

ABSTRACT

The phytochemical and bioactivity studies on *Mitrella kentii* species from Annonaceae family have been performed. The extraction of phytochemicals from dried and ground plants were performed using conventional method extractions; Soxhlet extractor and Cold-extraction. The isolation of pure compounds from crude extracts were carried out by using various chromatographic methods such as column chromatography (CC), thin layer chromatography (TLC) and preparative thin layer chromatography (PTLC). The structural elucidations of the isolated compounds were established based on the spectroscopic techniques such as UV-Vis, IR, MS, 1D-NMR ($^1\text{H-NMR}$ and $^{13}\text{C-NMR}$), 2D-NMR (COSY, DEPT, HSQC and HMBC) and single crystal X-ray diffraction analysis.

From this study, nine known compounds have been isolated and characterised from the stem bark of *Mitrella kentii*. These compounds are chalcones, desmosdumotin C **MK1** and its tautomer, 2'-cinnamoyl-3'-hydroxy-5'-methoxy-4',6',6'-trimethylcyclohexa-1',1-dienone **MK2**, terpenoids, stigmasta-4-en-3-one **MK3** and stigmasta-4,22-diene-3,25-dione **MK4**, flavanone, 6-hydroxy-5,7-dimethoxy-2-phenylchroman-4-one **MK5**, benzyl benzoate **MK6**, oxybis(ethane-2,1-diyl) dibenzoate **MK7**, oxoaporphine alkaloids, liriodenine **MK8** and atherospermidine **MK9**. All these compounds were isolated for the first time from *Mitrella kentii* species except for **MK8**. The isolated compounds were elucidated using spectroscopic techniques and by comparison of their spectral data with those previously reported in the literatures.

For the bioactivities study, only the major compound; **MK1** in this species was investigated further in the bioactivities. Firstly, the toxicity study on **MK1** showed that there are no abnormal physiological or behavioural changes, body weight alteration at any time of observation at the doses used during the 14 days. The anti-ulcer experiment was performed for the **MK1** and result showed that the rat pretreated with **MK1**, or omeprazole (standard) considerably reduced ulcer area formation compared to ulcer control group. **MK1** at doses of 5, 10, 20 mg/kg b.w, was observed to inhibit ulcer formation by 69.77%, 90.18% and 86.56%, respectively. **MK1** at doses 10 and 20 mg/kg protect stomach from ulceration significantly ($p < 0.05$) higher than that obtained by omeprazole at dose 20 mg/kg with 79.07%. Therefore, **MK1** demonstrated better result than omeprazole.

In vitro anti-*Helicobacter pylori* activity experiment also showed that **MK1** represents minimum inhibitory concentration MIC of >250 $\mu\text{g/ml}$ and minimum bactericidal concentration MBC >250 against *H. pylori* NCTC11637 strain and moderately MIC of 125 $\mu\text{g/ml}$ and MBC of 250 $\mu\text{g/ml}$ against *H. pylori* J99. Hence, **MK1** displayed good result for anti- *H. pylori* effect. For the FRAP experiment, **MK1** exhibited FRAP value of 120.7 ± 0.001 significantly ($p < 0.05$) which was lower than the positive controls used in this study; quercetin, 2046.7 ± 0.024 , gallic acid, 2562.7 ± 0.024 and ascorbic acid, 879.3 ± 0.005 , respectively. Meanwhile the DPPH assay did not show any significant inhibition in the dose dependant manner used in the study. Therefore, it can be concluded that **MK1** exerts its antiulcer effect through indirect antioxidant mechanism. In addition, **MK1** also maintained non protein sulfhydryl (NP-SH) content, glutathione (GSH) level, decreased malondialdehyde (MDA) level but didn't affect nitric oxide (NO) level or cyclooxygenase-2 (COX-2) activity. **MK1** also

showed an increase 70 kilodalton heat shock proteins (HSP-70) activity and decrease of control Bax proteins expression in ulcerated tissue.

ABSTRAK

Kajian fitokimia dan bioaktiviti terhadap spesis *Mitrella kentii* daripada famili Annonaceae telah dijalankan. Tumbuhan yang telah dikeringkan, dikisar, diekstrak sebatian dengan menggunakan pengekstrakan kaedah lama seperti pengekstrak Soxhlex dan rendaman. Kaedah pengasingan sebatian tulen daripada ekstrak mentah pula menggunakan pelbagai kaedah kromatografi seperti kromatografi turus (CC), kromatografi lapisan tipis (TLC) dan kromatografi persiapan lapisan nipis (PTLC). Seterusnya, kaedah spektroskopik seperti UV-Vis, IR, MS, 1D-NMR (¹H-NMR dan ¹³C-NMR), 2D-NMR (COSY, DEPT, HSQC, HMBC dan NOESY) dan analisis difraksi X-ray hablur tunggal digunakan untuk ilusidasi struktur sebatian.

