UNIVERSITI PUTRA MALAYSIA

PHYTOCHEMICAL CHARACTERIZATION OF *XEROSPERMUM NORONHIANUM*

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PHYTOCHEMICAL CHARACTERIZATION OF *XEROSPERMUM NORONHIANUM*

By

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PHYTOCHEMICAL CHARACTERIZATION OF *XEROSPERMUM NORONHIANUM*

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Chairman : Associate Professor Khozirah Shaari, Ph.D

Institute : Bioscience

Xerospermum noronhianum, locally known as *gigi buntal* (globe-fish's teeth) or *rambutan pachat* (leech's rambutan) is a species belonging to the Sapindaceae family. The Sapindaceae are generally well known as a prolific source of plant saponins. However, to date, there has been no systematic phytochemical and biological evaluation on this particular species. In the present study, several known compounds have been successfully isolated from various plant parts and solvent fractions of *Xerospermum noronhianum*. Three new triterpenoid saponins have been purified from the ethyl acetate and aqueous fractions of both stem and leaf of the plant. Through the use of high field NMR and mass spectroscopy, these saponins have been identified to be those of the oleanane-type, i.e 3-*O*-{*α*-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranosyl}-28-*O*-{[*α*-L-rhamnopyranosyl(1→2)][*α*-L-rhamnopyranosyl(1→6)]}-*β*-D-glucopyranosyl oleanolic acid, 3-*O*-{*α*-L-rhamnopyranosyl(1→3)-*β*-D-fucopyranosyl}-28-*O*-{*α*-L-rhamnopyranosyl(1→4)}-*β*-D-glucopyranosyl oleanolic acid and 3-*O*-{*α*-L-rhamnopyranosyl(1→2)-[3',4'-diacetoxy-*β*-D-fucopyranosyl]}-28-*O*-{*α*-L-

rhamnopyranosyl(1→2)}-*β*-D-glucopyranosyl oleanolic acid. Several known compounds have also been isolated and identified as the free triterpenes namely lupeol, oleanolic acid, friedelin, 3*β*-hydroxy-29-nor-20-lupenone, 3*β*-hydroxy-lup-20(29)en-30-al, mixture of 3*β*-hydroxyolean-12-en-11-one and 3*β*-hydroxyurs-12-en-11-one; the flavonoid glycosides kampferol-3-*O*-rhamnoside, quercetin-3-*O*-rhamnoside; the benzoic acid derivatives 3,5-dihydroxy-4-methoxybenzoic acid, 3,4-dihydroxybenzoic acid, 3,4,5-trihydroxybenzoic acid; as well as scopoletin, lupeol palmitate, and palmitic acid, mixture of *β*-sitosterol and stigmasterol and mixture of their glycosides. Oxidation of the major compound, lupeol, successfully yielded lupenone as the product.

The crude extracts and isolated pure compounds were also further evaluated for their anti-cholinesterase activity, against two enzymes; acetylcholinesterase and butyrylcholinesterase, using thin layer chromatography bioautographic method. The inhibition activities of the test samples were expressed as pMIQ values, which represents the negative logarithm of the minimal inhibitory quantity (in moles) that produced the spot with the least observable whiteness. Generally, the crude extracts of stem, leaf and bark parts, all showed inhibition in the preliminary screening by giving white observable inhibition spots against a yellow-coloured background. The enzyme inhibiting constituents were, in part, found to be due to the triterpenoidal saponins, the free triterpenes as well as the sterol and its glycosides. However, the tested compounds were considered to be weakly active due to their low pMIQ values. Compounds with pMIQ values $\geq$ 10.5 is considered to be bioactive. All of the tested compounds in this study gave pMIQ value in the range of 1.7 to 3.7.

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

PENGAJIAN KIMIA TUMBUHAN BAGI *XEROSPERMUM NORONHIANUM*

Oleh

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Disember 2008

Pengerusi : Profesor Madya Khozirah Shaari, Ph.D.

