



UNIVERSITI PUTRA MALAYSIA

**CHEMICAL CONSTITUENTS OF VITEX NEGUNDO AND
EVALUATION OF THEIR ANTI-INFLAMMATORY AND ANTIOXIDANT
ACTIVITIES**

FADZUREENA JAMALUDIN

IB 2008 10



**CHEMICAL CONSTITUENTS OF *VITEX NEGUNDO* AND EVALUATION OF
THEIR ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES**

By

FADZUREENA JAMALUDIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirement for the Degree of Doctor of Philosophy**

December 2008



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Doctor of Philosophy

**CHEMICAL CONSTITUENTS OF *VITEX NEGUNDO* AND EVALUATION OF
THEIR ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITIES**

By

FADZUREENA JAMALUDIN

December 2008

Chairman : Prof. Madya Dr. Khozirah Hj. Shaari , PhD

Institute : Bioscience

Leaves and stem of *Vitex negundo* were examined for phytochemicals using various techniques such as normal column chromatography, gel filtration on Sephadex LH-20 and radial chromatography. From the leaves, seven compounds were isolated and identified, by the use of various spectroscopic methods, to be mixture of the flavonoids luteolin, luteolin-3'-*O*-glucuronide, and isoorientin, the iridoid glycosides 2'-*p*-hydroxybenzoylmussaenosidic acid and agnuside, and *p*-hydroxyl benzoic acid as well as stigmasterol and β -sitosterol. Meanwhile, the stem yielded four lignans which were isolated for the first time from the plant, identified as 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde, vitedoin A, vitrofolal E and detetrahydroconidendrin.



Nitric oxide (NO) inhibitory assay using RAW 264.7 murine macrophage and soybean lipoxygenase inhibitory assay were carried out in the screening for anti-inflammatory properties of the crude methanolic extract, the hexane, dichloromethane and ethyl acetate soluble fractions of the plant. From the leaves, both the hexane and dichloromethane fractions were shown to strongly inhibit nitric oxide production with an IC_{50} of 14.00 $\mu\text{g/ml}$ and 20.00 $\mu\text{g/ml}$ respectively. Meanwhile, inhibition of soybean lipoxygenase activity was shown by the ethyl acetate fractions from both plant parts with IC_{50} of 56.38 $\mu\text{g/ml}$ and 63.94 $\mu\text{g/ml}$ respectively.

Further anti-inflammatory investigation on some of the isolated compounds showed that luteolin was significantly inhibited NO production with an IC_{50} of 41.50 $\mu\text{g/ml}$ (145.10 μM), and inhibited formation of (9Z, 11E)-(13S)-13-hydroxyoctadeca-9,11-dienoate with an IC_{50} of 1.55 $\mu\text{g/ml}$ (5.42 μM). Luteolin also exhibited high activity in PAF receptor binding assay with 70.20% inhibition at concentration of 18.2 $\mu\text{g/ml}$ and xanthine oxidase assay with 98.20% inhibition at concentration of 100 $\mu\text{g/ml}$. The antioxidant evaluation using DPPH radical scavenging assay showed that luteolin and 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde at a concentration of 250 $\mu\text{g/ml}$ exhibited significant inhibition at 96.2% and 94.7% respectively. The results indicated that luteolin may play a key factor in the plant's ability to reduce inflammation.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**KOMPONEN KIMIA SERTA PENILAIAN AKTIVITI ANTI-
INFLAMMASI DAN ANTIOKSIDAN DARIPADA *VITEX NEGUNDO***

Oleh

FADZUREENA JAMALUDIN

Disember 2008

Pengerusi : Prof. Madya Dr. Khozirah Hj. Shaari , PhD

Institut : Biosains

Kajian fitokimia telah dilakukan terhadap daun dan batang *Vitex negundo* melalui penggunaan berbagai teknik kromatografi seperti kromatografi turus biasa, filtrasi gel Sephadex LH-20 dan kromatografi radial. Tujuh sebatian telah berjaya dipencilkan dan dikenalpasti menggunakan pelbagai teknik spektroskopi daripada ekstrak daun iaitu daripada kumpulan flavonoid, luteolin, luteolin-3'-*O*-glukuronida dan isoorientin, daripada kumpulan iridoid glukosida, 2'-*p*-hidroksibenzoilmussaenosidik asid dan agnusida, juga sebatian yang dikenali sebagai *p*-hidroksibenzoik asid dan campuran stigmasterol and β -sitosterol. Daripada ekstrak batang, empat sebatian daripada kumpulan lignan berjaya dipencilkan pertama kali daripada tanaman ini yang dikenali sebagai 6-hidroksi-4-(4-hidroksi-3-metoksiphenil)3-hidroksimetil-7-metoksi-3,4-dihidro-2-naphthaldehid, vitedoin A, vitrofolal E and detetrahydroconidendrin.



