinase, and antidiabetic agents, while *E. phaseoloides* is useful as an antioxidant, anti-tyrosinase, and anticancer agents. *G. macrophyllus* and *P. cablin* can be used as antioxidant and anticancer agents. Hereinafter, *H. robusta* and *L. elliptica* could be applied as antioxidant, antidiabetic, and anticancer agents.

E. submonoceras ethanol extract was the one among all ten plants that showed the best activity of antioxidant, then was chosen to study further regarding isolation of the active compound. A greater amount of leaf material was then extracted successfully using four different polarities of solvents by Soxhlet extractor. The compound that isolated from ethyl acetate extract of this plant was known as gallic acid. Further, the biological activities and cytotoxicity effect were tested to the extracts and the compound. Ethyl acetate extract, methanol extract, and gallic acid are safe as antioxidant agents and can be applied on the surface of the skin (topical

application). All the extracts are promising as an antidiabetic agent in the presence of maltose substrate, while gallic acid is promising in the presence of both substrates (sucrose and maltose).

Proposal for future research

Information about the biological activities from this study could lead to further research regarding isolation and identification of active compounds related to their specific activities. Moreover, the mechanism and gene expression of the extracts directly into the cells (*in vitro*) and animal (*in vivo*) also need to be analyzed.

REFERENCES

Abarca-Vargas, R., Pena Malacara, C. F., & Petricevich, V. L. (2016). Characterization of chemical compounds with antioxidant and cytotoxic activities in *Bougainvillea* x *buttiana* Holttum and Standl, (var. Rose) extracts. *Antioxidants, 5*(4), 1-11.

Adedayo, B. C., Oboh, G., Oyeleye, S. I., Ejakpovi, I. I., Boligon, A. A., & Athayde, M. L. (2015). Blanching alters the phenolic constituents and in vitro antioxidant and anticholinesterases properties of fireweed (*Crassocephalum crepidioides*). *Journal of Taibah University Medical Sciences, 10*(4), 419-426.

Alabsi, A. M., Ali, R., Ali, A. M., Harun, H., Al-Dubai, S. A., Ganasegeran, K., . . . Abu Kasim, N. H. (2013). Induction of caspase-9, biochemical assessment and morphological changes caused by apoptosis in cancer cells treated with goniothalamin extracted from Goniothalamus macrophyllus. *Asian Pac J Cancer Prev, 14*(11), 6273-6280.

Alam, F., Najum Us Saqib, Q., & Waheed, A. (2017). Cytotoxic activity of extracts and crude saponins from *Zanthoxylum armatum* DC. against human breast (MCF-7, MDA-MB-468) and colorectal (Caco-2) cancer cell lines. *BMC Complementary and Alternative Medicine, 17*(1), 368-368.

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal, 21*(2), 143-152.

Albadawi, D. A., Mothana, R. A., Khaled, J. M., Ashour, A. E., Kumar, A., Ahmad, S. F., . . . Almusayeib, N. M. (2017). Antimicrobial, anticancer, and antioxidant compounds from *Premna resinosa* growing in Saudi Arabia. *Pharmaceutical Biology, 55*(1), 1759-1766.

Aniya, Y., Koyama, T., Miyagi, C., Miyahira, M., Inomata, C., Kinoshita, S., & Ichiba, T. (2005). Free radical scavenging and hepatoprotective actions of the medicinal herb, *Crassocephalum crepidioides* from the Okinawa Islands. *Biol Pharm Bull, 28*(1), 19-23.

Arung, E. T., Kusuma, I. W., Christy, E. O., Shimizu, K., & Kondo, R. (2009). Evaluation of

medicinal plants from Central Kalimantan for antimelanogenesis. *J Nat Med, 63*(4), 473-480.

Badhani, B., Sharma, N., & Kakkar, R. (2015). Gallic acid: a versatile antioxidant with promising therapeutic and industrial applications. *RSC Advances, 5*(35), 27540-27557.

Bae, S.-Y., Lee, E.-J., Son, R.-H., & Lee, Y.-H. (2009). The inhibitory effects of *Pogostemon cablin* Bentham extract on melanogenesis. *Journal of the Society of Cosmetic Scientists of Korea, 35*(1), 33-39.

Banerjee, M., Chattopadhyay, S., Choudhuri, T., Bera, R., Kumar, S., Chakraborty, B., & Mukherjee, S. K. (2016). Cytotoxicity and cell cycle arrest induced by andrographolide lead to programmed cell death of MDA-MB-231 breast cancer cell line. *Journal of Biomedical Science, 23*(1), 40.