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Xerospermum noronhianum, yang dikenali dengan nama tempatan ‘gigi buntal’ atau ‘rambutan pachat’ merupakan salah satu spesies dari keluarga tumbuhan Sapindaceae. Sapindaceae merupakan sumber yang kaya dengan sebatian saponin. Tiada kajian fitokimia mahupun biologi pernah dilakukan ke atas spesies *X. noronhianum,* setakat ini. Oleh yang demikian, kajian komposisi kimia dan aktiviti biologi telah dilakukan ke atas bahagian dahan, daun dan batang *X. noronhianum*. Hasil daripada kajian fitokimia, tiga sebatian dari jenis saponin triterpenoida telah dapat dipisahkan dari fraksi etil asetat dan akuas, bahagian dahan dan daun. Dengan menggunakan teknik Resonan Magnetik Nuklear (RMN) medan tinggi dan spektroskopi jisim, sebatian saponin berkenaan telah dikenalpasti sebagai 3-*O*-{*α*-L-ramnopiranosil(1→2)-*β*-D-fukopiranosil}-28-*O*-{[*α*-L-ramnopiranosil(1→2)][*α*-L-ramnopiranosil(1→6)]}-*β*-D-glukopiranosil oleanolik asid, 3-*O*-{*α*-L-ramnopiranosil(1→3)-*β*-D-fukopiranosil}-28-*O*-{*α*-L-ramnopiranosil(1→4)}-*β*-D-glukopiranosil oleanolik asid dan 3-*O*-{*α*-L-ramnopiranosil(1→2)-[3’,4’-diasetoksi-*β*-D-fukopiranosil]}-28-*O*-{*α*-L-ramnopiranosil(1→2)}-*β*-D-glukopiranosil oleanolik

asid. Ketiga-tiga sebatian saponin ini merupakan sebatian baru, iaitu diasingkan buat pertama kali dan belum pernah dikenapasti sebelum ini. Selain itu, beberapa sebatian lain yang agak biasa ditemui dalam tumbuhan juga telah berjaya dipisahkan daripada ekstrak pelbagai bahagian *X. noronhianum*. Antaranya ialah sebatian jenis triterpena bebas iaitu lupeol, asid oleanolik, friedelin, 3*β*-hidroksi-29-nor-20-lupenon, 3*β*-hidroksi-lup-20(29)en-30-al, campuran 3*β*-hidroksiolean-12-en-11-on dan 3*β*-hidroksiurs-12-en-11-on, sebatian jenis flavonoid glikosida iaitu kampferol-3-*O*-rhamnosida dan kuercetin-3-*O*-rhamnosida, sebatian terbitan asid benzoik iaitu asid 3,5-dihidroksi-4-metoksibenzoik, asid 3,4-dihidroksibenzoik dan asid 3,4,5-trihidroksibenzoik, skopoletin, lupeol palmitat dan asid palmitik serta campuran *β*-sitosterol dan stigmasterol dan campuran glikosidanya. Pengoksidaan lupeol, sebatian kimia major dari tumbuhan ini, menghasilkan lupenon. Aktiviti anti-kolinesterase bagi kesemua ekstrak dan sebatian kimia yang ditulenkan, juga dikaji. Perencatan aktiviti dua jenis enzim iaitu asetilkolinesterase dan butirilkolinesterase telah dikaji dengan menggunakan kaedah bioasai bioautografik lapisan nipis kromatografi. Aktiviti perencatan bagi sebatian kimia tulen diwakili oleh nilai pMIQ, iaitu logaritma negatif bagi minima kuantiti dalam mol yang merencatkan aktiviti. Secara keseluruhannya, semua ekstrak akuas methanol bagi ketiga-tiga bahagian tumbuhan tersebut menghasilkan zon perencatan yang berwarna putih di atas dasar yang berwarna kuning. Secara amnya, sebatian perencat aktiviti enzim di dalam ekstrak tumbuhan berkenaan adalah terdiri daripada jenis saponin triterpenoida, triterpena bebas, sterol dan sterol glikosida. Namun, kesemua sebatian bioaktif tersebut mempunyai aktiviti perencatan yang lemah secara individunya kerana nilai pMIQ masing-masing yang rendah, iaitu antara 1.7 hingga 3.7.