Daripada penialaian sifat anti-nflammasi tanaman ini telah diuji dengan model inflamasi seluler dan asai kinetic enzimatik. Asai perencatan nitric oksida (NO) menggunakan makrofag murin monositik (RAW 264.7) dan asai lipoxigenase kacang soya telah digunakan sebagai penabiran awal bagi ekstrak kasar methanol, fraksi heksana, fraksi diklorometana dan fraksi etil asetat. Hasilnya fraksi heksana dan fraksi diklorometana daripada daun menunjukkan aktiviti yang kuat merencat penghasilan nitric oksida, dengan nilai 50% perencatan 14.00 $\mu\text{g/ml}$ dan 20.00 $\mu\text{g/ml}$ setiap satu. Manakala penekanan lipoksigenase kacang soya hanya diperlihatkan oleh fraksi etil asetat daripada kedua-dua bahagian daipada tanaman ini dengan nilai 50% perencatan 56.38 $\mu\text{g/ml}$ dan 63.94 $\mu\text{g/ml}$ setiap satu.

Penyelidikan anti-inflamasi keatas sebahagian daripada sebatian hasil penulinan menunjukkan luteolin secara signifikan menekan pembebasan nitric oksida dengan nilai 50% perencatan 41.50 $\mu\text{g/ml}$ (145.10 μM), dan merencatan penghasilan (9Z, 11E)-(13S)-13-hidroksioktadeka-9,11-dienoat dengan nilai 50% perencatan 1.55 $\mu\text{g/ml}$ (5.42 μM). Luteolin juga didapati memberi nilai perencatan yang tinggi daripada asai penggabungan reseptor PAF dengan peratus perencatan sebanyak 70.20% pada kepekatan 18.2 $\mu\text{g/ml}$ dan pada asai xanthine oksida dengan peratus perencatan sebanyak 98.20% pada kepekatan 100 $\mu\text{g/ml}$.

Kajian anti-oksida yang telah dilakukan menggunakan asai pemusnah radikal DPPH mendapati dua sebatian iaitu luteolin and 6-hidroksi-4-(4-hidroksi-3-metoksiphenil)3-hidroksimetil-7-metoksi-3,4-dihidro-2-naphthaldehid pada



kepekatan 250 $\mu\text{g/ml}$ secara signifikan memberi peratus perencatan sebanyak 96.22% dan 94.7% setiap satu. Keputusan yang dipeolehi menunjukkan sebatian luteolin yang berjaya dipencilkan memainkan peranan penting dalam tanaman ini bagi merencat kesan inflammasi.



AKNOWLEDGEMENT

In the name of Allah, most Gracious and most Merciful.

All the great Merciful and Forgiveness of Allah who has bless me with the completion of this thesis even though with many barriers and obstacles I managed to complete this testament.

First and for most I would like to thank Forest Research Institute Malaysia (FRIM) and my programme director Dr. Rasadah Mat Ali for giving me the opportunity to further my study in this field of research.

I would like to express my sincere acknowledgement and deepest appreciation to my understanding supervisor, Assoc. Prof. Dr. Khozirah Hj. Shaari for her guidance, constructive criticism, never ending support and brilliant ideas throughout many discussions from the beginning of this research till the final manuscript.

I am also very grateful to members of my supervisory committee, Prof. Dr. Nordin Hj. Lajis, Prof. Dr. Daud Ahmad Israf Ali and again Dr. Rasadah Mat Ali for their discussion and professional assistance in my research.

Much appreciation goes to research officers; Mr. Salahudin, Puan Zurina and Puan Mazina for obtaining spectroscopy data. Thanks to my mentor Dr. Faridah Abas and Puan Mazura Pizar for her guidance and assistances throughout the research. Thanks to



all my labmates, especially Kak Rozi, Pauliena, Uwi, Kak Ana and Pei Jean. Thanks for the laughing, joking and cheerful day. I wish my special thanks to my beloved parents, sister and brothers for constant love, praying, support, sacrifice, motivation and encouragement. Last but not least, my deepest thank and love to my beloved husband Azry and my two princess Nuranees Zulaikha and Nuranees Zuhaira for the support, encouragement and understanding.