Barchan, A., Bakkali, M., Arakrak, A., Pagán, R., & Laglaoui, A. (2014). The effects of solvents polarity on the phenolic contents and antioxidant activity of three *Mentha* species extracts. *International Journal of Current Microbiology and Applied Sciences, 3*(11), 399-412.

Basumatary, S., Das, A. K., Nanjian, R., & Sharma, G. D. (2015). In vitro evaluation of antioxidant properties of *Hodgsonia heteroclita* (Cucurbitaceae) fruit. *Journal of Applied Pharmaceutical Science, 5*, 80-83.

Baurin, N., Arnoult, E., Scior, T., Do, Q. T., & Bernard, P. (2002). Preliminary screening of some tropical plants for anti-tyrosinase activity. *Journal of Ethnopharmacology, 82*(2), 155-158.

Benmehdi, H., Behilil, A., Memmou, F., & Amrouche, A. (2017). Free radical scavenging activity, kinetic behaviour and phytochemical constituents of *Aristolochia clematitis* L. roots. *Arabian Journal of Chemistry, 10*(1), S1402-S1408.

Beveridge, T. J. (1999). Structures of Gram-negative cell walls and their derived membrane vesicles. *Journal of Bacteriology, 181*(16), 4725-4733.

Biswas, S. K., Chowdhury, A., Das, J., Chowdhury, A., Raihan, S. Z., & Muhit, M. A. (2012). Phytochemical investigation with assessment of cytotoxicity and antibacterial activities of the ethanol extract of *Elaeocarpus serratus*. *American Journal of*

Plant Physiology, 7(1), 47-52.

Bowe, W. P., & Pugliese, S. (2014). Cosmetic benefits of natural ingredients. *Journal of Drugs in Dermatology, 13*(9), 1019-1027.

Bunrathep, S., Lockwood, G. B., Songsak, T., & Ruangrungsi, N. (2006). Chemical constituents from leaves and cell cultures of *Pogostemon cablin* and use of precursor feeding to improve patchouli alcohol level. *ScienceAsia, 32*(3), 293-296.

Caretto, S., Linsalata, V., Colella, G., Mita, G., & Lattanzio, V. (2015). Carbon fluxes between primary metabolism and phenolic pathway in plant tissues under stress. *International Journal of Molecular Sciences, 16*(11), 26378-26394.

Chand, L., Dasgupta, S., Chattopadhyay, S. K., & Ray, A. B. (1977). Chemical investigation of some *Elaeocarpus* species. *Planta Medica, 32*(06), 197-199.

Chang, T. S. (2009). An updated review of tyrosinase inhibitors. *Internatonal Journal of Molecular Sciences, 10*(6), 2440-2475.

Cheynier, V., Comte, G., Davies, K. M., Lattanzio, V., & Martens, S. (2013). Plant phenolics: recent advances on their biosynthesis, genetics, and ecophysiology. *Plant Physiology and Biochemistry 72*, 1-20.

Chung, R. C. K. (2001). Taxonomic notes on the Bornean *Helicia* and *Heliciopsis* (Proteaceae). *Journal of Tropical Forest Science, 13*(3), 534-547.

Coode, M. J. E. (2001). *Elaeocarpus* for flora Malesiana: The *E. stipularis* complex, *E. nitidus* group & *E. barbulatus*. *Kew Bulletin, 56*(3), 513-565.

Damjuti, W. (2013). *In vitro and in vivo bioactivities of Leptocarpus disjunctus ethanolic extract.* (Master). Chulalongkorn University, Bangkok, Thailand.

Dao, N. T., Jang, Y., Kim, M., Nguyen, H. H., Pham, D. Q., Dang, Q. L., . . . Hoang, V. D. (2019). Chemical constituents and anti-influenza viral activity of the leaves of Vietnamese plant *Elaeocarpus tonkinensis*. *Records of Natural Products, 13*(1), 71-80.

Do, Q. D., Angkawijaya, A. E., Tran-Nguyen, P. L., Huynh, L. H., Soetaredjo, F. E., Ismadji, S., & Ju, Y. H. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. *Journal of*

Food and Drug Analysis, 22(3), 296-302.

Duletić-Laušević, S., Alimpić Aradski, A., Šavikin, K., Knežević, A., Milutinović, M., Stević, T., . . . Marin, P. D. (2018). Composition and biological activities of Libyan *Salvia fruticosa* Mill. and *S. lanigera* Poir. extracts. *South African Journal of Botany, 117*, 101-109.

Elfahmi, Woerdenbag, H. J., & Kayser, O. (2014). Jamu: Indonesian traditional herbal medicine towards rational phytopharmacological use. *Journal of Herbal Medicine, 4*(2), 51-73.

