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}H-NMR and ^{13}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 µg/ml and minimum bactericidal concentration MBC >250 against *H. pylori* NCTC11637 strain and moderately MIC of 125 µg/ml and MBC of 250 µg/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 cycloxygenase-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 (^{1}H-NMR dan ^{13}C-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**, terpeniod, 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 pembetukan 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 μg/ml dan minimum kepekatan bakteria MBC >250 terhadap *H. pylori* ketegangan NCTC11637 dan sederhana terhadap MIC daripada 125 μg/ml dan MBC sebanyak 250 μg/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.

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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)

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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
MeOH/CH_3OH	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
J	Coupling constant
d	Doublet
s	Singlet

dd	Doublet of doublet
t	Triplet
m	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	Distortioness 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 (molecula r mass + 1)
$[M]^{+}$	Parent ion
MK	*Mitrella kentii*
AIDS	Acquired Immunodeficiency Syndrome
HIV	Human Immunodeficiency Virus