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I certify that an Examination Committee met on **3rd December 2008** to conduct the final examination of Tan Pei Jean on her thesis entitled "Phytochemical characterization of *Xerospermum noronhianum*" in accordance with the Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15th March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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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 or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

_____

TAN PEI JEAN

Date:

TABLE OF CONTENTS

		Page
ABSTRACT		i
ABSTRAK		iii
ACKNOWLEDGEMENTS		v
APPROVAL		vi
DECLARATION		viii
LIST OF TABLES		xii
LIST OF FIGURES		xv
LIST OF SCHEMES		xxii
LIST OF ABBREVIATIONS		xxiii
CHAPTER		
1	**INTRODUCTION**	
1.1	General	1
1.2	Plant Secondary Metabolites	3
1.3	Purpose of the Present Study	3
2	**LITERATURE REVIEW**	
2.1	The Sapindaceae	5
2.1.1	The genus *Xerospermum*	6
2.2	Chemistry of the Sapindaceae	7
2.2.1	Triterpenoidal Saponins	9
2.2.2	Terpenes	15
2.2.3	Flavonoids	17
2.2.4	Benzoic Acid Derivatives	18
2.2.5	Lipids and Fatty acids	19
2.3	Alzheimer's Disease and the Search for Cholinesterase Inhibitors	22
3	**METHODOLOGY**	
3.1	General Instrumentation	24
3.2	Chromatographic Techniques	25
3.2.1	Column Chromatography (CC)	25
3.2.2	Preparative Thin Layer Chromatography (PTLC)	25
3.2.3	Thin Layer Chromatography (TLC)	26
3.2.4	Centrifugal Thin Layer Chromatography (Chromatotron)	27
3.2.5	Liquid Chromatography-Mass Spectrometry (LC-MS)	27
3.2.6	Semi-preparative High Performance Liquid Chromatography (HPLC)	28
3.3	Plant materials	29
3.3.1	Collection and Preparation of Plant Materials	29
3.3.2	Extraction and Fractionation of *X. noronhianum*	29
3.3.3	Isolation of Compounds from *X. noronhianum*	30

3.3.4 Oxidation of Lupeol to Lupenone 39
3.3.5 Physical and spectral properties of isolated compounds 39
3.4 Bioassays
3.4.1 Cholinesterase 59
3.4.2 Cholinesterase Inhibition Assay 60