Fawole, O. A., Makunga, N. P., & Opara, U. L. (2012). Antibacterial, antioxidant and tyrosinase-inhibition activities of pomegranate fruit peel methanolic extract. *BMC Complementary and Alternative Medicine, 12*(200), 1-11.

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis, 24*(7), 1043-1048.

Fonseca-Santos, B., Corrêa, M. A., & Chorilli, M. (2015). Sustainability, natural and organic cosmetics: consumer, products, efficacy, toxicological and regulatory considerations. *Brazilian Journal of Pharmaceutical Sciences, 51*(1), 17-26.

Fu, R., Zhang, Y., Guo, Y., & Chen, F. (2014). Antioxidant and tyrosinase inhibition activities of the ethanol-insoluble fraction of water extract of *Sapium sebiferum* (L.) Roxb. leaves. *South African Journal of Botany, 93*, 98-104.

Garg, K., Goswami, K., & Khurana, G. (2012). A pharmacognostical review on *Elaeocarpus sphaericus*. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*(1), 3-8.

Geetha, D. H., Jayashree, I., & Rajeswari, M. (2015). Evaluation of in vitro anti-diabetic activity of *Elaeocarpus serratus* fruit. *International Journal of Pharmaceutical and Phytopharmacological Research, 5*(2), 26-29.

Geetha, D. H., Rajeswari, M., & Jayashree, I. (2013). Chemical profiling of *Elaeocarpus serratus* L. by GC-MS. *Asian Pacific Journal of Tropical Biomedicine, 3*(12), 985-987.

Gokulakrishnan, J., Kuppusamy, E., Shanmugam, D., Appavu, A., & Kaliyamoorthi, K.

(2013). Pupicidal and repellent activities of *Pogostemon cablin* essential oil chemical compounds against medically important human vector mosquitoes. *Asian Pacific Journal of Tropical Disease, 3*(1), 26-31.

Hairani, R. (2016). *Synthesis of Mansonone Derivatives as Antibacterial Agents.* (Master of Science). Chulalongkorn University, Bangkok, Thailand.

Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications. *African Journal of Pure and Applied Chemistry, 4*(8), 142-151.

Hardainiyan, S., Nandy, B. C., & Saxena, R. (2015). Phytochemical investigation of fruit extract of *Elaeocarpus ganitrus*. *International Journal of Pharmacy and Pharmaceutical Sciences, 7*(6), 415-418.

Hasibuan, I. (2016). *Valuasi Terhadap Potensi Tumbuhan di Cagar Alam Dolok Tinggi Raja Berdasarkan Persepsi Suku Batak Simalungun.* (Master). Institut Pertanian Bogor, Bogor, Indonesia. Retrieved from https://repository.ipb.ac.id/handle/123456789/82513

Hooshmand, S., Ghaderi, A., Yusoff, K., Thilakavathy, K., Rosli, R., & Mojtahedi, Z. (2014). Differentially expressed proteins in ER+ MCF7 and ER- MDAMB-231 human breast cancer cells by RhoGDI-α silencing and overexpression. *Asian Pacific Journal of Cancer Prevention, 15*(7), 3311-3317.

Hu, G., Peng, C., Xie, X., Zhang, S., & Cao, X. (2017). Availability, pharmaceutics, security, pharmacokinetics, and pharmacological activities of patchouli alcohol. *Evidence-Based Complementary and Alternative Medicine, 2017*(4850612), 1-9.

Ikegami, F., Sekine, T., Aburada, M., Fujii, Y., Komatsu, Y., & Murakoshi, I. (1989). Synthesis of entadamide A and entadamide B isolated from *Entada phaseoloides* and their inhibitory effects on 5-lipoxygenase. *Chemical and Pharmaceutical Bulletin, 37*(7), 1932-1933.

Ikegami, F., Sekine, T., Duangteraprecha, S., Matsushita, N., Matsuda, N., Ruangrungsi, N., & Murakoshi, I. (1989). Entadamide C, a sulphur-containing amide from *Entada phaseoloides*. *Phytochemistry, 28*(3), 881-882.

Juneja, K., Mishra, R., Chauhan, S., Gupta, S., Roy, P., & Sircar, D. (2019). Metabolite

profiling and wound-healing activity of *Boerhavia diffusa* leaf extracts using *in vitro* and *in vivo* models. *Journal of Traditional and Complementary Medicine*.

Khiangte, Z., & Lalramnghinglova, H. (2017). Inventorization of indigenous medicinal plants and practices in Mizoram, North East India. *Pleione, 11*(2), 268-276.

