UNIVERSITI TEKNOLOGI MARA

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF SYZYGIUM FILIFORME VAR. FILIFORME STEM BARK

MOHD HAFIZ BIN AHMAD

MSc

August 2015

ABSTRACT

The stem bark of Syzygium filiforme var. filiforme from family of Myrtaceae was investigated for its chemical constituents and biological activities. About 3 kg of the stem bark was extracted successively using solvent of different polarity. Various chromatographic methods were used to isolate and purify compounds from dichloromethane and methanol extracts such as vacuum liquid chromatography, glass column, centrifugal thin layer chromatography, thin layer chromatography and preparative thin layer chromatography. This study resulted in the isolation of five pentacyclic triterpenoids and two phytosterols which are 2α , 3β , 23-trihydroxyolean-12-en-28-oic acid (arjunolic acid, SF1), 2α , 3β -dihydroxylup-20(29)-en-28-oic acid (alphitolic acid, SF2), 3β -hydroxylup-20(29)-en-28-oic acid (betulinic acid, SF3), 3β hydroxyurs-12-en-28-oic acid (ursolic acid, SF4), ursolic acid 3-methyl ester (SF5), β -sitosterol (SF6) and stigmasterol (SF7). Structural identification was accomplished using spectroscopic methods such as ultraviolet (UV), infrared (IR), mass spectroscopy (MS), 1D and 2D nuclear magnetic resonance (NMR) (HMBC and HMQC). The methanolic extract showed moderate activities with IC₅₀ values of 44.7±6.42 µg/mL compared to ascorbic acid (IC₅₀ = 7.9±1.20 µg/mL) for DPPH assay but good activity for α -glucosidase assay with IC₅₀ values of 6.31±0.90 µg/mL compared to 1-deoxynojirimycin (IC₅₀ = $103.79\pm6.36 \ \mu g/mL$). Dichloromethane and hexane extracts showed weak activity on DPPH assay but dichloromethane extract showed good activity for α -glucosidase assay. Major compounds SF1, SF2, SF3 and SF4 were tested for DPPH and showed no promising activity. SF1, SF2, SF3 and SF4 were also tested for α -glucosidase assay but only SF1 and SF3 gave moderate activity with IC₅₀ values of 562.34 ± 11.80 and 501.19 ± 8.20 µM, respectively. The crude extracts and major compounds were also tested on antibacterial activity against Escherichia coli, Staphylococcus aureus and Bacillus subtilis. The methanolic and hexane extracts showed inhibition on tested organisms but no inhibition from dichloromethane extract. SF1 showed inhibition against E. coli, B. subtilis and S. aureus with minimum inhibition concentration at 1800 µg/mL, 900 µg/mL and 450 µg/mL, respectively. SF2 showed inhibition against S. aureus at 900 µg/mL. Then, SF4 showed inhibition against E. coli at 1800 μ g/mL and no inhibition showed by SF3 on tested bacteria. The MBC values of hexane extract, SF1 and SF4 against E.Coli. Methanol and hexane extracts showed inhibition on S. Aureus with MBC values of 1000 and 1100 µg/mL, respectively. Meanwhile, SF1 and SF2 showed MBC values of 560 and 1000 µg/mL, respectively against same bacteria. Finally, there are only hexane extract and SF1 showed inhibition at high concentration against B. subtilis with MBC values 1800 and 1100 µg/mL, respectively.

ACKNOWLEDGEMENT

In the name of Allah the Most Gracious, Most Merciful

Alhamdulillah. Thanks to Allah SWT, whom His blessing to complete this thesis entitled "Chemical Constituents and Biological Activities of *Syzygium filiforme* var. *filiforme* Stem Bark" as a requirement for the Degree of Masters of Science in the Faculty of Applied Sciences, Universiti Teknologi MARA.

Firstly, I would like to express my biggest gratitude to my supervisor, Prof. Dr. Nor Hadiani Ismail. I appreciate all her contributions and ideas to make my research experience more productive and valuable. Thank you also for providing me an excellent example as a successful woman, chemist and professor.

