UNIVERSITI TEKNOLOGI MARA

CHEMICAL CONSTITUENTS OF *Croton laevifolius* BLUME BARK

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Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Applied Sciences

April 2018

ABSTRACT

Phytochemical investigation on the bark of Malaysian *Croton laevifolius* Blume (Euphorbiaceae) was carried out with an intention of isolating and identifying its chemical constituents. The bark was successively extracted with the non polar (hexane), medium polar (dichloromethane) and polar (methanol) organic solvents. These extracts were evaluated for cytotoxicity, anti-inflammatory and antidiabetic activities. The hexane extract showed potential cytotoxicity against MCF-7 cell line and mild cytotoxicity against A549, WRL-68, PC-3 and A375 cells while dichloromethane (DCM) extract indicated mild and selective activity against A549 and A375 only. The hexane and DCM extracts were subjected to isolation and purification using various chromatographic techniques such as Medium Pressure Liquid Chromatography (MPLC), Radial Chromatography (RC) and Recycling Preparative High Performance Liquid Chromatography (RHPLC). Structures of the chemical compounds were elucidated using various spectroscopic techniques such as UV, IR, 1D-NMR (^{1}H, ^{13}C, DEPT and APT), 2D-NMR (COSY, HSQC, HMBC and NOESY) and mass spectrometry. This study has led to the isolation of fourteen compounds, in which seven new and one known clerodane type diterpene named crovatin, as well as one eudesmane-type sesquiterpene named crytomeridiol were isolated from the DCM extract. This is the first occurrence of cryptomeridiol in *Croton* species. The new diterpenes were deduced as laevifin A, laevifin B, laevifin C, laevifin D, laevifin E, laevifin F and laevifin G. Subsequently, the hexane extract yielded laevifin B, three oleanane triterpenes: β-amyrin, β-amyrone and acetyl aleuritolic acid; one steroid (β-sitostenone) and one flavonoid named pachypodol. The absolute configurations of the isolated clerodane diterpenes were established using Electronic Circular Dichroism (ECD) technique where the experimental ECD profiles of the compounds were compared to that of TDDFT calculated spectra. The absolute configuration of these diterpenes has led to the postulation of their biosynthetic pathways *via* a biosynthetic study. Selected compounds of sufficient quantity were further evaluated for their toxicity against MCF-7 and A375 cell lines and anti-inflammatory activity by LPS-induced NF-κB translocation inhibition in RAW 264.7 cells. The isolated compounds of sufficient amount were further tested for cytotoxicity. Compounds laevifins A, B and F displayed fair cytotoxicity with IC_{50} values of 115, 102 and 106 μM respectively while β-amyrone and β-sitostenone showed medium cytotoxicity against MCF-7 cell line with IC_{50} values of 73 and 94 μM respectively. In addition, β-amyrin and acetyl aleuritolic acid showed weak activities; sharing IC_{50} values of 115 μg/mL. Laevifin E, acetyl aleuritolic acid and β-sitostenone showed weak activities against A375 cell line with IC_{50} values of 152, 103 and 124 μM respectively. In anti-inflammatory evaluation, the hexane extract showed weak activity where compounds β-amyrin and acetyl aleuritolic acid of the hexane extract showed good anti-inflammatory activity at the concentration of 50 μg/mL.

ACKNOWLEDGEMENTS

All praises are due to Allah the most Gracious and most Merciful. I praise Him and seek for His forgiveness and guidance.

The work presented here would not have been possible without the support, encouragement, teaching and friendship of many people. Firstly, I would like to express my deepest appreciation to my supervisors, Professor Dr. Nor Hadiani Ismail, Professor Dr. Khalijah Awang and Associate Professor Dr. Norizan Ahmat for their invaluable supervision, advices, patience and guidance throughout this study. I ask Allah to multiply their rewards and bless them.

To my wife Aini Zahida Din, I deeply indebted to your sacrifices, encouragement, patience and understanding that have given me the strength throughout the completion of this study. Millions of thanks to my siblings Rahimah, Noor Rusyidah, Fatimah Hadani and Maryam Zahidah for your continuous support and prayers. I would like to express my appreciation to Atta-ur-Rahman Institute of Natural Products Discovery, UiTM, headed by Professor Dr. Nor Hadiani Ismail, the Institute's staffs and fellow associates for providing the outstanding facilities and assistance that I need. Dr. Anouar, Dr. Muhammad Taha, Dr. Nurul Huda, Dr. Hamizah, Dr. Fatimah, Dr. Che Puteh, Prof. Dr. J. F.F.Weber, Dr. Syed Adnan, Dr. Humera, Dr. Sadia, Dr. Shahrul Imran, Mr. Mohd Shukri, Mrs. Juliana Mrs. Azmah, Ms. Nur Shahidatul Shida, Mrs Amira, to name a few. Also my appreciations to the staffs of analytical laboratory, Faculty of Pharmacy, Universiti Teknologi MARA (UiTM) for the facilities and assistance provided.