Kobiela, T., Milner-Krawczyk, M., Pasikowska-Piwko, M., Bobecka-Wesołowska, K., Eris, I., Święszkowski, W., & Dulinska-Molak, I. (2018). The Effect of Anti-aging Peptides on Mechanical and Biological Properties of HaCaT Keratinocytes. *International Journal of Peptide Research and Therapeutics, 24*(4), 577-587.

Kong, D.-G., Zhao, Y., Li, G.-H., Chen, B.-J., Wang, X.-N., Zhou, H.-L., . . . Shen, T. (2015). The genus *Litsea* in traditional Chinese medicine: An ethnomedical, phytochemical and pharmacological review. *Journal of Ethnopharmacology, 164*, 256-264.

Koohsari, H., Ghaemi, E. A., Sadegh Sheshpoli, M., Jahedi, M., & Zahiri, M. (2015). The investigation of antibacterial activity of selected native plants from North of Iran. *J Med Life, 8*(Spec Iss 2), 38-42.

Krisyanella, Dachriyanus, & Marlina. (2011). Karakterisasi simplisia dan ekstrak serta isolasi senyawa aktif antibakteri dari daun Karamunting (*Rhodomyrtus tomentosa* (W.Ait) Hassk). [Characterization of simplicia and extract also isolation of antibacterial active compounds from Karamunting leaf (*Rhodomyrtus tomentosa* (W.Ait) Hassk)]. *Journal of Faculty of Pharmachy, Andalas University*, 1-17.

Kumar, T. S., Shanmugam, S., Palvannan, T., & Bharathi Kumar, V. M. (2008). Evaluation of antioxidant properties of *Elaeocarpus ganitrus* Roxb. leaves. *Iranian Journal of Pharmaceutical Research, 7*(3), 211-215.

Kurutas, E. B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal, 15*(1), 71.

Kustiawan, W. (2007). Medicinal plants of Kalimantan forest : A review. *Natural Life, 2*(1), 24-34.

Kusuma, I. W., Ainiyati, N., & Suwinarti, W. (2016). Search for biological activities from an invasive shrub species rose myrtle (*Rhodomyrtus tomentosa*). *Nusantara Bioscience, 8*(1), 55-59.

Lailati, M., & Ekasari, I. (2015). *Unique and interesting seeds of Cibodas botanic garden collections.* Paper presented at the Seminar Nasional & International Conference, Bandung, Indonesia.

Lallawmawma, H. (2016). *Antioxidant Potential of Few Selected Medicinal Plants of Mizoram and Their Anti-Ulcer Activity in Induced Albino Rats.* (Ph.D). Mizoram University, Aizawl, Mizoram, India. Retrieved from http://mzuir.inflibnet.ac.in/bitstream/123456789/148/1/H.%20Lallawmawma%20(Biotechnology).pdf

Leffingwell, J. C. (2001). Chemical constituents of tobacco leaf and differences among tobacco types. *Chemistry Preprint Archive, 2001*(2), 173-232.

Lei, J.-C., Yang, C.-X., Yang, Y., Zhang, W., & Yu, J.-Q. (2015). Antioxidant and antitumour activities of extracts from *Patrinia villosa* and its active constituents. *Journal of Functional Foods, 16*, 289-294.

Li, H., Zhang, D., Tan, L. H., Yu, B., Zhao, S. P., & Cao, W. G. (2017). Comparison of the antioxidant properties of various solvent extracts from *Dipsacus asperoides* and identification of phenolic compounds by LC-ESI-QTOF-MS–MS. *South African Journal of Botany, 109*, 1-8.

Limsuwan, S., Subhadhirasakul, S., & Voravuthikunchai, S. P. (2009). Medicinal plants with significant activity against important pathogenic bacteria. *Pharmaceutical Biology, 47*(8), 683-689.

Lin, F. J., Yen, F. L., Chen, P. C., Wang, M. C., Lin, C. N., Lee, C. W., & Ko, H. H. (2014). HPLC-fingerprints and antioxidant constituents of *Phyla nodiflora*. *The Scientific World Journal, 2014*(528653), 1-8.

Liu, H.-X., Tan, H.-B., & Qiu, S.-X. (2016). Antimicrobial acylphloroglucinols from the leaves of *Rhodomyrtus tomentosa*. *Journal of Asian Natural Products Research, 18*(6), 535-541.

Liyanaarachchi, G. D., Samarasekera, J. K. R. R., Mahanama, K. R. R., & Hemalal, K. D. P.