I certify that a Thesis Examination Committee has met on 10th December 2008 to conduct the final examination of Fadzureena binti Jamaludin on her thesis entitled “Chemical Constituents of *Vitex negundo* and the Evaluation of their Anti-inflammatory and Antioxidant Activities” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Degree of Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

GWENDOLINE EE CHENG LIAN, PhD

Associate Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

AMIN ISMAIL, PhD

Associate Professor
Faculty of Medicine & Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

MUHAMMAD NAZRUL HAKIM ABDULLAH, PhD

Associate Professor
Faculty of Medicine & Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

LAILY DIN, PhD

Professor, Dato’
School of Chemical Science and Technology
Faculty of Science & Technology
Universiti Kebangsaan Malaysia
(External Examiner)

BUJANG KIM HUAT, PhD

Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :



This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

KHOZIRAH SHAARI, PhD

Associate Professor
Institute Bioscience
Universiti Putra Malaysia
(Chairman)

DAUD AHMAD ISRAF ALI, PhD

Professor
Institute Bioscience
Universiti Putra Malaysia
(Member)

RASADAH MAT ALI, PhD

Dr.
Forest Research Institute Malaysia
(Member)

NORDIN HJ. LAJIS, PhD

Professor
Institute Bioscience
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date :



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged . I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

FADZUREENA JAMALUDIN

Date :



TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	iv
ACKNOWLEDGEMENT	vii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvii
GLOSSARY OF ABBREVIATIONS	xxiii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	
2.1 Botany, Distribution and Ethnobotany of <i>Vitex</i> species	
2.1.1 The Genus <i>Vitex</i>	6
2.1.2 <i>Vitex negundo</i> Linn.	7
2.2 A review of Previous Investigation on the <i>Vitex</i> species	
2.2.1 Chemical constituents from <i>Vitex</i> species	9
2.2.2 Chemical constituents from <i>Vitex negundo</i> Linn.	19
2.3 Biologically Active Constituents from <i>Vitex</i> species	23
2.4 Anti-inflammatory and antioxidant properties of <i>Vitex negundo</i> Linn.	25
2.5 Inflammation	27
2.6 Mediators of Inflammation	29
2.7 Lipoygenase and inhibition of leukotriene biosynthesis	30
2.8 Nitric Oxide	33
2.9 Platelet Activating Factor	34
2.10 Xanthine Oxidase	35
2.11 Phytochemicals which have anti-inflammatory actions and possible mechanism	36
2.12 Antioxidant	38
2.13 Free Radicals	38
2.14 Plant Antioxidant	39
3 METHODOLOGY	
3.1 General Instrumentation	41
3.2 Chromatographic Methods	41
3.3 Solvents	42
3.4 Isolation of Compounds from <i>Vitex negundo</i>	
3.4.1 Plant material	42
3.4.2 Extraction and Isolation of compounds from crude methanolic extract of <i>Vitex negundo</i> leaves	43



3.4.3	Extraction and Isolation of compounds from crude methanolic extract of <i>Vitex negundo</i> stem	47
3.4.4	Spectral data of isolated compounds	50
3.5	Anti-inflammatory Assay	56
3.5.1	Chemical and reagents	58
3.5.2	Preparation of test samples	59
3.5.3	Methodology	
	<i>In-vitro</i> enzymatic lipoxygenase inhibition assay	60
	<i>In-vitro</i> inhibition of nitric oxide production assay	63
	Cell culture	63
	Griess Assay	63
	Cell viability	64
	Data analysis	64
	Platelet activating factor receptor binding inhibitory assay	66
	<i>In-vitro</i> enzymatic xanthine oxidase inhibition assay	69
3.6	Antioxidant Activity	
3.6.1	Chemical and reagents	71
3.6.2	Preparation of test samples	72
3.6.3	Methodology	
	DPPH radical scavenging assay	72
	Xanthine / Xanthine oxidase superoxide scavenging assay	73
4	RESULTS AND DISCUSSION	
4.1	Isolation and characterization of compounds isolated from <i>Vitex negundo</i>	76
4.1.1	Flavonoid and Flavonoid glycosides	
	Luteolin	77
	Isoorientin	88
	Luteolin 3'- <i>O</i> -glucuronide	97
4.1.2	Iridoid glycoside	
	Agnuside	107
	2'- <i>p</i> -hydroxybenzoylmussaenosidic acid	125
4.1.3	Lignans	
	6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-	147
	Vitedoin A	162
	Vitrofolal E	173
	Detetrahyrdoconidendrin	183
4.1.4	Other compounds	
	mixture of β -sitosterol and stigmasterol	195
	<p>-hydroxybenzoic acid</p>	198
4.2	Anti-inflammatory properties of <i>Vitex negundo</i>	
4.2.1	Inhibition of Soybean Lipoxygenase by the crude Extract and solvent fractions of <i>Vitex negundo</i>	202

