# UNIVERSITI TEKNOLOGI MARA

# PHYTOCHEMICAL AND ANTIOXIDANT STUDIES OF MALAYSIAN MEDICINAL PLANTS *SYZYGIUM POLYANTHUM* AND *OCTOMELES SUMATRANA*

**SALWA MOHAMMED RAWEH ABDULLAH AL-FAQEER**

Thesis submitted in fulfilment
of the requirement for the degree of
**Doctor of Philosophy**

**Faculty of Pharmacy**

January 2017

# AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This topic has not been submitted to any other academic institution or non-academic institution for any other degree or qualification.

I, herby, acknowledge that I have been supplied with Academic Rules and Regulations for post Graduate of Universiti Teknologi MARA, regulating the conduct of my study and research.

| | | |
|---|---|---|
| Name of Student | : | Salwa Mohammed Raweh Abdullah Al-Faqeer |
| Student's ID No | : | 2008278202 |
| Programme | : | Doctor of Philosophy (Pharmaceutical chemistry) PH-990 |
| Faculty | : | Pharmacy |
| Thesis Title | : | Phytochemical and antioxidant studies of Malaysian medicinal plants *Syzygium polyanthum* and *Octomeles sumatrana.* |
| Signature of Student | : | |
| Date | : | January 2017 |

# ABSTRACT

The leaves of *S. polyanthum* (Myrtaceae) and barks of *O. sumatrana* (Datiscaceae) were investigated for their chemical constituents, antioxidant and cytoprotective activities. Their aqueous extracts were first subjected to acidic hydrolysis and the organic layers were dissolved in water and partitioned using hexane, ethyl acetate (EtOAc) and *n*-butanol (BuOH). Six compounds (betulinic acid, ellagic acid, kaempferol, myricetin, quercetin, and β-sitosterol) were isolated and identified from the EtOAc and BuOH extracts of *S. polyanthum* and four compounds (quercetin, kaempferol, rutin, bryonolic acid) were purified from the *n*-butanol extract of *O. sumatrana* by means of MPLC and HPLC. The structures of the above compounds were determined by comparing their NMR and LCMS-TOF data with reported values. The structure of bryonolic acid was further confirmed by X-ray crystallography. Eleven essential oil components (α-caryophyllene, β-caryophyllene, caryophyllene oxide, 1,8-cineole, β-elemene, eugenol, eugenol acetate, isoeugenol, α-pinene, β-pinene, terpinen-4-ol) were identified from *S. polyanthum* and four fatty acid derivatives (linoleic acid, methyl linoleate, myristic acid, palmitic acid) and three steroids [5,6-dihydroegosterol, ergosta-5,8(14)-dien-3β-ol, ergosta-5-en-3β-ol] were determined from *O. sumatrana* by GC-MS analysis of their hexane extracts. The *n*-hexane, EtOAc and BuOH extracts were subjected to DPPH, FRAP and cytoprotective activities. The EtOAc and BuOH extracts of both plants showed potent DPPH activity with the $EC_{50}$ values of 159.12 ± 0.11μg/mL and 186.40 ± 0.58 μg/mL in *S. polyanthum* and 125.3 ± 0.17 μg/mL and 136.4 ± 0.17 μg/mL in *O. sumatrana,* respectively. It was found that bryonolic acid ($EC_{50}$ = 26.7 ± 0.74) only marginally quenched DPPH radical but ellagic acid, myricetin, quercetin, rutin and kaempferol (92.4 ± 3.82, 74.1 ± 1.29, 76.04 ± 2.63, 76.8 ± 1.11 and 71.22 ± 1.09 (μM), respectively) showed strong DPPH radical scavenging activity. Then, the isolated compounds from *S. polyanthum* and *O. sumatrana* (myricetin, ellagic acid, betulinic acid, β-sitosterol, rutin, quercetin, kaempferol and bryonolic acid) were tested for their cytotoxic effects towards three types of cells including normal human embryonic liver (WRL-68), normal green monkey kidney (Vero) and human hepatocarcinoma (HepG2) cell lines. The cells were treated with different concentrations of the compounds and the results showed that the compounds from *S. polyanthum* and *O. sumatrana* were non-toxic towards normal cells. However, betulinic acid and bryonolic acid had high cytotoxicity towards HepG2 cells. Next, the cytoprotective effects of the isolated compounds against hydrogen peroxide-induced WRL-68 and Vero cells were investigated. Quercetin, kaempferol, myricetin, ellagic acid, betulinic acid, β-sitosterol and bryonolic acid showed significant protective effects compared to control against oxidative stress-induced WRL-68 and Vero cells. Furthermore, betulinic acid and bryonolic acid showed higher protective effect compared to ellagic acid, kaempferol, myricetin and quercetin and the activities of the antioxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT) were enhanced in a dose-dependent manner. In conclusion, this study demonstrated that most compounds from *S. polyanthum* and *O. sumatrana* were cytoprotective against oxidative stress induced by $H_2O_2$ with betulinic acid and bryonolic acid having the highest potential to be developed to be used as anticancer candidates and alternative medicine.

# ACKNOWLEDGEMENTS

"IN THE NAME OF ALLAH THE MOST BENEFICENT THE MOST MERCIFUL"

All praises for the most omnipotent, omnipresent and omniscient Almighty Allah, who blessed me to accomplish my PhD research work. All respect for his last Holy Prophet (Peace be upon him), whose teaching inspired me to wider my thoughts and deliberate the things deeply.

Deep obligation and indebtedness and most sincere gratitude are offered to my honourable supervisors, Prof. Dr. Jean-Frédéric Faizal Weber Abdullah and Prof. Dr. Aishah Adam, whose dexterous and prime supervisions and emboldening help have enabled me to complete this work. I am especially grateful that they always being with me for advice and assistance.

Any sense of gratitude will be incomplete without giving glory and words of thanks to my co-supervisors, Dr. Humera Naz, and Dr. Mizaton Hazizul Hasan for their continuous support, guidance, kindness, and encouragement.

I would like to express my special gratitude to Sana'a University, Yemen, for the support and trust granted to me through a study scholarship. I am also thankful to Faculty of Pharmacy and Universiti Teknologi MARA (UiTM) for giving me opportunity to complete my research in well equipped laboratories.

My grateful thanks to my sweet late parents whose teaching always supported me in all what I have done in my life and who are always with me. I would like to thanks my sisters Fatima, Radhia, Dhikra, Ghada and my brothers Mura'ad, Faisal, Yasser and their families for their moral support and care during my research.

I would like to express special thanks to all my friends Dr. Anouar El Hassan, Syahrul Imran, Hafiz Ahmad, Raudhalujannah (UiTM, Perlis), Nurul Jannah, Ummu Amira, Hakimah, Syaza, Shazwani, Nik Khirunisa, Nuraini Che Aziz and colleagues from my mother country for their assistance, continuing support, and friendship during this study. I am also thankful to Dr. Syed Adnan Ali Shah for his suggestion regarding NMR experiments and Dr. Sadia Sultan for her valuable comments during this thesis writing. Special thanks to Mr. Rahimi and Mr. Ezalee from the Analytical Unit for their help and cooperation.

In the end, I want to express my sincere gratitude to everyone who helped me directly or indirectly for completion of this research.

# TABLE OF CONTENTS