(2018). Tyrosinase, elastase, hyaluronidase, inhibitory and antioxidant activity of Sri Lankan medicinal plants for novel cosmeceuticals. *Industrial Crops and Products, 111*, 597-605.

Maalik, A., Khan, F. A., Mumtaz, A., Mehmood, A., Azhar, S., Atif, M., . . . Tariq, I. (2014). Pharmacological applications of quercetin and its derivatives: A short review *Tropical Journal of Pharmaceutical Research, 13*(9), 1561-1566.

Mapunya, M. B., Nikolova, R. V., & Lall, N. (2012). Melanogenesis and antityrosinase activity of selected South African plants. *Evidence-Based Complementary and Alternative Medicine, 2012*(374017), 1-6.

Matius, P., Tjwa, S. J. M., Raharja, M., Sapruddin, Noor, S., & Ruslim, Y. (2018). Plant diversity in traditional fruit gardens (*munaans*) of Benuaq and Tunjung Dayaks tribes of West Kutai, East Kalimantan, Indonesia *Biodiversitas, 19*(4), 1280-1288

Moon, J., Yim, E., Song, G., Lee, N. H., & Hyun, C. (2010). Screening of elastase and tyrosinase inhibitory ctivity from Jeju Island plants. *Eurasian Journal of Biosciences, 4*(1), 41-53.

Mulyoutami, E., Rismawan, R., & Joshi, L. (2009). Local knowledge and management of *simpukng* (forest gardens) among the Dayak people in East Kalimantan, Indonesia. *Forest Ecology and Management, 257*(10), 2054-2061.

Muniandy, K., Gothai, S., Tan, W. S., Kumar, S. S., Mohd Esa, N., Chandramohan, G., . . . Arulselvan, P. (2018). In vitro wound healing potential of stem extract of *Alternanthera sessilis*. *Evidence-Based Complementary and Alternative Medicine, 2018*, 3142073-3142073.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*(5), 1523-1542.

Ngearnsaengsaruay, C., Middleton, D., & Chayamarit, K. (2011). A revision of the genus *Litsea* Lam. (Lauraceae) in Thailand. *Thai Forest Bulletin (Botany), 39*, 40-119.

Nowak, D., Goslinski, M., Wojtowicz, E., & Przygonski, K. (2018). Antioxidant properties and phenolic compounds of vitamin C-rich juices. *J Food Sci, 83*(8), 2237-2246.

Nyemb, J. N., Tchinda, A. T., Talla, E., Nanga, E. B., Ngoudjou, D. T., Henoumont, C., . . .

Mbafor, J. T. (2018). Vitellaroside, a new cerebroside from V*itellaria paradoxa* (sapotaceae) and its bioactivities. *Natural Products Chemistry & Research, 6*(1), 306.

Olszowy, M., Dawidowicz, A. L., & Jóźwik-Dolęba, M. (2019). Are mutual interactions between antioxidants the only factors responsible for antagonistic antioxidant effect of their mixtures? Additive and antagonistic antioxidant effects in mixtures of gallic, ferulic and caffeic acids. *European Food Research and Technology, 245*(7), 1473-1485.

Patwardhan, B., Vaidya, A. D. B., & Chorghade, M. (2004). Ayurveda and natural products drug discovery. *Current Science, 86*(6), 789-799.

Pientaweeratch, S., Panapisal, V., & Tansirikongkol, A. (2016). Antioxidant, anti-collagenase and anti-elastase activities of *Phyllanthus emblica*, *Manilkara zapota* and silymarin: An in vitro comparative study for anti-aging applications. *Pharmaceutical Biology, 54*(9), 1865-1872.

Pillai, S., Oresajo, C., & Hayward, J. (2005). Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation - a review. *International Journal of Cosmetic Science, 27*(1), 17-34.

Prasad, K. N., Yang, B., Dong, X., Jiang, G., Zhang, H., Xie, H., & Jiang, Y. (2009). Flavonoid contents and antioxidant activities from Cinnamomum species. *Innovative Food Science & Emerging Technologies, 10*(4), 627-632.

Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry, 269*, 337-341.

Prihantini, A. I., Tachibana, S., & Itoh, K. (2014). Evaluation of antioxidant and alpha-glucosidase inhibitory activities of some subtropical plants. *Pak J Biol Sci, 17*(10), 1106-1114.

Prihantini, A. I., Tachibana, S., & Itoh, K. (2015). Antioxidant active compounds from *Elaeocarpus sylvestris* and their relationship between structure and activity.

Procedia Environmental Sciences, 28, 758-768.

Ramadhan, R. (2015). *Exploration of Bioactive Compounds for Diabetes Therapy from Selected East Kalimantan Flora.* (Ph.D. Dissertation). Chulalongkorn University, Bangkok, Thailand.

