

## TABLE OF CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGEMENT</b>	<b>iii</b>
<b>ENGLISH ABSTRACT</b>	<b>iv</b>
<b>THAI ABSTRACT</b>	<b>vii</b>
<b>LIST OF TABLES</b>	<b>xii</b>
<b>LIST OF FIGURES</b>	<b>xiv</b>
<b>LIST OF SCHEME</b>	<b>xvi</b>
<b>ABBREVIATIONS AND SYMBOLS</b>	<b>xviii</b>
<b>CHAPTER 1 INTRODUCTION</b>	<b>1</b>
<b>CHAPTER 2 REVIEW OF LITERATURES</b>	<b>8</b>
2.1 Taxa and Classification of <i>Acalypha indica</i> Linn., <i>Bridelia retusa</i> (L.) A. Juss. and <i>Cleidion javanicum</i> BL.	8
2.2 Review of chemical composition and bioactivities of <i>Acalypha indica</i> Linn., <i>Bridelia retusa</i> (L.) A. Juss. and <i>Cleidion javanicum</i> BL.	10
<b>CHAPTER 3 EXPERIMENTAL</b>	<b>17</b>
3.1 Source and Authentication of the Plant Materials	17
3.2 General Techniques	17
3.2.1 Chemicals	17
3.2.2 Chromatography	17
3.3 Analysis of essential oil	19
3.4 Antioxidant activity	19
3.4.1 ABTS Method	20
3.4.2 DPPH Method	21
3.4.3 Total phenolic contents	22
3.4.4 Total flavonoid contents	23
3.5 Biological Activity	23

## TABLE OF CONTENTS (CONTINUED)

	<b>Page</b>
3.5.1 Antimicrobial activity	23
3.5.2 Cell toxicity on cell lines	24
3.6 Extraction and Isolation	26
3.6.1 Isolation of <i>Acalypha indica</i> Linn.	26
3.6.2 Isolation of <i>Cleidion javanicum</i> BL.	35
3.6.3 Isolation of <i>Bridelia retusa</i> (L.) A. Juss.	41
<b>CHAPTER 4 RESULTS AND DISCUSSION</b>	<b>46</b>
4.1 The percentage yield of extracts and essential oils	46
4.2 Determination of antioxidant activities of the essential oils and extracts	48
4.3 Determination of biological activities	57
4.4 Determination of cytotoxic activity	59
4.5 Analysis of the essential oil	65
4.6 Isolation of the bioactive extracts	74
4.6.1 Isolation of <i>A. indica</i> constituent	74
4.6.2 Isolation of <i>C. javanicum</i> constituent	85
4.6.3 Isolation of <i>B. retusa</i> constituent	89
<b>CHAPTER 5 CONCLUSION</b>	<b>91</b>
<b>REFERENCES</b>	<b>95</b>
<b>APPENDIX</b>	<b>105</b>
<b>VITA</b>	<b>118</b>

## LIST OF TABLES

<b>Table</b>	<b>Page</b>
4.1 The amount and percentage yields of the extracts from three medicinal plants: <i>A. indica</i> , <i>C. javanicum</i> and <i>B. retusa</i>	46
4.2 The percentage yields of the essential oil from three medicinal plants: <i>A. indica</i> , <i>C. javanicum</i> and <i>B. retusa</i>	47
4.3 Percentage inhibition of Trolox concentration serie for ABTS screening method	48
4.4 Antioxidant activities by ABTS assay (essential oil)	49
4.5 Antioxidant activities by ABTS assay (crude extract)	49
4.6 The percentage inhibition of each concentration of the trolox standard solution	51
4.7 Antioxidant activities by DPPH assay (essential oil)	52
4.8 Antioxidant activities by DPPH assay (crude extract)	52
4.9 Total phenol contents of the extracts	54
4.10 Total flavonoid contents of the extracts	56
4.11 Antibacterial activities of the extracts	61
4.12 Antifungal activities of the exteacts	62
4.13 MIC values of the selective essential oils of three medicinal plants	63
4.14 Cytotoxic activities of extracts and essential oils	64
4.15 Volatile components in aerial parts of <i>A. indica</i>	66
4.16 Chemical composition of the leaves essential oil of <i>C. javanicum</i>	68
4.17 Chemical composition of the fruits essential oil of <i>C. javanicum</i>	69
4.18 Chemical composition of the leaves essential oil of <i>B. retusa</i>	71
4.19 Chemical composition of the fruits essential oil of <i>B. retusa</i>	73
4.20 <sup>1</sup> H-NMR data of L-quebrachitol in CD <sub>3</sub> OD	77