4.2.2	Inhibition of soybean LO by isolated compounds	205
4.2.2	Inhibition of nitric oxide production by the crude extract and solvent fractions of <i>Vitex negundo</i>	207
4.2.4	Inhibition of nitric oxide production by isolated compounds	211
4.2.5	Inhibition of platelet activating factor (PAF) receptor binding by isolated compounds	212
4.2.6	Inhibition of xanthine oxidase by isolated compounds	213
4.3	Antioxidant assays	
4.3.1	Inhibition of DPPH radical scavenging assay by the crude methanolic and compounds isolated from <i>Vitex negundo</i>	214
4.3.2	Inhibition of xanthine oxidase superoxide scavenging assay by the crude methanolic and compounds isolated from <i>Vitex negundo</i>	215
5	CONCLUSION	218
	BIBLIOGRAPHY	220
	BIODATA OF THE STUDENT	



LIST OF TABLES

Table	Page
1 Flavones isolated from <i>Vitex</i> species	19
2 Anti-inflammatoty actions and possible mehanism of some Phytochemicals	37
3 Antioxidant actions and possible mehanism of some phytochemicals	39
4 Compounds isolated from <i>V. negundo</i>	76
5 The assignment of protons and carbons of luteolin (64)	79
6 The assignments of protons and carbons for isoorientin (69)	90
7 The assignment of protons and carbons of luteolin 3'- <i>O</i> -glucuronide (92)	99
8 The assignment of protons and carbons of agnuside (6)	111
9 The assignment of protons and carbons of 2'- <i>p</i> -hydroxybenzoyl mussaenosidic acid (15)	129
10 The assignment of protons and carbons of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75)	150
11 The assignment of protons and carbons of vitedoin A (76)	164
12 The assignment of protons and carbons of vitrofolal E (54)	175
13 The proton assignment of detetrahydroconidendrin (56)	186
14 Inhibition of lipoxygenase by crude extracts and solvent fractions of <i>Vitex negundo</i>	204
15 Inhibition of lipoxygenase by compounds isolated from <i>Vitex negundo</i>	206
16 Inhibition of NO-released from RAW 264.7 murine macrophage stimulated with E.coli LPS (10 µg/ml) and IFN-γ (200 U/ml) by the crude extracts and various solvent fractions of <i>Vitex negundo</i>	210
17 Inhibition of NO-released from RAW 264.7 murine macrophage stimulated with E.coli LPS (10 µg/ml) and IFN-γ (200 U/ml) by compounds isolated from <i>Vitex negundo</i>	211



18	Inhibitory effects of the isolated compounds on PAF receptor binding to platelets at a test concentration of 18.2 µg/ml.	212
19	Xanthine Oxidase Inhibitory effects of the isolated compounds at test concentration of 100 µg/ml.	213
20	Inhibitory effects of the isolated compounds on DPPH radical scavenging assay at concentration of 250 µg/ml.	214
21	Inhibitory effects of the isolated compounds on xanthine oxidase superoxide scavenging assay at test concentration of 250 µg/ml.	215