4 RESULTS AND DISCUSSIONS
4.1 Extraction and Isolation of compounds from *Xerospermum noronhianum* 64
4.2 Triterpenoids Isolated from *Xerospermum noronhianum* 66
4.2.1 Characterization of XnPES1 as lupeol (**31**) 66
4.2.2 Characterization of XnPES1a as lupenone (**61**) 81
4.2.3 Characterization of XnPEB1 as lupeol palmitate (**62**) 91
4.2.4 Characterization of XnPEB5 as 3*β*-hydroxy-29-nor-20-lupenone (**63**) 103
4.2.5 Characterization of XnPEB4a as 3*β*-hydroxy-lup-20(29)-en-30-al (**64**) 113
4.2.6 Characterization of XnPEB3 as friedelin (**65**) 123
4.2.7 Characterization of XnHS3a as oleanolic acid (**30**) 136
4.2.8 Characterization of XnPEB4b as mixture of 3*β*-hydroxyolean-12-en-11-one (**66**) and 3*β*-hydroxyurs-12-en-11-one (**67**) 148
4.3 Saponins Isolated from *Xerospermum noronhianum* 166
4.3.1 LCMS Analysis of Saponin Fraction 166
4.3.2 Characterization of XnES2 as 3-*O*-{*α*-L-rhamnopyranosyl(1→2)-*β*-D-fucopyranosyl} -28-*O*-{[*α*-L-rhamnopyranosyl(1→2)][*α*-L-rhamnopyranosyl(1→6)]}-*β*-D-glucopyranosyl oleanolic acid (**68**) 170
4.3.3 Characterization of XnESP2 as 3-*O*-{*α*-L-rhamnopyranosyl(1→3)-*β*-D-fucopyranosyl} -28-*O*-{*α*-L-rhamnopyranosyl(1→4)}-*β*-D-glucopyranosyl oleanolic acid (**69**) 188
4.3.4 Characterization of XnESP3 as 3-*O*-{*α*-L-rhamnopyranosyl(1→2)-[3',4'-diacetoxy-*β*-D-fucopyranosyl]}-28-*O*-{*α*-L-rhamnopyranosyl(1→2)} -*β*-D-glucopyranosyl oleanolic acid (**70**) 204
4.4 Miscellaneous Types of Compounds from *X. noronhianum* 220
4.4.1 Characterization of XnEL3 as 3, 5-dihydroxy-4-methoxybenzoic acid (**71**) 220
4.4.2 Characterization of XnEL7 as 3, 4, 5-trihydroxybenzoic acid (**45**) 227
4.4.3 Characterization of XnEL5 as 3, 4-dihydroxybenzoic acid (**72**) 233
4.4.4 Characterization of XnEL1 as kaempferol 3-*O*-rhamnoside (**73**) 239

4.4.5 Characterization of XnEL2 as quercetin 3-*O*-rhamnoside (**74**) 251
4.4.6 Characterization of XnD3 as scopoletin (**75**) 263
4.4.7 Characterization of XnHL3 as palmitic acid (**76**) 272
4.4.8 Characterization of XnHS2 as mixture of *β*-sitosterol (**77**) and stigmasterol (**78**) 280
4.5 Evaluation of Anti-cholinesterase Activity 283

5 CONCLUSION 287

REFERENCES 290
BIODATA OF THE STUDENT 295
LIST OF PUBLICATIONS 296

LIST OF TABLES

Table		Page
3.1	Gradient Solvent System used for LCMS Analysis of Saponins Fractions	28
3.2	Yield of Crude Extracts from Various Parts of *X. noronhianum*	29
3.3	Amount of Fractions from Different Parts of *X. noronhianum*	30
3.4	^{1}H NMR Data of Lupane-skeleton Triterpenes	42
3.5	^{13}C NMR Data of Lupane-skeleton Triterpenes	44
3.6	^{1}H NMR Data of Oleanane-skeleton Triterpenes	46
3.7	^{13}C NMR Data of Oleanane-skeleton Triterpenes	47
3.8	^{1}H NMR Data of Saponins	50
3.9	^{13}C NMR Data of Saponins	53
4.1	Compounds Isolated from Various Parts of *X. noronhianum*	64
4.2	^{1}H-^{13}C Correlations of XnPES1 (**31**) Based on HSQC and HMBC Experiments	70
4.3	Comparison of XnPES1 (**31**) ^{1}H and ^{13}C NMR Data with Literature Values	71
4.4	^{1}H-^{13}C Correlations of XnPES1a (**61**) Based on HSQC and HMBC Experiments	83
4.5	Comparison of XnPES1a (**61**) ^{13}C NMR Data with Literature Values	84
4.6	^{1}H-^{13}C Correlations of XnPEB1 (**62**) Based on HSQC and HMBC Experiments	94
4.7	Comparison of XnPEB1 (**62**) ^{1}H NMR Data with Literature Values	95
4.8	^{1}H-^{13}C Correlations of XnPEB5 (**63**) Based on HSQC and HMBC	

