UNIVERSITI PUTRA MALAYSIA

COMPARATIVE ANALYSES OF ANTIMICROBIAL ACTIVITIES OF Bauhinia purpurea L., Dicranopteris linearis (Burm.f.) Underw., Melastoma malabathricum L. AND Muntingia calabura L. METHANOLIC EXTRACTS

CHUAH EE LEY

FPSK (M) 2014 7

COMPARATIVE ANALYSES OF ANTIMICROBIAL ACTIVITIES OF *Bauhinia purpurea* L., *Dicranopteris linearis* (Burm.f.) Underw., *Melastoma malabathricum* L. AND *Muntingia calabura* L. METHANOLIC EXTRACTS

By

CHUAH EE LEY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

August 2014

© COPYRIGHT UPM

COPYRIGHT

All material contained within the thesis, including without limitation text, logos, icons, photographs, and all other artwork, is copyright material of Universiti Putra Malaysia unless otherwise stated. Use may be made of any material contained within the thesis for non-commercial purposes from the copyright holder. Commercial use of material may only be made with the express, prior, written permission of Universiti Putra Malaysia.

Copyright©Universiti Putra Malaysia

© COPYRIGHT UPM

DEDICATION

This thesis is dedicated to the following individuals who have accompanied me through thick and thin in completing this study:

To my parents - Thank you for encouraging me all the way.

To my brother - Thank you for your support.

To my supervisor and co-supervisors - Thank you for believing in me.

© COPYRIGHT UPM

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

COMPARATIVE ANALYSES OF ANTIMICROBIAL ACTIVITIES OF *Bauhinia purpurea* L., *Dicranopteris linearis* (Burm.f.) Underw., *Melastoma malabathricum* L. AND *Muntingia calabura* L. METHANOLIC EXTRACTS

By

CHUAH EE LEY

August 2014

Chairman: Assoc. Prof. Mohd. Nasir bin Mohd. Desa, PhD
Faculty: Medicine and Health Sciences

Microbial infections are common issues that happen in the society. However, the emergence of multidrug-resistant microbials have caused complications in diagnosing the effective treatments for patients to overcome the infections. The efficacy of antimicrobial agents available in the market against such resistant isolates have been compromised, aside from the side effects to human health caused by prolonged use of these drugs. The vast usage of traditional medicines in folklore era has triggered interest to seek for alternatives from plant sources in battling against these increasing multidrug- resistant microbials. This research aimed to compare a few assays in determining the antimicrobial activities of plant extracts. This study utilised disc diffusion assay, broth microdilution assay (visual turbidity inspection and spectrophotometric analysis) and colorimetric resazurin microtiter assay (REMA) to analyse the antimicrobial activities of methanolic leaf extracts of *Bauhinia purpurea* (BPME), *Dicranopteris linearis* (DLME), *Melastoma malabathricum* (MMME) and *Muntingia calabura* (MCME) against four American Type Culture Collection (ATCC®) bacterial strains, which were *Escherichia coli* ATCC® 25922™, *Pseudomonas aeruginosa* ATCC® 27853™, *Staphylococcus aureus* ATCC® 25923™ and *Staphylococcus aureus* ATCC® 700699™. Comparative analyses showed that MMME and MCME elicited greater antimicrobial activities compared to BPME and DLME, with Gram-positive strains showing greater susceptibility patterns. Interestingly, the methicillin-resistant *Staphylococcus aureus*/vancomycin-intermediate *S. aureus* (MRSA/VISA) strain employed in this study showed the greatest susceptibility pattern among the tested bacterial strains. Comparative analyses revealed that REMA would be a more accurate method to determine the minimum inhibitory concentration (MIC) values as the absence of colour change of resazurin may not signify the non-viability of bacterial cells, but rather the bacteriostatic phase of cells due to inhibitory effect of antimicrobial agents (plant extracts). On the other hand, conventional plating method on solid growth media and observation of bacterial growth after overnight incubation would be a more precise way to determine the minimum bactericidal concentration (MBC) values due to the bacterial growth can be observed easily by observing any presence of single colonies on the surface of solid media. Growth indicator which is usually

© COPYRIGHT UPM

employed in determining the MBC values may not be the most accurate way to determine the MBC values. This is so as it was observed that the bacterial suspension treated with methanolic leaf extract which changed the colour of resazurin from blue to purple did not harbour any bacterial growth upon plated on solid growth media. This may be due to the toxicity of antimicrobial agents which might have impaired the cell's viability and its ability to proliferate. This probably resulted the reduced capability of the cell to reduce resazurin (blue pigments) to resorufin (pink pigments). Disc diffusion assay can be employed as a preliminary screening for antimicrobial activities of potential antimicrobial agents before further tests are carried out, whereas spectrophotometric analysis can be employed as a supplementive measurement to observe the susceptibility pattern of microbials when treated with antimicrobial agents.

