

CONTENTS

	Page
ABSTRACT IN THAI.....	iv
ABSTRACT IN ENGLISH.....	v
ACKNOWLEDGEMENTS.....	vi
CONTENTS.....	vii
LIST OF TABLES.....	ix
LIST OF FIGURES.....	x
LIST OF SCHEMES.....	xiii
LIST OF ABBREVIATIONS.....	xiv
CHAPTER	
I INTRODUCTION.....	1
1.1 Thai natural products as leads for drug candidates	1
1.2 Plants in the genus <i>Harrisonia</i>	2
1.2.1 Botanical characteristics of <i>Harrisonia perforata</i>	2
1.2.2 Secondary metabolites of the genus <i>Harrisonia</i>	2
1.3 Aim and scope of the present study	7
II EXPERIMENTS.....	9
2.1 Plant material	9
2.2 General experimental Procedures.....	9
2.2.1 Fourier transform infrared spectrophotometer (FT-IR)	9
2.2.2 Mass spectrometer (MS)	9
2.2.3 Melting point.....	9
2.2.4 Nuclear magnetic resonance spectrometer (NMR)....	9
2.2.5 Optical rotation.....	9
2.2.6 Ultraviolet-visible spectrophotometer (UV-vis).....	10

	Page
2.2.7 X-ray crystallography.....	10
2.2.8 Microplate spectrophotometer.....	10
2.2.9 CO ₂ Cell Culture Incubator.....	10
2.2.10 Biosafety cabinet	10
2.3 Chemicals.....	10
2.3.1 Solvents.....	10
2.3.2 Other chemicals.....	10
2.4 Extraction and Isolation.....	11
2.5 Biological activity.....	17
2.5.1 Nitric oxide inhibitory assay.....	17
2.5.2 Cytotoxicity assay.....	17
2.5.3 Western Blot Analysis.....	18
III RESULTS AND DISCUSSION	19
3.1 Isolated compounds from the roots and fruits.....	19
of <i>H. perforata</i>	
3.2 Structure elucidation of isolated compounds.....	21
3.2.1 Structure elucidation of compound 1	21
3.2.2 Structure elucidation of compound 2	24
3.2.3 Structure elucidation of compound 3	27
3.2.4 Structure elucidation of compound 4	31
3.2.5 Structure elucidation of compound 5	36
3.2.6 Structure elucidation of compound 6	39
3.2.7 Structure elucidation of compound 7	42
3.2.8 Structure elucidation of compound 8	44
3.2.9 Structure elucidation of compound 9	47
3.3 Anti-inflammatory activity of isolated compounds.....	49
IV CONCLUSION.....	52
REFERENCES.....	54
APPENDIX.....	57
VITA.....	88

LIST OF TABLES

Table	Page
1.1 Taxonomy of <i>Harrisonia perforata</i>	2
3.1 The NMR data of compound 1 and harrisonin (CDCl_3).....	23
3.2 The NMR data of compound 2 (CDCl_3 , 400 MHz).....	26
3.3 The NMR data of compound 3 (CDCl_3 , 400 MHz).....	29
3.4 Crystal data and structure refinement for compound 3	30
3.4 The NMR data of compound 4 (CDCl_3 , 400 MHz).....	34
3.6 Crystal data and structure refinement for compound 4	35
3.7 The NMR data of compound 5 and (+)-vouacapenic acid.....	38
3.8 The NMR data of compound 6 and harrisonol A (CDCl_3 , 400 MHz)...	41
3.9 The NMR data of compound 7 and peucenin-7-methyl ether (CDCl_3 , 400 MHz).....	43
3.10 The NMR data of compound 8 (CDCl_3 , 400 MHz).....	46
3.11 The NMR data of compound 9 and braylin I (CDCl_3 , 400 MHz).....	48
3.12 Inhibitory effect of isolated compounds on NO production..... in LPS-induced macrophages.....	50