	Experiments	105
4.9	Comparison of XnPEB5 (**63**) ^{1}H and ^{13}C NMR Data with Literature Values	106
4.10	^{1}H-^{13}C Correlations of XnPEB4a (**64**) Based on HSQC and HMBC Experiments	116
4.11	^{1}H-^{13}C Correlations of XnPEB3 (**65**) Based on HSQC and HMBC Experiments	126
4.12	Comparison of XnPEB3 (**65**) ^{1}H and ^{13}C NMR Data with Literature Values	127
4.13	^{1}H-^{13}C Correlations of XnHS3a (**30**) Based on HSQC and HMBC Experiments	140
4.14	Comparison of XnHS3a (**30**) ^{1}H and ^{13}C NMR Data with Literature Values	141
4.15	^{1}H-^{13}C Correlations of XnPEB4b (**66**) (3β-hydroxyolean-12-en-11-one) Based on HSQC and HMBC Experiments	153
4.16	^{1}H-^{13}C Correlations of XnPEB4b (**67**) (3β-hydroxyurs-12-en-11-one) Based on HSQC and HMBC Experiments	154
4.17	Comparison of XnPEB4b (**67**) (3β-hydroxyurs-12-en-11-one) ^{1}H NMR Data with Literature Values	155
4.18	^{1}H-^{13}C Correlations of XnES2 (**68**) Based on HSQC and HMBC Experiments	175
4.19	^{1}H-^{13}C Correlations of XnESP2 (**69**) Based on HSQC and HMBC Experiments	191
4.20	^{1}H-^{13}C Correlations of XnESP3 (**70**) Based on HSQC and HMBC Experiments	208

4.21 ^{1}H-^{13}C Correlations of XnEL1 (**73**) Based on HSQC and HMBC Experiments 241

4.22 Comparison of XnEL1 (**73**) ^{13}C NMR Data with Literature Values 242

4.23 ^{1}H-^{13}C Correlations of XnEL2 (**74**) Based on HSQC and HMBC Experiments 253

4.24 Comparison of XnEL2 (**74**) ^{13}C NMR Data with Literature Values 254

4.25 Comparison of XnD3 (**75**) ^{1}H and ^{13}C NMR Data with Literature Values 265

4.26 Comparison of XnHL3 (**76**) ^{13}C NMR Data with Literature Values 273

LIST OF FIGURES

Figure		Page
3.1	Mechanism of Acetylcholinesterase Inhibition Assay	61
4.1	EIMS spectrum of XnPES1 (**31**)	72
4.2	IR spectrum of XnPES1 (**31**)	72
4.3	^{1}H NMR spectrum of XnPES1 (**31**)	73
4.4	^{13}C NMR spectrum of XnPES1 (**31**)	74
4.5	HSQC spectrum of XnPES1 (**31**)	75
4.6	HMBC spectrum of XnPES1 (**31**)	76
4.7	HMBC spectrum (expansion) of XnPES1 (**31**)	77
4.8	HMBC spectrum (expansion) of XnPES1 (**31**)	78
4.9	HMBC spectrum (expansion) of XnPES1 (**31**)	79
4.10	COSY spectrum of XnPES1 (**31**)	80
4.11	EIMS spectrum of XnPES1a (**61**)	85
4.12	IR spectrum of XnPES1a (**61**)	85
4.13	UV spectrum of XnPES1a (**61**)	86
4.14	^{1}H NMR spectrum of XnPES1a (**61**)	87
4.15	^{13}C NMR spectrum of XnPES1a (**61**)	88
4.16	HMBC spectrum of XnPES1a (**61**)	89
4.17	HMBC spectrum (expansion) of XnPES1a (**61**)	90
4.18	EIMS spectrum of XnPEB1 (**62**)	96
4.19	IR spectrum of XnPEB1 (**62**)	96
4.20	UV spectrum of XnPEB1 (**62**)	97