Besides that, I would also like to express my grateful acknowledgement to my cosupervisor, Dr. Humera Naz for her advice, supervision, and crucial contribution. She always kindly grants me her time to answer some of my unintelligent questions about isolation, purification and elucidation techniques.

My sincerest thanks to my friends in Atta-ur-Rahman laboratory, Sharil, Nazif, Hafizan, Syukri, Lan, Fifi, Heri, Imran, Dila, Ummu, Jannah, Salwa, Fatimah, Hakimah, Syaza and Wani for their encouragement, constructive suggestion and full of support throughout the duration of my study from the beginning until the end. Thank you to all Atta-ur-Rahman staff for their kindness. Never to forget to my best friends, Syukri, Aza, Hafiz, Amin and Che Lah for their advices and moral supports when I was down.

Last but not least, my deepest gratitude to my beloved parents, Mr. Ahmad Bin Hj. Tahir and l for their supports and prayers. They are the one who sincerely raised me with their caring and gently love. Thanks mom and dad for your love, care and support that make me more matured to face off the reality of life. For dad, this is the biggest gift from me for you and your spirit and strength are always with me even though you are no longer around. Al-fatihah.

TABLE OF CONTENTS

AUTHOR'S DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
TABLE OF CONTENTS	V
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF SCHEMES	xi
LIST OF SYMBOLS	xii
LIST OF ABBREVIATIONS	xiii

CHAPTER ONE: INTRODUCTION	1
1.1 Research Background	1
1.2 Problem Statement	2
1.3 Objectives of Study	3

СН	APTER TWO: LITERATURE REVIEWS	4
2.1	The Family Myrtaceae	4
	2.1.1 The Botany and Morphology of the Myrtaceae	5
	2.1.2 Traditional and Medicinal Uses of Myrtaceae Plants	6
2.2	The Botany, Morphology and Medicinal Uses of Syzygium Genus	8
2.3	Syzygium filiforme var. filiforme	10
2.4	Chemical Constituents of Syzygium Species	11
	2.4.1 Terpenoids	11
	2.4.2 Flavonoids	16
2.5	Biological Activity of Syzygium Species	18
2.6	Biosynthesis of Triterpenoids	21

CHAPTER ONE INTRODUCTION

1.1 RESEARCH BACKGROUND

Phytochemicals are chemical constituents or substances present in plants. They usually have characteristic pharmacological or biological activities. Over the years, these natural products have been used in traditional medicines as well as developed into modern drugs. Drugs derived from plant sources have been used in treatment of various human disorders for thousands of years in the form of the traditional Ayurvedic and Chinese medicine. Some popular examples of drugs from natural products are aspirin from willow bark and morphine from opium poppy. During 1960's, the studies of natural products served as the principle driving force for the new discovery of chemical reactivity (Bankova, 2007). These studies help natural product chemists to find out the main compounds that play a role as bioactive substances. In search bioactive compounds, research in phytochemistry focusing on medicinal plants are actively pursued in many countries all over the world.

The benefits of medicinal plants to treat diseases are also a reason for phytochemist to use plant as a source of medicinal agents. In the Quran and the Bible, about 20 and 125 plants are mentioned, respectively, as being used as medicinal agents to treat various ailments Jantan (2004). The gifts from the Creator are very invaluable and interesting because the diversity of living organisms in different part of the world. There are more than 35,000 plant species have being used in various human cultures around the world for medicinal purposes (Philip *et al.*, 2009). Malaysia is a beautiful country with variety of flora and fauna. It's location near the equator results in constantly warm and damp climate throughout the year. About 1,200 species of higher plants in Peninsular Malaysia and 2,000 species in Sabah and Sarawak are reported to have medicinal value and have been used for generations in various traditional health care systems (Philip *et al.*, 2009). The importance of Malaysia's diverse medicinal plants lies not only in their chemotherapeutic value in traditional health care but also in their potential as source of new chemical entities for drug discovery (Rain et al., 2007). This has spurred a lot of research interest on the