Ramadhan, R., Worawalai, W., & Phuwapraisirisan, P. (2018). New onoceranoid xyloside from *Lansium parasiticum*. *Natural Product Research, Nov*(5), 1-8.

Ramakrishna, D., Pavan, K. K., Mukkanti, K., & Abedulla, K. K. (2008). Antiulcer activity of the seeds of *Entada phaseoloides*. *Pharmacologyonline, 3*, 93-98.

Ray, A. B., Dutta, S. C., & Dasgupta, S. (1976). Flavonoids of *Elaeocarpus lanceofolius*. *Phytochemistry, 15*(11), 1797-1798.

Rezk, A. (2015). *From Ethnomedicine to Application: Biological Activities and Cytotoxicity of Leaf Extracts from Plants of the Genus Rhododendron.* (Ph.D). Jacobs University, Bremen.

Ritto, D., Tanasawet, S., Singkhorn, S., Klaypradit, W., Hutamekalin, P., Tipmanee, V., & Sukketsiri, W. (2017). Astaxanthin induces migration in human skin keratinocytes via Rac1 activation and RhoA inhibition. *Nutrition research and practice, 11*(4), 275-280.

Rivelli, A. R., Caruso, M. C., Maria, S. D., & Galgano, F. (2017). Vitamin C content in leaves and roots of horseradish (*Armoracia rusticana*): seasonal variation in fresh tissues and retention as affected by storage conditions. *Emirates Journal of Food and Agriculture, 29*(10), 799-806.

Runtunuwu, A. E. (2013). *Studi Etnoekologi Pemanfaatan Tumbuhan Obat oleh Masyarakat Suku Dayak Tunjung Linggang di Kabupaten Kutai Barat, Provinsi Kalimantan Timur.* (Bachelor). Sanata Dharma University, Yogyakarta, Indonesia. Retrieved from http://repository.usd.ac.id/1895/

Saising, J., Ongsakul, M., & Voravuthikunchai, S. P. (2011). *Rhodomyrtus tomentosa* (Aiton) Hassk. ethanol extract and rhodomyrtone: a potential strategy for the treatment of biofilm-forming staphylococci. *Journal of Medical Microbiology, 60*(Pt 12), 1793-1800.

Sarkar, R., Arora, P., & Garg, K. V. (2013). Cosmeceuticals for hyperpigmentation: What is

available? *Journal of Cutaneous and Aesthetic Surgery 6*(1), 4-11.

Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods, 42*(4), 321-324.

Semwal, D. K., Semwal, R. B., Combrinck, S., & Viljoen, A. (2016). Myricetin: A dietary molecule with diverse biological activities. *Nutrients, 8*(2), 90.

Setyowati, F. M. (2010). Etnofarmakologi dan pemakaian tanaman obat suku Dayak Tunjung di Kalimantan Timur. [Ethnopharmacology and usage of medicinal plant in Dayak Tunjung tribe, East Kalimantan]. *Media Litbang Kesehatan, XX*(3), 104-112.

Setyowati, F. M., & Wardah. (2007). Keanekaragaman tumbuhan obat masyarakat Talang Mamak di sekitar Taman Nasional Bukit Tigapuluh, Riau [Diversity of medicinal plant by Talang Mamak tribe in surrounding of Bukit Tiga Puluh National Park, Riau]. *Biodiversitas, 8*(3), 228-232.

Subramanian, A. P., John, A. A., Vellayappan, M. V., Balaji, A., Jaganathan, S. K., Supriyanto, E., & Yusof, M. (2015). Gallic acid: Prospects and molecular mechanisms of its anticancer activity. *RSC Advances, 5*(45), 35608-35621.

Sugimoto, S., Matsunami, K., & Otsuka, H. (2018). Biological activity of *Entada phaseoloides* and *Entada rheedei*. *Journal of Natural Medicines, 72*(1), 12-19.

Susiarti, S., Rahayu, M., & Rugayah. (2018). Diversity of Indonesian medicinal plant in the lowland forest, Bodogol and its surrounding of Mount Gede-Pangrango National Park, West Java. *IOP Conference Series: Earth and Environmental Science, 166*(012021).

Talukdar, N., Dutta, A. M., Chakraborty, R., & Das, K. (2017). Screening of phytochemicals, antioxidant and inhibitory effect on alpha-amylase by ethanolic extract of *Elaeocarpus ganitrus* (bark). *International Journal of Pharmaceutical Sciences and Research, 8*(12), 5270-5275.