LIST OF FIGURES

Figure	Page
1.1 Thai medicinal plant drugs	2
1.2 Parts of <i>Harrisonia perforata</i>	3
3.1 The chemical structures of isolated compounds from <i>H. perforata</i>	19
3.2 Compound 1.....	21
3.3 HMBC (a) and COSY (b) correlations of compound 1.....	22
3.4 Compound 2.....	24
3.5 HMBC (a) and COSY (b) correlations of compound 2.....	25
3.6 Compound 3.....	27
3.7 HMBC (a) and COSY (b) correlations of compound 3.....	28
3.8 ORTEP diagram of compound 3.....	28
3.9 Compound 4.....	31
3.10 HMBC (a) and COSY (b) correlations of compound 4.....	32
3.11 ORTEP diagram of compound 4.....	33
3.12 Proposed biosynthetic pathway between compound 4..... and harperforin F.....	33
3.13 Compound 5.....	36
3.14 HMBC (a) and COSY (b) correlations of compound 5.....	37
3.15 Compound 6.....	39
3.16 HMBC (a) and COSY (b) correlations of compound 6.....	40
3.17 Compound 7.....	42
3.18 Compound 8.....	44
3.19 HMBC (a) and COSY (b) correlations of compound 8.....	45
3.20 Compound 9.....	47
3.21 HMBC (a) and COSY (b) correlations of compound 9.....	48
3.22 Effect of harperfolide (2) on cell viability.....	50
3.23 Effect of harperfolide (2) on the expression of iNOS protein..... in LPS-induced macrophages.....	51
A.1 ^1H NMR (400 MHz) spectrum of compound 1 (CDCl_3).....	58

Figure	Page
A.2 ^{13}C NMR (400 MHz) spectrum of compound 1 (CDCl_3).....	58
A.3 COSY (400 MHz) spectrum of compound 1 (CDCl_3).....	59
A.4 HSQC (400 MHz) spectrum of compound 1 (CDCl_3).....	59
A.5 HMBC (400 MHz) spectrum of compound 1 (CDCl_3).....	60
A.6 IR spectrum of compound 1 (KBr).....	60
A.7 ^1H NMR (400 MHz) spectrum of compound 2 (CDCl_3).....	61
A.8 ^{13}C NMR (400 MHz) spectrum of compound 2 (CDCl_3).....	61
A.9 COSY (400 MHz) spectrum of compound 2 (CDCl_3).....	62
A.10 HSQC (400 MHz) spectrum of compound 2 (CDCl_3).....	62
A.11 HMBC (400 MHz) spectrum of compound 2 (CDCl_3).....	63
A.12 IR spectrum of compound 2 (KBr).....	63
A.13 HRESIMS Mass spectrum of compound 2	64
A.14 ^1H NMR (400 MHz) spectrum of compound 3 (CDCl_3).....	65
A.15 ^{13}C NMR (400 MHz) spectrum of compound 3 (CDCl_3).....	65
A.16 COSY (400 MHz) spectrum of compound 3 (CDCl_3).....	66
A.17 HSQC (400 MHz) spectrum of compound 3 (CDCl_3).....	66
A.18 HMBC (400 MHz) spectrum of compound 3 (CDCl_3).....	67
A.19 IR spectrum of compound 3 (KBr).....	67
A.20 ^1H NMR (400 MHz) spectrum of compound 4 (CDCl_3).....	68
A.21 ^{13}C NMR (400 MHz) spectrum of compound 4 (CDCl_3).....	68
A.22 COSY (400 MHz) spectrum of compound 4 (CDCl_3).....	69
A.23 HSQC (400 MHz) spectrum of compound 4 (CDCl_3).....	69
A.24 HMBC (400 MHz) spectrum of compound 4 (CDCl_3).....	70
A.25 IR spectrum of compound 4 (KBr).....	70
A.26 HRESIMS Mass spectrum of compound 4	71
A.27 ^1H NMR (400 MHz) spectrum of compound 5 (CDCl_3).....	72
A.28 ^{13}C NMR (400 MHz) spectrum of compound 5 (CDCl_3).....	72
A.29 COSY (400 MHz) spectrum of compound 5 (CDCl_3).....	73
A.30 HSQC (400 MHz) spectrum of compound 5 (CDCl_3).....	73
A.31 HMBC (400 MHz) spectrum of compound 5 (CDCl_3).....	74

