UNIVERSITI TEKNOLOGI MARA

BIOLOGICAL ACTIVITY STUDIES OF SOME RUBIACEOUS PLANTS (SUBFAMILY RUBIOIDEAE) AND PHYTOCHEMICAL INVESTIGATION OF *HEDYOTIS PHILIPPINENSIS*

EMI NORZEHAN BINTI MOHAMAD MAHBOB

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

Faculty of Applied Sciences

May 2010

ABSTRACT

In this study, the biological activities of 13 Rubioideae plants were investigated with the aim of discovering its bioactive constituents. The assays employed were ferric thiocyanate (FTC), thiobarbituric acid (TBA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods for antioxidant potential as well as Griess assay for the measurement of nitric oxide (NO) inhibition in lipopolysaccharide (LPS) and interferon-γ (IFN-γ)-treated RAW 264.7 cells. In the *in vitro* antioxidant assays including FTC, TBA and DPPH, all the tested plants exert strong antioxidant potential. *Hedyotis philippinensis* (leaves and stems), *Spermacoce exilis* and *Spermacoce latifolia* showed potent inhibitory activity on NO production in LPS and IFN-γ-induced RAW 264.7 cells in the anti-inflammatory assay. For the second part of this study, phytochemical investigation of the leaves and stems of selected *Hedyotis* species, namely *H. philippinensis* was carried out using various chromatographic and solid phase extraction techniques to yield the major iridoid glycoside, asperuloside along with scopoletin and astragalin. Structures of the compounds were established by means of various spectroscopic techniques including nuclear magnetic resonance (NMR) and mass spectroscopy. The major compound, asperuloside was found to inhibit LPS/IFN-γ-induced NO production by 63% at a concentration of 100 μM (45% cell viability) in a dose-dependent manner with an IC_{50} value of 75.45±2.25 μM. Towards a chemotaxonomic study of Rubiaceous plants, the detection of marker compounds of the subfamily Rubioideae was attempted by employing the isolated asperuloside as the marker. Asperuloside detection on 13 species of subfamily Rubioideae comprising six genera (*Argostemma*, *Chasalia*, *Hedyotis*, *Lasianthus*, *Rennellia* and *Spermacoce*) was carried out using thin layer chromatography (TLC). In addition, a simple liquid chromatography mass spectrometry (LC-MS) profiling method was successfully developed to detect and quantify asperuloside in the samples. The asperuloside content was found to be significantly varied among five species, ranging from 1.46-24.64 ppm with *H. philippinensis* containing the highest amount. However, the compound was undetectable in the other eight species. In conclusion, this study has found that Rubioideae plants possessed constituents associated with various pharmacological activities such as anti-inflammatory, antioxidant and are mostly devoid of cytotoxic effects. This study has also shown that asperuloside is a good chemotaxonomic marker of Rubiaceous plants. Phytochemical investigation on these plants would likely reveal more active principles which could be potentially important markers.

ACKNOWLEDGEMENTS

Alhamdulillah is the only word to express my biggest gratefulness to Allah s.w.t for giving me the strength upon completion of my thesis. Nevertheless, without the encouragement of all, I am surely cannot finish the thesis alone. Hence, I would like to state my deep appreciations and thanks to all persons involved directly or indirectly.

My gratefulness to Assoc. Prof. Dr. Rohaya Ahmad as the main supervisor. She gave her best in all aspects throughout the field of natural products-phytochemistry that is a new frontier for me. Thanks also go to Assoc. Prof. Dr. Zainon Mohd. Noor as the co-supervisor with her impressive knowledge and thoughtfulness.

Last but not least, my greatest debt is to my parents, my siblings and my husband. They gave me full support, encouragement and prayers throughout my study. From them, I learned the value of life and hardship, and also the importance of education.

There are no such words that are too valuable only Alhamdulillah and for all, thank you again for make all this possible.

TABLE OF CONTENTS

CHAPTER 1

PLANTS: IMPORTANT SOURCE OF MEDICINAL NATURAL PRODUCTS

1.1 General Introduction

Since time immemorial, mankind has sourced the flora and fauna around him to ameliorate disease. In the past 100 years, this dependence was irreversibly broken for two reasons. Firstly, advances in biology began to provide insight into the molecular mechanisms underlying diseases, suggesting rational targets for therapeutic intervention. Secondly, advances in organic chemistry enabled the design and synthesis of sophisticated drug molecules. By the 1950s, such purely synthetic medicines were on an equal footing with their natural product counterparts (Ortholand and Ganesan, 2004).

In the past 20 years, approaches to the study of biologically active natural products have changed so dramatically that one is almost tempted to say that a new science has been born. Some of the areas in which strategies for the conduct of natural product chemistry have changed include plant selection, collection, isolation techniques, structure elucidation, biological evaluation, semisynthesis, dereplication, and biosynthesis (Cordell, 1995).

Plants are a valuable source of new natural products. Despite the availability of different approaches for the discovery of therapeuticals, natural products still remain as one of the best reservoirs of new structural types (Hostettmann, 1998). In the past decade, the essential oils and various extracts of plants have provoked interest as