**LIST OF TABLES (CONTINUED)**

<b>Table</b>	<b>Page</b>
4.21 Selection of mobile phase and flow rate of standard <i>L</i> -quebrachitol	79
4.22 Precision study for <i>L</i> -quebrachitol	81
4.23 Recovery study of <i>L</i> -quebrachitol	81
4.24 System suitability for RP-HPLC determination of <i>L</i> -quebrachitol	82
4.25 Determination of <i>L</i> -quebrachitol by standard addition method.	84
4.26 Chemical composition of DfH7.2* fraction	85
4.27 Chemical composition of DfH7.3.2* fraction	87
4.28 <sup>1</sup> H-NMR spectral data of stigmasterol in CDCl <sub>3</sub>	88
4.29 <sup>1</sup> H-NMR and <sup>13</sup> C-NMR spectral data of benzoic acid (compounds: DfM8.5*) in CDCl <sub>3</sub>	90

## LIST OF FIGURES

<b>Figure</b>	<b>Page</b>
1.1 <i>Acalypha indica</i> Linn.	3
1.2 <i>Bridelia retusa</i> (L.) A. Juss.	5
1.3 <i>Cleidion javanicum</i> BL.	7
2.1 Structures of some active chemical compounds of <i>A. indica</i>	12
2.2 Structure of some active chemical compounds of <i>B. retusa</i>	13
2.3 Structure of some active chemical compounds of <i>C. javanicum</i> (1).	15
2.4 Structure of some active chemical compounds of <i>C. javanicum</i> (2).	16
3.1 A linear regression ( $R^2 = 0.9953$ ) of trolox (ABTS assay)	20
3.2 A linear regressions ( $R^2$ ) of Trolox (DPPH assay)	22
4.1 Concentration-response curve for the absorbance at 734 nm for ABTS <sup>•+</sup> as a function of concentration of standard trolox	48
4.2 Calibration curve for the absorbance at 540 nm of DPPH method as a function of the concentration of trolox standard solution	51
4.3 A linear regression ( $R^2 = 0.9994$ ) of gallic acid	54
4.4 A linear regression ( $R^2 = 0.9995$ ) of quercetin	56
4.5 Total ion chromatogram of <i>A. indica</i> aerial parts essential oil	65
4.6 Total ion chromatogram of <i>C. javanicum</i> leaves essential oil	68
4.7 Total ion chromatogram of <i>C. javanicum</i> fruits essential oil	69
4.8 Total ion chromatogram of <i>B. retusa</i> leaves essential oil	71
4.9 Total ion chromatogram of <i>B. retusa</i> fruits essential oil	72
4.10 Crystalline white powder of L-quebrachitol	76
4.11 Chemical structures of compounds: L-quebrachitol	76
4.12 Key HMBC correlations of L-quebrachitol	76

**LIST OF FIGURES (CONTINUED)**

<b>Figure</b>	<b>Page</b>
4.13 The optimum wavelength of L-quebrachitol	78
4.14 Linearity of L-quebrachitol standard response (peak area) over the concentration range of 3.91-1000 µg/mL (the y-error bars represent the standard deviation of the response at each concentration)	80
4.15 HPLC chromatogram of L-quebrachitol standard, obtained using a mobile phase composed of methanol:acetonitrile:tetrahydrofuran in 79.50 : 20.40 : 0.10 (v/v), at a flow rate 0.3 mL/min, column temperature 25 °C and DAD operated at 206 nm	83
4.16 Standard addition curve for the determination of L-quebrachitol	84
4.17 Total ion chromatogram of DfH7.2* fraction	85
4.18 Total ion chromatogram of DfH7.3.2* fraction	86
4.19 Chemical structure of compound: stigmasterol	88
4.20 Chemical structure of compound: benzoic acid	89