4.21	^{1}H NMR spectrum of XnPEB1 (**62**)	98
4.22	^{13}C NMR spectrum of XnPEB1 (**62**)	99
4.23	HSQC spectrum of XnPEB1 (**62**)	100
4.24	HMBC spectrum of XnPEB1 (**62**)	101
4.25	HMBC spectrum (expansion) of XnPEB1 (**62**)	102
4.26	EIMS spectrum of XnPEB5 (**63**)	107
4.27	IR spectrum of XnPEB5 (**63**)	107
4.28	UV spectrum of XnPEB5 (**63**)	108
4.29	^{1}H NMR spectrum of XnPEB5 (**63**)	109
4.30	^{13}C NMR spectrum of XnPEB5 (**63**)	110
4.31	HMBC spectrum of XnPEB5 (**63**)	111
4.32	HMBC spectrum (expansion) of XnPEB5 (**63**)	112
4.33	EIMS spectrum of XnPEB4a (**64**)	117
4.34	HREIMS spectrum of XnPEB4a (**64**)	117
4.35	IR spectrum of XnPEB4a (**64**)	118
4.36	UV spectrum of XnPEB4a (**64**)	118
4.37	^{1}H NMR spectrum of XnPEB4a (**64**)	119
4.38	^{13}C NMR spectrum of XnPEB4a (**64**)	120
4.39	HMBC spectrum of XnPEB4a (**64**)	121
4.40	HMBC spectrum (expansion) of XnPEB4a (**64**)	122
4.41	EIMS spectrum of XnPEB3 (**65**)	128
4.42	IR spectrum of XnPEB3 (**65**)	128
4.43	UV spectrum of XnPEB3 (**65**)	129
4.44	^{1}H NMR spectrum of XnPEB3 (**65**)	130

4.45	^{13}C NMR spectrum of XnPEB3 (**65**)	131
4.46	HSQC spectrum of XnPEB3 (**65**)	132
4.47	HMBC spectrum of XnPEB3 (**65**)	133
4.48	HMBC spectrum (expansion) of XnPEB3 (**65**)	134
4.49	COSY spectrum of XnPEB3 (**65**)	135
4.50	EIMS spectrum of XnHS3a (**30**)	142
4.51	IR spectrum of XnHS3a (**30**)	142
4.52	^{1}H NMR spectrum of XnHS3a (**30**)	143
4.53	^{13}C NMR spectrum of XnHS3a (**30**)	144
4.54	HSQC spectrum of XnHS3a (**30**)	145
4.55	HMBC spectrum of XnHS3a (**30**)	146
4.56	HMBC spectrum (expansion) of XnHS3a (**30**)	147
4.57	EIMS spectrum of XnPEB4b (**66, 67**)	156
4.58	IR spectrum of XnPEB4b (**66, 67**)	156
4.59	UV spectrum of XnPEB4b (**66, 67**)	157
4.60	^{1}H NMR spectrum of XnPEB4b (**66, 67**)	158
4.61	^{1}H NMR spectrum (expansion) of XnPEB4b (**66, 67**)	159
4.62	^{13}C NMR spectrum of XnPEB4b (**66, 67**)	160
4.63	^{13}C NMR spectrum (expansion) of XnPEB4b (**66, 67**)	161
4.64	HSQC spectrum of XnPEB4b (**66, 67**)	162
4.65	HMBC spectrum of XnPEB4b (**66, 67**)	163
4.66	HMBC spectrum (expansion) of XnPEB4b (**66, 67**)	164
4.67	HMBC spectrum (expansion) of XnPEB4b (**66, 67**)	165
4.68	Total Ion Chromatogram (left up) and Liquid Chromatogram	168