Dalam kajian ini, sembilan sebatian lama telah dipisahkan dari kulit batang stem *Mitrella kentii*. Sebatian tersebut ialah calkon, desmosdumotin C **MK1** dan tautomer, 2'-sinamoil-3'-hidroksi-5'-metoksi-4',6',6'-trimetilsikloheksana-1',1'-dienon **MK2**, terpenoid, stigmasta-4-en-3-on **MK3** and stigmasta-4,22-dien-3,25-dion **MK4**, flavanon, 6-hidroksi-5,7-dimetoksi-2-fenil-2,3-dihidro-4H-kroma-4-on **MK5**, benzil benzoat **MK6**, oksibis(etana-2,1-diil) dibenzoat **MK7**, oksoaporfina, liriodinina **MK8** and aterospermidina **MK9**. Kesemua sebatian-sebatian tersebut adalah kali pertama dipisahkan daripada spesis *Mitrella kentii* kecuali **MK8**. Sebatian-sebatian yang telah dipisahkan tersebut dielusidasikan dengan menggunakan teknik spektroskopik serta kaedah perbandingan literatur.

Untuk kajian bioaktiviti pula, hanya sebatian utama; **MK1** dalam spesis ini digunakan untuk kajian yang selanjutnya. Untuk kajian ketoksikan, **MK1** telah

menunjukkan tiada sebarang perubahan ketidaknormalan terhadap fizikal atau kelakuan, berat badan diperhatikan sepanjang eksperimen dijalankan mengikut dos yang digunakan selama 14 hari. Eksperimen anti-ulser telah dijalankan terhadap sebatian **MK1** dan keputusan telah menunjukkan bahawa tikus yang diberikan dengan **MK1** atau omeprazole (kawalan) menunjukkan pengurangan dengan ketara kawasan pembentukan ulser berbanding dengan kumpulan kawalan ulser. Pada dos 5, 10, 20 mg/kg **MK1**, masing-masing telah menunjukkan pengurangan pembentukan ulser iaitu 69.77%, 90.18% dan 86.56%. Pada dos 10 dan 20 mg/kg **MK1** dapat melindungi perut daripada ulser ($p < 0.05$) ketara lebih tinggi daripada yang diperolehi oleh omeprazole pada dos 20 mg/kg dengan 79.07%. Oleh itu, **MK1** telah memberikan hasil keputusan yang lebih baik daripada omeprazole.

Dalam eksperimen *in vitro* anti-*Helicobacter pylori* aktiviti, **MK1** juga mewakili MIC kepekatan perencatan minimum $>250 \mu\text{g/ml}$ dan minimum kepekatan bakteria MBC >250 terhadap *H. pylori* ketegangan NCTC11637 dan sederhana terhadap MIC daripada $125 \mu\text{g/ml}$ dan MBC sebanyak $250 \mu\text{g/ml}$ terhadap *H. pylori* J99. Oleh itu, **MK1** menunjukkan hasil yang baik untuk kesan anti-*H. pylori*. Untuk eksperimen FRAP, **MK1** memberikan nilai FRAP 120.7 ± 0.001 , ($p < 0.05$) ketara lebih rendah daripada kawalan positif yang digunakan dalam kajian ini dan mempamerkan nilai seperti berikut; quercetin, 2046.7 ± 0.024 , asid gallic, 2562.7 ± 0.024 dan asid askorbik, 879.3 ± 0.005 . Sementara itu, untuk eksperimen DPPH, **MK1** tidak menunjukkan sebarang perencatan yang ketara untuk dos yang digunakan dalam kajian ini. Oleh itu, **MK1** telah memberikan kesan anti-ulser melalui mekanisme antioksidan secara tidak langsung. Di samping itu, **MK1** juga mengekalkan kandungan non protein sulfhydryl (NP-SH), tahap glutathione (GSH), menurunkan tahap malondialdehid (MDA) tetapi

tidak menjejaskan tahap nitrik oksi (NO) atau siklooksigenas-2 (COX-2). **MK1** juga menunjukkan peningkatan 70 kilodalton protein kalis haba (HSP-70) aktiviti dan pengurangan kawalan Bax protein dalam penghasilan tisu pengulseran.