LIST OF FIGURES

Figure		Page
1	Schematic flow of process used in obtaining bioactive substances from plants	2
2	<i>Vitex rotundifolia</i> (a) and <i>Vitex trifolia</i> (b)	7
3	<i>Vitex negundo</i> Linn	9
4	Inflammation or the immune response	28
5	The biosynthesis and chemical structures of leukotrienes	31
6	Isolation scheme for compounds from leaves of <i>V. negundo</i>	46
7	Isolation scheme for compounds from stem of <i>V. negundo</i>	49
8	Color reaction involved in NO assay	57
9	Metabolisation of MTT to a formazan salt by viable cells	57
10	Flowchart for the enzymatic lipoxygenase inhibition assay	62
11	Flowchart of nitric oxide assay	65
12	Flowchart for PAF receptor binding assay	68
13	Flowchart for the enzymatic xanthine oxidase inhibition assay	70
14	Flowchart for the scavenging of DPPH free radical assay	73
15	Flowchart for the xanthine/ xanthine oxidase superoxide scavenging assay	75
16	Mechanism of mass fragmentation for luteolin (64)	80
17	EIMS spectrum of luteolin (64)	81
18	IR spectrum of luteolin (64)	82
19	¹ H-NMR spectrum of luteolin (64) in CD ₃ OD	83
20	COSY spectrum of luteolin (64) in CD ₃ OD	84
21	¹³ C-NMR spectrum of luteolin (64) in CD ₃ OD	85



22	HSQC spectrum of luteolin (64) in CD ₃ OD	86
23	HMBC spectrum of luteolin (64) in CD ₃ OD	87
24	LCMS spectrum of isoorientin (69)	91
25	¹ H-NMR spectrum of isoorientin (69) in CD ₃ OD	92
26	COSY spectrum of isoorientin (69) in CD ₃ OD	93
27	¹³ C-NMR spectrum of isoorientin (69) in CD ₃ OD	94
28	HMBC spectrum of isoorientin (69) in CD ₃ OD	95
29	HMBC spectrum of isoorientin (69) in CD ₃ OD	96
30	¹ H-NMR spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	100
31	¹³ C-NMR spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	101
32	HSQC spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	102
33	HMBC spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	103
34	HMBC spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	104
35	HMBC spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	105
36	HMBC spectrum of luteolin 3'- <i>O</i> -glucuronide (92) in CD ₃ OD	106
37	Iridoid moiety as a partial structure	110
38	LCMS spectrum of agnuside (6)	112
39	¹ H-NMR spectrum of agnuside (6) in CD ₃ OD	113
40	¹³ C-NMR spectrum of agnuside (6) in CD ₃ OD	114
41	HSQC spectrum of agnuside (6) in CD ₃ OD	115
42	HSQC spectrum of agnuside (6) in CD ₃ OD	116
43	HSQC spectrum of agnuside (6) in CD ₃ OD	117
44	COSY spectrum of agnuside (6) in CD ₃ OD	118
45	COSY spectrum of agnuside (6) in CD ₃ OD	119



46	HMBC spectrum of agnuside (6) in CD ₃ OD	120
47	HMBC spectrum of agnuside (6) in CD ₃ OD	121
48	HMBC spectrum of agnuside (6) in CD ₃ OD	122
49	HMBC spectrum of agnuside (6) in CD ₃ OD	123
50	HMBC spectrum of agnuside (6) in CD ₃ OD	124
51	¹ H-NMR spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	130
52	¹³ C-NMR spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	131
53	HSQC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	132
54	HSQC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	133
55	COSY spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	134
56	COSY spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	135
57	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	136
58	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	137
59	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	138
60	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	139
61	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	140
62	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	141



63	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	142
64	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	143
65	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	144
66	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	145
67	HMBC spectrum of 2'- <i>p</i> - hydroxybenzoyl mussaenosidic acid (15) in CD ₃ OD	146
68	IR spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75)	151
69	UV spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75)	151
70	EIMS spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75)	152
71	¹ H-NMR spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75) in CD ₃ OD	153
72	¹ H-NMR spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75) in CD ₃ OD	154
73	¹³ C-NMR spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75) in CD ₃ OD	155
74	COSY spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75) in CD ₃ OD	156
75	HSQC spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75) in CD ₃ OD	157
76	HSQC spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2- naphthaldehyde (75) in CD ₃ OD	158