(right up) of EtOAc Stem Saponins Fraction with Selected Ion Mass Spectrums

4.69 Liquid Chromatogram of Leaf EtOAc (top) and Aqueous Fraction (bottom) with UV Profile (middle) 169

4.70 APCI-MS spectrum of XnES2 (**68**) 177

4.71 HRMS spectrum of XnES2 (**68**) 178

4.72 ^{1}H NMR spectrum of XnES2 (**68**) 179

4.73 ^{1}H NMR spectrum (expansion) of XnES2 (**68**) 180

4.74 ^{13}C NMR spectrum of XnES2 (**68**) 181

4.75 ^{13}C NMR spectrum (expansion) of XnES2 (**68**) 182

4.76 COSY spectrum of XnES2 (**68**) 183

4.77 TOCSY spectrum of XnES2 (**68**) 184

4.78 HSQC spectrum of XnES2 (**68**) 185

4.79 HMBC spectrum of XnES2 (**68**) 186

4.80 HMBC spectrum (expansion) of XnES2 (**68**) 187

4.81 APCI-MS spectrum of XnESP2 (**69**) 193

4.82 HRMS spectrum of XnESP2 (**69**) 194

4.83 ^{1}H NMR spectrum of XnESP2 (**69**) 195

4.84 ^{1}H NMR spectrum (expansion) of XnESP2 (**69**) 196

4.85 ^{13}C NMR spectrum of XnESP2 (**69**) 197

4.86 ^{13}C NMR spectrum (expansion) of XnESP2 (**69**) 198

4.87 COSY spectrum of XnESP2 (**69**) 199

4.88 TOCSY spectrum of XnESP2 (**69**) 200

4.89 HSQC spectrum of XnESP2 (**69**) 201

4.90	HMBC spectrum of XnESP2 (**69**)	202
4.91	HMBC spectrum (expansion) of XnESP2 (**69**)	203
4.92	APCI-MS spectrum of XnESP3 (**70**)	210
4.93	HRMS spectrum of XnESP3 (**70**)	211
4.94	^{1}H NMR spectrum of XnESP3 (**70**)	212
4.95	^{13}C NMR spectrum of XnESP3 (**70**)	213
4.96	^{13}C NMR spectrum (expansion) of XnESP3 (**70**)	214
4.97	COSY spectrum of XnESP3 (**70**)	215
4.98	TOCSY spectrum of XnESP3 (**70**)	216
4.99	HSQC spectrum of XnESP3 (**70**)	217
4.100	HMBC spectrum of XnESP3 (**70**)	218
4.101	HMBC spectrum (expansion) of XnESP3 (**70**)	219
4.102	IR spectrum of XnEL3 (**71**)	222
4.103	UV spectrum of XnEL3 (**71**)	223
4.104	EIMS spectrum of XnEL3 (**71**)	223
4.105	^{1}H NMR spectrum of XnEL3 (**71**)	224
4.106	^{13}C NMR spectrum of XnEL3 (**71**)	225
4.107	HMBC spectrum of XnEL3 (**71**)	226
4.108	IR spectrum of XnEL7 (**45**)	228
4.109	UV spectrum of XnEL7 (**45**)	228
4.110	EIMS spectrum of XnEL7 (**45**)	229
4.111	^{1}H NMR spectrum of XnEL7 (**45**)	230
4.112	^{13}C NMR spectrum of XnEL7 (**45**)	231
4.113	HMBC spectrum of XnEL7 (**45**)	232