ACKNOWLEDGEMENT

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

I acknowledge the Almightyness of Allah who creates all things. Thank you Ya Allah for the best way from the beginning until the end...In Shaa Allah...Allahuakhbar!!

First and foremost, my greatest appreciation is to my supervisor, the late Prof Datuk Dr. A. Hamid A. Hadi for his excellent supervision, advice, guidance and encouragement. I will be forever grateful for his advice. I learned so many techniques and had a good experience working with him. Prof, you are always in my heart. I'll remember what you are always said to me whether direct or indirect. You are really awesome. I'll miss you forever Prof. For you we need to work hard to success. Thanks for everything. Thanks to my second supervisor, Dr. Kamal Aziz Ketuly, who brought me to join Prof Hamid's group and helping me to change the field from biodiesel to natural products. He is a teacher to me in the chromatography field including HPLC, GC and column chromatography. Everything his teaches me until really confident and knows everything about chromatography. Both of them always supported me and gave me all opportunity of work, facilities necessary, and his spontaneous attitude made working conditions as pleasant as possible. They also gave me opportunity to learn all the instruments under HIR grant. I felt so thankful to both of you because gave me for everything; knowledge, money and even for love. I really love both of you. I don't know how to say thank you for both of you. I'll try my best to continue my future life later.

Thanks so much also to my new supervisor Prof Dr. Hapipah Mohd Ali and my new co-supervisor Dr. Najihah Mohd Hashim for kindly take me as their new student,

even though just for awhile but you are really helping me and I can't forget that. Thank you so much again for everything and hopefully we will work together later.

Thanks also to the all staff in the Chemistry Department: En. Mohamad, Mr. Siew and Puan Asmah (LC-MS, GC-MS and GC-FID), Cik Norzalida, En. Fateh and En. Nordin (NMR), En. Hashim for the columns and his best friend En. Mohamad Ismail, Puan Lela (FTIR), Puan Dara Fiona and En. Hakim (UV-Vis), Puan Aida (auto GC-FID), En. Hazri (Office) and all staff at Chemistry Department. Thanks a lot because help me to finish my study.

Thanks with hugs and kisses to my labmate working at C-110 and HIR lab: my dear Mr. Mehran Fadaeinasab (Mehran), Puan Hairin Taha (Kak Hairin), Puan Hanita Omar (Kak Nita). Thanks so much because always help me, give me advices and everything. We are laugh and also cry together. You all are like my family and always in my heart. Thanks so much for everything. I hope Allah can give the best for all of you.

Thanks so much to my beloved friends: Mr Vicit Ryzal (Vicit), Mr Woo Jun Onn (Woo), Mr Chin Guo Feng (Kino), Ms Yew Han Choi (Han Choi), Cik Linahafizza (Lina), Puan Saidatun Nafisah (Sai), En. Afiq Azil (Afiq), Cik Hazrati Wazir (Atie), Prof Dr. Misni Misran, En. Muhamad Najib Abd Rahman (Najib), Cik Shahidatul Shida (Shida), En. Muhamad Faizal Abd Halim (Faizal), En. Mohd Faisal Ismail (Chan), En. Mohd Zahari Husain (Zari), Ms Jane, Ms Evelyn Lim and Ms Tiew Tshu Xian and Colloid's lab member. My ex-roommate Puan Nor Faraliza Mizan (Fara) and my

housemate, Cik Salmiza Sani (Sal) and Cik Norhafizah Abu Bakar (Fizah). Thanks so much for everything my dear friends.

Last but definitely not least, I want to express my gratitude to my beloved parents abah and mama, my brother (Mohd Azwan Azizan), my sister in law (Salmi Mohamed Razif), my nephew (Muhammad Adyan Ramadhan) and my sister (Ainnul Addawiyah Syahadah Azizan), whose love was an invaluable source of inspiration and energy. Thanks so much for everything my beloved family. I love you all so much!!!!!!

To all friends, also giving an essential support for me, I just can repeat the same: thank you very much.

One group of people have not been mentioned yet but they also contributed considerably to the successful realisation of this study. I am very grateful to all of you. I really don't know how to impress my thanks for all of you. This can't say by the word, I'm speechless. I hope Allah can give the best for all of you.