Taylor, J. L. S., Rabe, T., McGaw, L. J., Jäger, A. K., & van Staden, J. (2001). Towards the scientific validation of traditional medicinal plants. *Plant Growth Regulation, 34*(1), 23-37.

Tung, N. H., Ding, Y., Choi, E. M., Van Kiem, P., Van Minh, C., & Kim, Y. H. (2009). New anthracene glycosides from *Rhodomyrtus tomentosa* stimulate osteoblastic differentiation of MC3T3-E1 cells. *Archives of Pharmacal Research, 32*(4), 515-520.

Uji, T. (2004). Keanekaragaman jenis, plasma nutfah, dan potensi buah-buahan asli Kalimantan. [Species diversity, genetic resources, and potential of the indigenous fruits in Kalimantan]. *BioSMART, 6*(2), 117-125.

Vinayagam, R., Jayachandran, M., & Xu, B. (2016). Antidiabetic effects of simple phenolic acids: A comprehensive review. *Phytotherapy Research, 30*(2), 184-199.

Voss, F. (1982). Atlas of East Kalimantan, Indonesia. East Kalimantan Transmigration Area Development Project (TAD).

Wang, R., Wang, J., Dong, T., Shen, J., Gao, X., & Zhou, J. (2019). Naringenin has a chemoprotective effect in MDA-MB-231 breast cancer cells via inhibition of caspase-3 and -9 activities. *Oncology Letters, 17*(1), 1217-1222.

Wangthong, S. (2010). *Biological Activities of Thanaka Hesperethusa crenulata Stem Bark.* (Ph.D). Chulalongkorn University, Bangkok, Thailand.

Wattanapiromsakul, C., Wangsintaweekul, B., Sangprapan, P., Itharat, A., & Keawpradub, N. (2005). Goniothalamin, a cytotoxic compound, isolated from *Goniothalamus macrophyllus* (Blume) Hook. f. & Thomson var. *macrophyllus*. *Songklanakarin Journal of Science and Technology, 27*, 479-487.

WHO. (2009). *Traditional medicine in Republic of Indonesia, in conference on traditional medicine in ASEAN countries*. Bangkok: World Health Organization Retrieved from http://www.searo.who.int/entity/medicines/topics/traditional_medicines_in_republic_of_indonesia.pdf

Wilson, D. W., Nash, P., Buttar, H. S., Griffiths, K., Singh, R., De Meester, F., . . . Takahashi, T. (2017). The role of food antioxidants, benefits of functional foods, and influence of feeding habits on the health of the older person: An overview. *Antioxidants (Basel), 6*(4), 1-20.

Wu, Y., Bai, J., Zhong, K., Huang, Y., Qi, H., Jiang, Y., & Gao, H. (2016). Antibacterial

activity and membrane-disruptive mechanism of 3-p-trans-coumaroyl-2-hydroxyquinic acid, a novel phenolic compound from Pine Needles of *Cedrus deodara*, against *Staphylococcus aureus*. *Molecules (Basel, Switzerland), 21*(8), 1-12.

Yadav, S., Trivedi, N. A., & Bhatt, J. D. (2015). Antimicrobial activity of fresh garlic juice: an in vitro study. *Ayu, 36*(2), 203-207.

Yang, D. J., Moh, S. H., Son, D. H., You, S., Kinyua, A. W., Ko, C. M., . . . Kim, K. W. (2016). Gallic acid promotes wound healing in normal and hyperglucidic conditions. *Molecules (Basel, Switzerland), 21*(7), 899.

Yusro, F., Mariani, Y., Diba, F., & Ohtani, K. (2014). Inventory of medicinal plants for fever used by four Dayak sub ethnic in West Kalimantan, Indonesia. *Kuroshio Science, 8*(1), 33-38.

Zhang, Y.-L., Zhou, X.-W., Wu, L., Wang, X.-B., Yang, M.-H., Luo, J., . . . Kong, L.-Y. (2017). Isolation, structure elucidation, and absolute configuration of syncarpic acid-conjugated terpenoids from *Rhodomyrtus tomentosa*. *Journal of Natural Products, 80*(4), 989-998.

Zhuang, L., Chen, L.-F., Zhang, Y.-B., Liu, Z., Xiao, X.-H., Tang, W., . . . Li, M.-M. (2017). Watsonianone A from *Rhodomyrtus tomentosa* fruit attenuates respiratory-syncytial-virus-induced inflammation in vitro. *Journal of Agricultural and Food Chemistry, 65*(17), 3481-3489.