77	HMBC spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde (75) in CD ₃ OD	159
78	HMBC spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde (75) in CD ₃ OD	160
79	HMBC spectrum of 6-hydroxy-4-(4-hydroxy-3-methoxyphenyl)3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde (75) in CD ₃ OD	161
80	¹ H-NMR spectrum of vitedoin A (76) in CD ₃ OD	165
81	¹ H-NMR spectrum of vitedoin A (76) in CD ₃ OD	166
82	¹³ C-NMR spectrum of vitedoin A (76) in CD ₃ OD	167
83	HSQC spectrum of vitedoin A (76) in CD ₃ OD	168
84	HSQC spectrum of vitedoin A (76) in CD ₃ OD	169
85	HMBC spectrum of vitedoin A (76) in CD ₃ OD	170
86	HMBC spectrum of vitedoin A (76) in CD ₃ OD	171
87	HMBC spectrum of vitedoin A (76) in CD ₃ OD	172
88	¹ H-NMR spectrum of vitrofolal E (54) in CD ₃ OD	176
89	¹ H-NMR spectrum of vitrofolal E (54) in CD ₃ OD	177
90	¹³ C-NMR spectrum of vitrofolal E (54) in CD ₃ OD	178
91	HMBC spectrum of vitrofolal E (54) in CD ₃ OD	179
92	HMBC spectrum of vitrofolal E (54) in CD ₃ OD	180
93	HMBC spectrum of vitrofolal E (54) in CD ₃ OD	181
94	HMBC spectrum of vitrofolal E (54) in CD ₃ OD	182
95	EIMS spectrum of detetrahydroconidendrin (56)	187
96	¹ H-NMR spectrum of detetrahydroconidendrin (56) in CD ₃ OD	188
97	¹³ C-NMR spectrum of detetrahydroconidendrin (56) in CD ₃ OD	189



98	HMBC spectrum of detetrahydroconidendrin (56) in CD ₃ OD	190
99	HMBC spectrum of detetrahydroconidendrin (56) in CD ₃ OD	191
100	HMBC spectrum of detetrahydroconidendrin (56) in CD ₃ OD	192
101	HMBC spectrum of detetrahydroconidendrin (56) in CD ₃ OD	193
102	HMBC spectrum of detetrahydroconidendrin (56) in CD ₃ OD	194
103	EIMS spectrum of mixture of β -sitosterol and stigmasterol (90)	196
104	¹ H-NMR spectrum of mixture of β -sitosterol and stigmasterol (90) in CHCl ₃	197
105	IR spectrum of <i>p</i> -hydroxybenzoic acid (91)	199
106	EIMS spectrum of <i>p</i> -hydroxybenzoic acid (91)	200
107	¹ H-NMR spectrum of <i>p</i> -hydroxybenzoic acid (91) in CD ₃ OD	201
108	Inhibition (%) of lipoxygenase by extracts of <i>Vitex negundo</i>	203
109	Inhibition (%) of lipoxygenase by solvent fractions of <i>Vitex negundo</i>	204
110	Inhibition of NO released from RAW 264.7 murine macrophage cell line by leaf MeOH extract of <i>Vitex negundo</i>	208
111	Inhibition of NO released from RAW 264.7 murine macrophage cell line by stem MeOH extract of <i>Vitex negund</i>	209
112	Inhibition of NO released from RAW 264.7 murine macrophage cell line by hexane fraction from leaves of <i>Vitex negundo</i>	209
113	Inhibition of NO released from RAW 264.7 murine macrophage cell line by DCM fraction from leaves of <i>Vitex negundo</i>	210



GLOSSARY OF ABBREVIATIONS

MRSA	Methicilin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicilin-sensitive <i>Staphylococcus aureus</i>
PAMPs	Pathogen-associated molecular patterns
IL-1	Interleukin-1
TNF- α	Tumor necrosis factor- α
PG	Prostaglandin
LT	Leukotrien
PAF	Platelet-activating factor
LOX	Lipoxygenase
HLO	Human lipoxygenase
AA	Arachidonic acid
FLAP	5-lipoxygenase activating protein
5-HPETE	5S-hydroperoxy-6,8-trans-11,14- <i>cis</i> -eicosatetraenoic acid
LTA ₄	Leukotriene A ₄
LTB ₄	Leukotriene B ₄
LTC ₄	Leukotriene C ₄
LTD ₄	Leukotriene D ₄
LTE ₄	Leukotriene E ₄
NO	Nitric oxide
NOS	Nitric oxide synthase
XO	Xanthine Oxidase
ROS	Reactive oxygen species
UV	Ultraviolet
EIMS	Electron Spray Impact Mass Spectrometry
LCMS	Liquid Chromatography Mass Spectrometry
FTIR	Fourier Transform Infra Red
NMR	Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Spectroscopy



TLC	Thin Layer Chromatography
MTT	3-(4,5)-dimethyl-thiazol-2-yl)2,5-diphenyltetrazolium bromide
DPPH	1,2-diphenyl-2-picrylhydrazyl
AOP	Antioxidant Potential
NBT	Nitro blue tetrazolium
OD	Optical density