I gratefully acknowledge the Ministry of High Education (MOHE) for the HIR-MOHE grant (F000009-21001) and the University of Malaya grant UPGP (PG064-2012B).

Dedicated to my beloved supervisor: Prof Datuk Dr. A. Hamid A. Hadi

You Raise Me Up

*When I am down and oh my soul, so weary
When troubles come and my heart burdened be
Then, I am still and wait here in the silence
Until you come and sit awhile with me
You raise me up, so I can stand on mountains
You raise me up, to walk on stormy seas
I am strong, when I am on your shoulders
You raise me up.....to me than I can be*

Thanks for everything.....

Al-Fatihah

(10th June 1953-12th June 2013)

TABLE OF CONTENTS

LIST OF CONTENTS	Page
ABSTRACT	ii
ACKNOWLEDGEMENT	viii
TABLE OF CONTENTS	xii
LIST OF SCHEMES	xviii
LIST OF FIGURES	xix
LIST OF TABLES	xxii
LIST OF ABBREVIATIONS	xxiii
CHAPTER ONE	
INTRODUCTION	1
1.1 GENERAL	1
1.2 ANNONACEAE: DISTRIBUTION AND HABITAT	6
1.3 BOTANICAL ASPECT OF ANNONACEAE	8
1.4 CLASSIFICATION OF GENUS	8
1.4.1 Genus <i>Mitrella</i>	8
1.4.2 <i>Mitrella kentii</i> (Bl) Miq (KL 4139)	9
1.4.3 Physical Description of <i>Mitrella kentii</i> (Bl) Miq	9
1.5 OBJECTIVES OF THE STUDY	10
CHAPTER TWO	
GENERAL CHEMICAL ASPECTS AND BIOLOGICAL ACTIVITIES	11
2.1 GENERAL	11
2.2 ALKALOIDS	12

2.2.1	Classification of Alkaloids	13
2.2.2	Simple isoquinolines	13
2.2.3	Tetrahydrobenzylisoquinolines	14
2.2.4	Aporphines Alkaloids	14
2.2.5	Oxoaporphine	16
2.2.6	Example of alkaloids extracted from Annonaceae species	17
	2.2.6.1 Aporphine Alkaloids of <i>Fissistigma poilanei</i>	17
	2.2.6.2 Oxoaporphine and pyrimidine- β -Carboline Alkaloids of <i>Annona foetida</i>	19
	2.2.6.3 Isoquinoline Alkaloids from <i>Rollinia mucosa</i>	19
2.3	TERPENOIDS	21
	2.3.1 Classification of Terpenoids	21
	2.3.2 Example of Terpenoids from <i>Mangifera indica</i>	22
2.4	PHENOLIC COMPOUNDS	26
	2.4.1 Definition of Phenolic Compounds	28
	2.4.2 Flavonoids	30
	2.4.3 Major Sub-Classes of Flavonoids	33
	2.4.4 Minor Sub-Classes of Flavonoids	35
	2.4.4.1 Chalcone	36
	2.4.5 Pharmacology of Flavonoids	38
	2.4.6 Example of flavonoids extracted from Annonaceae species	38
	2.4.6.1 Flavonoids from <i>Goniothalamus gardneri</i> and <i>Goniothalamus thawaitesii</i>	38
	2.4.6.2 Flavonoids from genus <i>Desmos</i>	40
2.5	CHEMICAL CONSTITUENTS OF <i>MITRELLA KENTII</i> SPECIES	42
2.6	BIOLOGICAL ACTIVITIES OF ANNONACEAE PLANTS	46

2.6.1	Antiulcer	46
2.6.2	Antioxidant	47
CHAPTER THREE		
RESULTS AND DISCUSSION		
		49
PART A: PHYTOCHEMICALS		
		49
3.1	Structure Elucidation of Compound MK1	50
3.2	Structure Elucidation of Compound MK2	59
3.3	Structure Elucidation of Compound MK3	66
3.4	Structure Elucidation of Compound MK4	72
3.5	Structure Elucidation of Compound MK5	78
3.6	Structure Elucidation of Compound MK6	87
3.7	Structure Elucidation of Compound MK7	94
3.8	Structure Elucidation of Compound MK8	102
3.9	Structure Elucidation of Compound MK9	107
PART B: BIOACTIVITY		
		113
3B.1	INTRODUCTION	113
3B.2	RESULTS	116
	3B.2.1 Toxicity study	116
	3B.2.2 Gross evaluation	117
	3B.2.3 Gastric mucus content, pH and biochemical analysis	117
	3B.2.4 Gastric tolerability	117
	3B.2.5 Histological evaluation	120
	3B.2.6 HSP-70 and Bax immunohistochemistry	120
	3B.2.7 Effect of MK1 on GSH level in gastric homogenate	125