Figure	Page
A.32 IR spectrum of compound 5 (KBr).....	74
A.33 ^1H NMR (400 MHz) spectrum of compound 6 (CDCl_3).....	75
A.34 ^{13}C NMR (400 MHz) spectrum of compound 6 (CDCl_3).....	75
A.35 COSY (400 MHz) spectrum of compound 6 (CDCl_3).....	76
A.36 HSQC (400 MHz) spectrum of compound 6 (CDCl_3).....	76
A.37 HMBC (400 MHz) spectrum of compound 6 (CDCl_3).....	77
A.38 IR spectrum of compound 6 (KBr).....	77
A.39 ^1H NMR (400 MHz) spectrum of compound 7 (CDCl_3).....	78
A.40 ^{13}C NMR (400 MHz) spectrum of compound 7 (CDCl_3).....	78
A.41 IR spectrum of compound 7 (KBr).....	79
A.42 ^1H NMR (400 MHz) spectrum of compound 8 (CDCl_3).....	80
A.43 ^{13}C NMR (400 MHz) spectrum of compound 8 (CDCl_3).....	80
A.44 COSY(400 MHz) spectrum of compound 8 (CDCl_3).....	81
A.45 HSQC (400 MHz) spectrum of compound 8 (CDCl_3).....	81
A.46 HMBC (400 MHz) spectrum of compound 8 (CDCl_3).....	82
A.47 IR spectrum of compound 8 (KBr).....	82
A.48 HRESIMS Mass spectrum of compound 8	83
A.49 ^1H NMR (400 MHz) spectrum of compound 9 (CDCl_3).....	84
A.50 ^{13}C NMR (400 MHz) spectrum of compound 9 (CDCl_3).....	84
A.51 COSY (400 MHz) spectrum of compound 9 (CDCl_3).....	85
A.52 HSQC (400 MHz) spectrum of compound 9 (CDCl_3).....	85
A.53 HMBC (400 MHz) spectrum of compound 9 (CDCl_3).....	86
A.54 IR spectrum of compound 9 (KBr).....	86
A.55 HRESIMS Mass spectrum of compound 9	87

LIST OF SCHEMES

Scheme	Page
2.1 The extraction procedure of <i>H. perforata</i> roots (April 2010)	11
2.2 The isolation from fraction B of EtOAc extract of <i>H. perforata</i> roots (collected in April 2010)	13
2.3 The isolation of EtOAc extract of <i>H. perforata</i> roots from fraction E (collected in March 2012)	14
2.4 The isolation of EtOAc extract of <i>H. perforata</i> roots from fraction C and F (collected in March 2012).	15
2.5 The extraction and isolation procedure of <i>H. perforata</i> fruits.	16

LIST OF ABBREVIATIONS

<i>J</i>	Coupling constant
δ	Chemical shift
δ_H	Chemical shift of proton
δ_C	Chemical shift of carbon
s	Singlet (for NMR spectra)
d	Doublet (for NMR spectra)
dd	Doublet of doublet (for NMR spectra)
t	Triplet (for NMR spectra)
m	Multiplet (for NMR spectra)
q	Quartet (for NMR spectra)
brs	Broad singlet (for NMR spectra)
brd	Broad doublet (for NMR spectra)
calcd.	Calculated
1H NMR	Proton nuclear magnetic resonance
^{13}C NMR	Carbon-13 nuclear magnetic resonance
2D NMR	Two dimensional nuclear magnetic resonance
1H - 1H COSY	Homonuclear (proton-proton) correlation spectroscopy
HSQC	Heteronuclear single quantum coherence
HMBC	Heteronuclear multiple bond correlation
ORTEP	Oak ridge thermal ellipsoid plot
HPLC	High performance liquid chromatography
HRESIMS	High resolution electrospray ionization mass spectrometry
CC	Column chromatography
TLC	Thin layer chromatography
IC ₅₀	Half maximal inhibitory concentration
CDCl ₃	Deuterated chloroform
MeOH	Methanol
CHCl ₃	Chloroform
CH ₂ Cl ₂	Dichloromethane

EtOAc	Ethyl acetate
DMSO	Dimethylsulfoxide
KBr	Potassium bromide
SiO ₂	Silicon dioxide
g	Gram (s)
mg	Milligram (s)
mL	Milliliter (s)
μg	Microgram (s)
μL	Microliter (s)
μM	Micromolar
mM	Millimolar
L	Liter (s)
M	Molar
min	Minute
h	Hour
rpm	Round per minute
m	Meter (s)
mm	Millimeter (s)
cm	Centimeter (s)
nm	Nanometer
Hz	Hertz
MHz	Megahertz
cm ⁻¹	Reciprocal centimeter (unit of wave number)
ppm	part per million
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
IR	Infared
UV	Ultraviolet
m.p.	Melting point
α	Alpha
β	Beta

m/z	Mass to charge ratio
$[M+H]^+$	Protonated molecule
$[M+Na]^+$	Pseudomolecular ion
$[\alpha]_D^{20}$	Specific rotation at 20 °C and sodium D line (589 nm)
λ_{\max}	Wavelength of maximum absorption
c	Concentration
ϵ	Molar extinction coefficient
Å	Angstrom
°C	Degree celcius
deg.	Degree
sp.	Species
No.	Number