4.114 IR spectrum of XnEL5 (**72**) 234

4.115 UV spectrum of XnEL5 (**72**) 235

4.116 EIMS spectrum of XnEL5 (**71**) 235

4.117 ^{1}H NMR spectrum of XnEL5 (**72**) 236

4.118 ^{13}C NMR spectrum of XnEL5 (**72**) 237

4.119 HMBC spectrum of XnEL5 (**72**) 238

4.120 IR spectrum of XnEL1 (**73**) 243

4.121 UV spectrum of XnEL1 (**73**) 243

4.122 APCI-MS spectrum of XnEL1 (**73**) 244

4.123 ^{1}H NMR spectrum of XnEL1 (**73**) 245

4.124 ^{13}C NMR spectrum of XnEL1 (**73**) 246

4.125 COSY spectrum of XnEL1 (**73**) 247

4.126 HSQC spectrum of XnEL1 (**73**) 248

4.127 HMBC spectrum of XnEL1 (**73**) 249

4.128 HMBC spectrum (expansion) of XnEL1 (**73**) 250

4.129 IR spectrum of XnEL2 (**74**) 255

4.130 UV spectrum of XnEL2 (**74**) 255

4.131 APCI-MS spectrum of XnEL2 (**74**) 256

4.132 ^{1}H NMR spectrum of XnEL2 (**74**) 257

4.133 ^{13}C NMR spectrum of XnEL2 (**74**) 258

4.134 COSY spectrum of XnEL2 (**74**) 259

4.135 HSQC spectrum of XnEL2 (**74**) 260

4.136 HMBC spectrum of XnEL2 (**74**) 261

4.137 HMBC spectrum (expansion) of XnEL2 (**74**) 262

4.138 EIMS spectrum of XnD3 (**75**) 265

4.139 IR spectrum of XnD3 (**75**) 266

4.140 UV spectrum of XnD3 (**75**) 266

4.141 ^{1}H NMR spectrum of XnD3 (**75**) 267

4.142 ^{13}C NMR spectrum of XnD3 (**75**) 268

4.143 COSY spectrum of XnD3 (**75**) 269

4.144 HSQC spectrum of XnD3 (**75**) 270

4.145 HMBC spectrum of XnD3 (**75**) 271

4.146 EIMS spectrum of XnHL3 (**76**) 274

4.147 IR spectrum of XnHL3 (**76**) 274

4.148 ^{1}H NMR spectrum of XnHL3 (**76**) 275

4.149 ^{13}C NMR spectrum of XnHL3 (**76**) 276

4.150 COSY spectrum of XnHL3 (**76**) 277

4.151 HSQC spectrum of XnHL3 (**76**) 278

4.152 HMBC spectrum of XnHL3 (**76**) 279

4.153 IR spectrum of XnHS2 (**77, 78**) 281

4.154 EIMS spectrum of XnHS2 (**77, 78**) 281

4.155 ^{1}H NMR spectrum of XnHS2 (**77, 78**) 282

LIST OF ABBREVIATIONS

µg	microgram
mg	milligram
mM	millimolar
µl	microliter
ml	milliliter
℃	Degree in Celcius
mp	Melting point
NMR ^{1}H	Proton Nuclear Magnetic Resonance
NMR ^{13}C	Carbon-13 Nuclear Magnetic Resonance
δ	Chemical shift
J	Coupling constant
Hz	Hertz
o	Ortho
m	Meta
α	Alpha
β	Beta
d	Doublet
dd	Doublet of doublet
ddd	Doublet of double of doublet
dt	Doublet of triplet
s	Singlet
t	Triplet

q	Quartet
m	Multiplet
br	Broad
COSY	Correlation spectroscopy
DEPT	Distortionless Enhancement Polarization Transfer
HMBC	Heteronuclear Multiple Bond Correlation
HSQC	Heteronuclear Single Quantum Correlation
NOESY	Nuclear Overhauser Effect Spectroscopy
TOCSY	Total Correlation Spectroscopy
IR	Infrared
υ	Wave number
GC-MS	Gas Chromatography-Mass Spectrometry
EI-MS	Electron Impact Mass Spectrometry
m/z	Mass-charged ratio
LC-MS	Liquid Chromatography-Mass Spectrometry
APCI-MS	Atmospheric Pressure Chemical Ionization Mass Spectrometry
UV	Ultra-violet
PDA	Photo Diode Array
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography
CC	Column Chromatography
SPE	Solid Phase Extraction
IC_{50}	Concentration for 50% inhibition
[α]	Specific rotation