3B.2.8 Effects of MK1 on lipid peroxidation	125
3B.2.9 <i>In vitro</i> effect on NP-SH compounds	125
3B.2.10 Nitric oxide (NO) and COX-[2 inhibitory activity	125
3B.2.11 <i>In vitro</i> anti- <i>Helicobacter pylori</i> activity	127
3B.2.12 Antioxidant evaluation of MK1	127
3B.3 DISCUSSIONS	127
CHAPTER FOUR	
CONCLUSION	133
CHAPTER FIVE	
EXPERIMENTAL	134
PART A: PHYTOCHEMICAL	134
5.1 GENERAL EXPERIMENT PROCEDURES	134
5.2 CHROMATOGRAPHY	134
5.2.1 Column Chromatography (CC)	134
5.2.2 Thin Layer Chromatography (TLC)	135
5.2.3 Preparative Thin Layer Chromatography (PTLC)	135
5.3 REAGENTS	135
5.3.1 Mayer's Reagent	136
5.3.2 Dragendorff's Reagent	136
5.4 PLANT MATERIAL	137
5.5 EXTRACTION OF THE STEM BARK OF <i>M. KENTII</i>	137
5.5.1 Hexane extraction	137
5.5.2 Acid-base extraction of alkaloids	137

5.5.3	Dichloromethane extraction	138
5.5.4	Methanol Extraction	138
5.6	ISOLATION OF THE CRUDES	138
5.6.1	Hexane crude	138
5.6.2	Alkaloid crude	139
5.6.3	Dichloromethane crude	139
	PART B: BIOACTIVITIES	145
5B.1	MATERIALS AND METHODS	145
5B.1.1	Drugs and chemicals	145
5B.1.2	Animals	145
5B.1.3	Acute toxicity study	145
5B.2.4	Induction of acute gastric lesion	146
5B.2.5	Measurement of gastric juice acidity, mucus content and the biochemical parameters	147
5B.2.6	Gastroprotective Assessments	147
5B.2.7	Gastric tolerability test	147
5B.2.8	Histological evaluation	148
5B.2.9	Effect of MK1 on tissue homogenate contents	148
4.2.9.1	Glutathione levels	149
5B.2.10	Thiobarbituric Acid reactive substance assay	149
5B.2.11	Determination of nitric oxide	149
5B.2.12	Estimation of nonprotein sulfhydryls (NP-SH)	150
5B.2.13	<i>In vitro</i> evaluation of COX-2 inhibitory activity	150
5B.2.14	<i>In vitro</i> anti- <i>Helicobacter pylori</i> activity	150
5B.2.15	Ferric-reducing antioxidant power (FRAP) assay	151
5B.2.16	DPPH assay method	152

5B.2.17 Statistical analysis	152
5.3 Physical and Spectral Data of Isolated Compounds	153
REFERENCES	156
APPENDICES	171

LIST OF SCHEMES

Scheme	Page
1.1 The order of Magnoliidae	7
1.2 Species in Genus <i>Mitrella</i>	9
1.3 Example of <i>Mitrella kentii</i> (Bl) Miq (KL 4139)	10
2.4.1 General classification of plant phenolics	26
2.4.2 Phenolic compounds biosynthesis	30
5.1 Extraction and isolation of chemical constituents from <i>M. kentii</i>	141
5.2 Isolation and purification of chemical constituents from <i>M. kentii</i>	142
5.3 Isolation and purification of chemical constituents from <i>M. kentii</i>	143
5.4 Isolation and purification of chemical constituents from <i>M. kentii</i>	144