Zoremsiami, J. (2017). *Investigation of the Anticancer Potential of Pasaltakaza, Helicia nilagirica Bedd.* (Ph.D). Mizoram University, Aizawl, Mizoram, India. Retrieved from http://14.139.116.8:8080/jspui/handle/123456789/323

APPENDIX

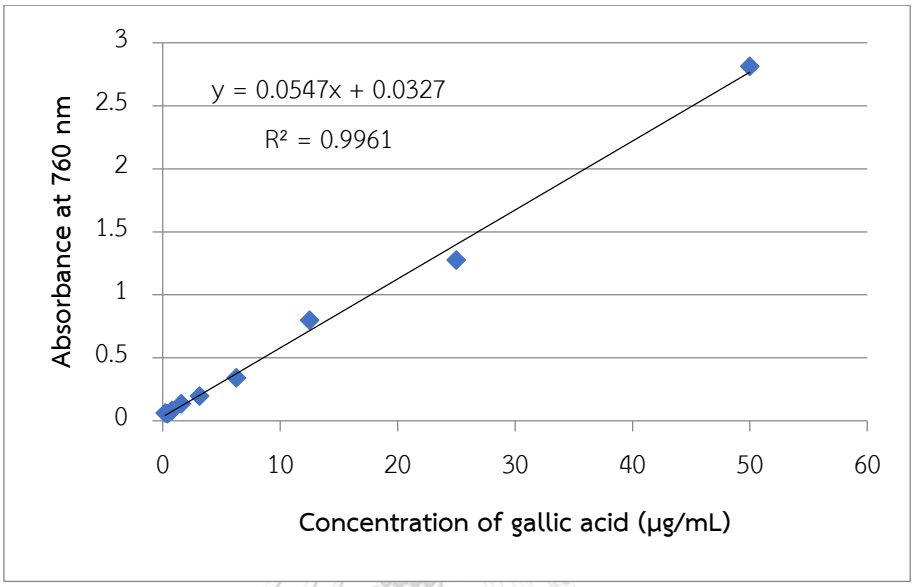


Figure A.1 Standard curve of gallic acid

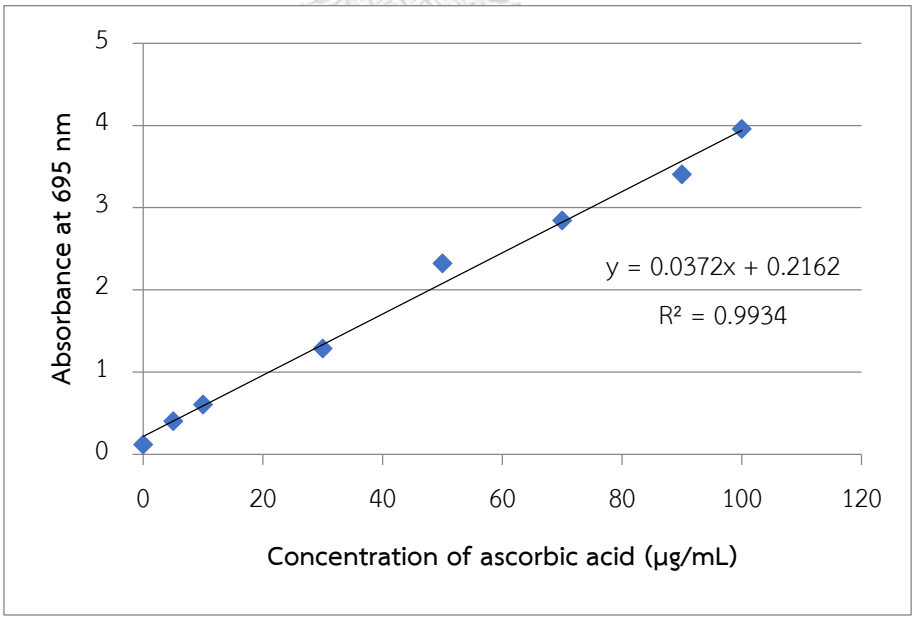


Figure A.2 Standard curve of ascorbic acid

VITA

NAME	Franxisca Mariani
DATE OF BIRTH	22 December 1980
PLACE OF BIRTH	Jakarta, Indonesia
INSTITUTIONS ATTENDED	Bachelor degree of Forestry from Department of Forest Product, Technology, Faculty of Forestry, Mulawarman University, East Kalimantan, Indonesia in 2003. Master degree of Agriculture from Department of Agricultural Product Technology, Faculty of Agricultural Technology, Brawijaya University, East Java, Indonesia in 2009.
HOME ADDRESS	Perumahan Sempaja Lestari Indah, Samarinda, East Kalimantan Province, Indonesia.