It is my pleasure to thank Professor Dr. Muhammad Iqbal Chowdhary of the ICCBS, University of Karachi, Pakistan as well as Professor Dr. Dulcie A. Mulholland and Dr. Moses K. Langat of the University of Surrey, UK for the short research attachment opportunities at your respective institutions. My gratitude are extended to the staffs of the University of Malaya (UM) especially Dr. Siti Nadiah for the X-ray crystallography, Dr. Looi Chung Yeng for the cytotoxicity and anti-inflammatory assays, as well as the Chemistry Department's herbarium team; Mr. Din, Mr. Teo and Mr. Rafly. Not to forget, many thanks to the staffs and colleagues at the School of Fundamental Science, Universiti Malaysia Terengganu; Professor Dr. Hamdan Suhaimi, Professor Dr. Nakisah, Associate Professor Dr. Marinah, Associate Professor Dr. Marinah, Associate Professor Dr. Ku Halim, Associate Professor Dr. Wan Mohd Khairul, Associate Professor Dr. Habsah, Dr. Juriffah, Dr. Asnuzilawati, Dr. Soraya, Dr. Nurul Huda, Dr. Md Uwaisulqarni, Professor Dr. Nora'aini, Associate Professor Dr. Marzuki, Ms. Noorasiah Moidu, Mrs Rozita, Mrs. Rohaida Awang, among others for your assistance and support during my study leave and post study leave.

Last but not least, my special thanks to my fellow UiTM and UM phytochemistry lab members; Edayah, Asmah, Hafiz, Noraini, Vicky, Nurunajah, Farah (Ann), Fauziah, Halimatun, Nik Khairunnisa, Khadijah, Fadzli, Nooraimi, Hazrina (Nana), Devi, Norsita, Chan Gomathi, Omer, Ahmed Kaleem, Kee, Azmi, Shelly, Joey, Rosalind, Azrul, Hafiz, Azeana, Nurul, Hanita and Nadia, among others. Your assistance, support, kindness and sweet memories will always be cherished in my heart. Alhamdulillah.

TABLE OF CONTENTS

CHAPTER ONE
INTRODUCTION

Humans have benefited plants for nutrition and as commodity since time immemorial and throughout the history (Staniek *et al.*, 2014). It is in lateral to the use of natural products from plants for the treatment of various kinds of diseases and illnesses, as well as in traditional beliefs. Natural products chemistry deals with the chemical compounds deriving from plants and animals from both terrestrial and marine sources which nowadays have turned into an interdisciplinary science engaging close collaboration with biologists, pharmacologists and clinicists. Natural products also complement the synthetic molecules in terms of composition, functional groups, weight, size, as well as architectural and stereochemical complexity (Colegate & Molyneux, 2007). Research in natural products chemistry combined with pharmacological screening has come out with the discovery of a vast array of bioactive secondary metabolites and useful leads for drug discovery *via* (Dias *et al.*, 2012; Tandon & Verma, 2009). In addition, plentiful of these metabolites have turned to be drug candidates (Dias *et al.*, 2012). This has made natural products research as one of the principal approach of discovering bioactive compounds (Colegate & Molyneux, 2007).

There are approximately 298,000 species of plants on earth, of which 215,644 have been described and catalogued and about one-third of these plant species have yet to be discovered (Mora *et al.*, 2011; Cseke *et al.*, 2011). Collectively, Asian tropical flora remains one of the least studied and known (Webb, Slik and Triono, 2010). As one of the twelve "megadiversity" countries of the world, there are about 19,548 plant species recorded in Malaysia as of 5th September 2009, of which 130,304 plant specimens have been catalogued. Of the recorded species, an approximate of 12,500 species is the flowering plants and more than 1,100 species are of ferns and fern allies (Webb, Slik and Triono, 2010, Ministry of Science *et al.*, 1998). Not to mention the reported plants with therapeutic properties, these figures reflect on so many species in this country that have not being studied chemically and medicinally, thus making it an interesting site in search for medicinal and new bioactive molecules.