LIST OF FIGURES

Figure	Page
2.2.1 Skeleton I: General structure of aporphine alkaloids	15
2.4.3 The skeleton structure of the flavones (a class of flavonoids), with rings named and positions numbered	32
2.5.2 Major subclasses of flavonoids. Classification is based on variations in the heterocyclic C-ring	35
3.1.1 Important Correlations on COSY and HMBC of MK1	53
3.1.2 X-ray crystallographic of MK1	53
3.1.3 The MS Spectrum of MK1	53
3.1.4 ¹ H-NMR Spectrum of MK1	54
3.1.5 ¹³ C-NMR Spectrum of MK1	54
3.1.6 DEPT-135 Spectrum of MK1	55
3.1.7 COSY Spectrum of MK1	56
3.1.8 HSQC Spectrum of MK1	57
3.1.9 HMBC Spectrum of MK1	58
3.2.1 Possible tautomers of MK1 , MK2(B) and MK2(C)	63
3.2.2 The MS Spectrum of MK2	63
3.2.3 ¹ H-NMR Spectrum of MK2	64
3.2.4 ¹³ C-NMR Spectrum of MK2	64
3.2.5 DEPT-135 Spectrum of MK2	65
3.3.1 GC-TOFMS Spectrum of MK3	70
3.3.2 ¹ H NMR Spectrum of MK3	70
3.3.3 ¹³ C-NMR Spectrum of MK3	71
3.3.4 DEPT-135 Spectrum of MK3	71
3.4.1 GC-TOFMS Spectrum of MK4	76

3.4.2	¹ H-NMR Spectrum of MK4	76
3.4.3	¹³ C-NMR Spectrum of MK4	77
3.4.4	DEPT-135 Spectrum of MK4	77
3.5.1	Important Correlations on COSY and HMBC of MK5	80
3.5.2	The MS Spectrum of MK5	82
3.5.3	¹ H-NMR Spectrum of MK5	82
3.5.4	¹³ C-NMR Spectrum of MK5	83
3.5.5	DEPT-135 Spectrum of MK5	83
3.5.6	COSY Spectrum of MK5	84
3.5.7	HSQC Spectrum of MK5	85
3.5.8	HMBC Spectrum of MK5	86
3.6.1	Important Correlations on COSY and HMBC of MK6	89
3.6.2	GC-MS Spectrum of MK6	90
3.6.3	¹ H-NMR Spectrum of MK6	90
3.6.4	¹³ C-NMR Spectrum of MK6	91
3.6.5	DEPT-135 Spectrum of MK6	91
3.6.6	COSY Spectrum of MK6	92
3.6.7	HMBC Spectrum of MK6	93
3.7.1	Important Correlations on COSY and HMBC of MK7	96
3.7.2	The MS Spectrum of MK7	97
3.7.3	¹ H-NMR Spectrum of MK7	97
3.7.4	¹³ C-NMR Spectrum of MK7	98
3.7.5	DEPT-135 Spectrum of MK7	98
3.7.6	COSY Spectrum of MK7	99
3.7.7	HSQC Spectrum of MK7	100

3.7.8	HMBC Spectrum of MK7	101
3.8.1	The MS Spectrum of MK8	105
3.8.2	¹ H-NMR Spectrum of MK8	105
3.8.3	¹³ C-NMR Spectrum of MK8	106
3.9.1	The MS Spectrum of MK9	111
3.9.2	¹ H-NMR Spectrum of MK9	111
3.9.3	¹³ C-NMR Spectrum of MK9	112
3B.1	Macroscopic appearance of the gastric mucosa of the rats pretreated with MK1 at doses 5, 10, 20 mg/kg (2C, 2D and 2E) or omeprazole 20mg/kg (2B) showed reduced lesion formation in compare with the ulcer control rats (2A)	119
3B.2	Macroscopic evaluation of the gastric mucosa of the rats pretreated with MK1 at doses 5, 10, 20 mg/kg (C-E) or omeprazole (B) showed improved histological appearance compared to ulcer control rats (A) which have extensive visible hemorrhagic necrosis of the gastric mucosa with edema and leucocytes infiltration of submucosa. (H & E stain 20x)	121
3B.3	Effect of MK1 gastric tissue glycoprotein-PAS staining in ethanol-induced gastric ulcer in rats. (A) Normal group, (B) Ulcer group, (C, D) treated MK1 group at doses 10, 20 mg/kg respectively (PAS stain 20x)	122
3B.4	Immunohistochemical analysis of Bax protein expression in the stomachs of rats with ethanol-induced gastric mucosal lesions (A) normal control group, ulcer control group (B) and the treated group with MK1 at 10 and 20 mg/kg (C, D), respectively (20x)	123
3B.5	Immunohistochemical analysis of HSP-70 protein expression in the stomachs of rats with ethanol-induced gastric mucosal lesions. (A) Normal control group, ulcer control group (B), the treated groups with MK1 at doses 10 and 20 mg/kg, (C, D) respectively (20x)	124