## LIST OF SCHEMES

<b>Scheme</b>	<b>Page</b>
3.1 Extraction scheme of <i>Acalypha indica</i> Linn.	26
3.2 Isolation scheme of methanol extract of <i>A. indica</i> aerial parts (PAM)	27
3.3 Isolation scheme of compound PA5.1	28
3.4 Isolation scheme of compound PA6.1	28
3.5 Isolation scheme of compound PA7.1	29
3.6 Isolation scheme of compound PA8.1	29
3.7 Isolation scheme of compound PA9.1	30
3.8 Isolation scheme of compound PA11a, PA11b and PA11d	30
3.9 Isolation scheme of compound PA13.3.1, PA13.3.2, PA13.3.3, PA13.3.4	31
3.10 Isolation scheme of compound PA21.9*	32
3.11 Extraction of <i>C. javanicum</i> leaves	35
3.12 Extraction of <i>C. javanicum</i> stems	35
3.13 Extraction of <i>C. javanicum</i> fruits	35
3.14 Isolation scheme of hexane extract of <i>C. javanicum</i> fruits (DfH)	36
3.15 Isolation scheme of compound DfH7.2*, DfH7.3.2*, DfH7.3.3*, DfH7.3.4*, DfH7.3.5* and DfH7.4*	37
3.16 Isolation scheme of methanol extract of <i>C. javanicum</i> fruits (DfM)	38
3.17 Isolation scheme of compound DfM6.5*	38
3.18 Isolation scheme of compound DfM7.3*	39
3.19 Isolation scheme of compound DfM8.2*	39
3.20 Isolation scheme of compound DfM9.3*	40
3.21 Isolation scheme of compound DfM10.2* and DfM10.4*	40
3.22 Extraction of <i>B. retusa</i> leaves	41
3.23 Extraction of <i>B. retusa</i> stems	41
3.24 Extraction of <i>B. retusa</i> fruits	41
3.25 Isolation scheme of methanol extract of <i>B. retusa</i> fruits (TfM)	42

**LIST OF SCHEME (CONTINUED)**

<b>Scheme</b>	<b>Page</b>
3.26 Isolation scheme of compound TfM8.5*	43
3.27 Isolation scheme of methanol extract of <i>B. retusa</i> leaves (TLM)	43
3.28 Isolation scheme of compound TLM4.3*	44
3.29 Isolation scheme of compound TLM5.4*	44
3.30 Isolation scheme of compound TLM6.2*	45
3.31 Isolation scheme of compound TLM8.3*	45

**ABBREVIATIONS AND SYMBOLS**

<i>brd</i>	Broad doublet
<i>brs</i>	Broad singlet
°C	Degree Celsius
CDCl <sub>3</sub>	Deuterated chloroform
cm	Centimeter
CO <sub>2</sub>	Carbon dioxide
δ	Chemical shift
<i>d</i>	Doublet
<i>dd</i>	Doublet of doublet
DCM	Dichloromethane
dm	Decimeter
DMEM	Dulbelco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
ED <sub>50</sub>	50% Effective dose
EIMS	Electron impact mass spectrum
EtOAc	Ethyl acetate
EtOH	Ethanol
FID	Flame ionization detector
g	Gram
GC	Gas chromatography
<sup>1</sup> H-NMR	Proton nuclear magnetic resonance
h	Hours
Hz	Hertz
IC <sub>50</sub>	50% Inhibition concentration
i.d.	Internal diameter
IR	Infrared
<i>J</i>	Coupling constant
Kg	Kilogram
λ	wavelength

l	Liter
LD <sub>50</sub>	50% Lethality dose
µg	Microgram
µL	Microliter
µm	Micrometer
m	Meter
MeOH	Methanol
mg	Milligram
MHz	Megahertz
MIC	Minimum inhibition concentration
min	Minutes
mL	Milliliter
mm	Millimeter
mM	Milimolar
MS	Mass spectrum
$\nu_{\max}$	Wavenumber at maximum absorption
NMR	Nuclear magnetic resonance
No.	Number
ppm	Part per million
RI	Retention index
RT	Retention time
s	Singlet
SDS	Sodium dodecyl sulfate
TLC	thin-layer chromatography
UV	Ultraviolet