LIST OF TABLES

Table	Page
2.3.1 Classification of Terpenoids	22
2.4 Classification of phenolic compounds	27
3.1 ¹ H-NMR (400 MHz), ¹³ C-NMR (100 MHz) and HMBC data of MK1	52
3.2 ¹ H-NMR (400 MHz) and ¹³ C-NMR (100 MHz) of MK2(B)	62
3.3 ¹ H-NMR (400 MHz) and ¹³ C-NMR (100 MHz) of MK3	69
3.4 ¹ H-NMR (600 MHz) and ¹³ C-NMR (150 MHz) of MK4	75
3.5 ¹ H-NMR (400 MHz) and ¹³ C-NMR (100 MHz) of MK5	81
3.6 ¹ H-NMR (400 MHz) and ¹³ C-NMR (100 MHz) of MK6	89
3.7 ¹ H-NMR (400 MHz) and ¹³ C-NMR (100 MHz) of MK7	96
3.8 ¹ H NMR (400 MHz) and ¹³ C NMR spectral data (100 MHz) of MK8	104
3.9 ¹ H NMR (400 MHz) and ¹³ C-NMR spectral data (100 MHz) of MK9	110
3B.1 Gastroprotective effect of MK1 against ethanol induced ulceration and observed liver function test	118
3B.2 Effects of MK1 on the level of malondialdehyde (MDA), glutathione (GSH), nonprotein sulfhydryl (NP-SH) group, nitric oxide (NO) and COX-2 inhibition	126

LIST OF ABBREVIATIONS

α	alpha
β	beta
λ_{\max}	maximum wavelength
δ	chemical shift in ppm
mM	milimolar
μM	micromolar
mg/mL	miligram per mililiter
mL	mililitre
m	meter
nm	nanometer
cm^{-1}	per centimeter
MHz	Mega Hertz
Hz	Hertz
m/z	Mass to charge ratio
UV	Ultraviolet
IR	Infrared
MS	Mass spectrum
HREI-MS	High-Resolution Electron Impact Mass Spectroscopy

NMR	Nuclear Magnetic Resonance
ppm	Part per million
eV	Electron Volt
TLC	Thin Layer Chromatography
PTLC	Preparative Thin Layer Chromatography
CC	Column Chromatography
CDCl_3	Deuterated chloroform
CH_2Cl_2	Dichloromethane
$\text{MeOH}/\text{CH}_3\text{OH}$	Methanol
CH_3COCH_3	Acetone
HCl	Hydrochloric acid
NH_3	Ammonia
CH_3	Methyl group
OCH_3	Methoxy group
OCH_2O	Methylenedioxy group
OH	Hydroxy group
Aq.	Aqueous
conc.	Concentration
PUD	Peptic Ulcer Disease

NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
NO	Nitric Oxide
ROS	Reactive Oxygen Species
TPTZ	2,4,6-Tri(2-pyridyl)-s-triazine
pH	power of hydrogen
I %	Inhibition Percentage
µm	micrometer
GSH	glutathione
DTNB	5,5-ditiobis-2-nitrobenzoic acid
TBARS	Thiobarbituric Acid reactive substance
MDA	malondialdehyde
SDS	Sodium Dodecyl Sulphate
NP-SH	nonprotein sulfhydryls
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
FRAP	Ferric Reducing Antioxidant properties
<i>J</i>	Coupling constant
<i>d</i>	Doublet
<i>s</i>	Singlet

<i>dd</i>	Doublet of doublet
<i>t</i>	Triplet
<i>m</i>	Multiplet
1D-NMR	One dimension nuclear magnetic resonance
2D-NMR	Two dimension nuclear magnetic resonance
¹ H-NMR	Proton nuclear magnetic resonance
¹³ C-NMR	Carbon 13 nuclear magnetic resonance
COSY	2D homonuclear chemical shift correlation spectroscopy
DEPT	Distortionless enhancement by polarization transfer
HSQC	Heteronuclear single quantum coherence
HMBC	Heteronuclear multiple bond coherence
LC-MS	Liquid Chromatography Mass Spectroscopy
[M+H] ⁺	Pseudo-molecular ion (molecular mass + 1)
[M] ⁺	Parent ion
MK	<i>Mitrella kentii</i>
AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency Virus