© COPYRIGHT UPM

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains.

ANALISIS PERBANDINGAN AKTIVITI ANTIMIKROB EKSTRAK METANOL *Bauhinia purpurea* L., *Dicranopteris linearis* (Burm.f.) Underw., *Melastoma malabathricum* L. DAN *Muntingia calabura* L.

Oleh

CHUAH EE LEY

Ogos 2014

Pengerusi: Prof. Madya Mohd. Nasir bin Mohd. Desa, PhD
Fakulti: Perubatan dan Sains Kesihatan

Jangkitan mikrob adalah isu biasa yang berlaku di kalangan masyarakat. Namun begitu, kemunculan mikrob tahan ubat telah mengakibatkan kerumitan dalam mengdiagnosis rawatan yang berkesan untuk pesakit bagi mengatasi jangkitan tersebut. Keberkesanan agen antimikrob yang terdapat di pasaran terhadap isolat tahan ubat sebegini telah dikompromi, selain daripada kesan-kesan sampingan pada kesihatan manusia akibat daripada penggunaan ubat-ubatan dalam jangka masa yang panjang. Penggunaan ubat-ubatan tradisional yang meluas pada zaman dahulu telah mencetuskan minat untuk mencari alternatif daripada sumber-sumber tumbuhan dalam memerangi mikrob penentang-multiubatan yang semakin bertambah. Penyelidikan ini bertujuan untuk membandingkan beberapa kaedah dalam menentukan aktiviti antimikrob ekstrak tumbuhan. Kajian ini menggunakan ujian difusi cakera, ujian mikropencairan 'broth' (pemeriksaan kekeruhan secara pemerhatian dengan mata kasar dan analisis spektrofotometrik) dan ujian kolorimetrik resazurin mikrotiter (REMA) untuk menganalisis aktiviti antimikrob ekstrak metanol daun *Bauhinia purpurea* (BPME), *Dicranopteris linearis* (DLME), *Melastoma malabathricum* (MMME) dan *Muntingia calabura* (MCME) terhadap empat 'American Type Culture Collection' (ATCC®) strain bakteria, iaitu *Escherichia coli* ATCC® 25922™, *Pseudomonas aeruginosa* ATCC® 27853™, *Staphylococcus aureus* ATCC® 25923™ dan *Staphylococcus aureus* ATCC® 700699™. Analisis perbandingan menunjukkan bahawa MMME dan MCME menghasilkan aktiviti antimikrob yang lebih tinggi berbanding dengan BPME dan DLME, di mana strain bakteria Gram-positif menunjukkan corak kecenderungan yang lebih tinggi. Yang menariknya, strain 'methicillin-resistant *Staphylococcus aureus*/vancomycin-intermediate *S. aureus*' (MRSA/VISA) yang digunakan di dalam kajian ini menunjukkan corak kecenderungan yang tertinggi di kalangan strain-strain bakteria yang diuji. Analisis perbandingan menunjukkan bahawa REMA adalah kaedah yang lebih tepat untuk menentukan nilai kepekatan minimum perencatan (MIC) kerana ketiadaan penukaran warna resazurin tidak bererti sel-sel bakteria telah mati, sebaliknya sel-sel berkemungkinan berada di fasa bakteriostatik disebabkan oleh kesan perencatan oleh agen antimikrob (ekstrak tumbuhan). Di samping itu, kaedah 'plating' secara konvensional pada media pertumbuhan pepejal

© COPYRIGHT UPM

dan pemerhatian pertumbuhan bakteria selepas inkubasi semalaman adalah kaedah yang lebih tepat untuk menentukan nilai kepekatan minimum 'bactericidal' (MBC) kerana pertumbuhan bakteria boleh diperhatikan dengan lebih mudah dengan pemerhatian terhadap sebarang pertumbuhan koloni bakteria pada permukaan media pepejal. Penanda pertumbuhan yang biasa digunakan untuk menentukan nilai MBC mungkin bukan kaedah yang paling tepat dalam penentuan nilai MBC. Hal ini yang demikian kerana pemerhatian mendapati tiada pertumbuhan koloni bakteria apabila suspensi bakteria yang dirawat dengan ekstrak metanol daun yang menukarkan warna resazurin dari biru ke ungu diselaputkan pada permukaan media pertumbuhan pepejal. Ini mungkin disebabkan oleh ketoksikan agen antimikrob yang berkemungkinan telah menjejaskan keaktifan sel dan kebolehannya untuk tumbuh. Hal ini mungkin telah mengurangkan keupayaan sel untuk menukarkan resazurin (pigmen biru) kepada resorufin (pigmen merah jambu). Ujian difusi cakera boleh digunakan sebagai pemeriksaan asas untuk aktiviti antimikrob bagi agen antimikrob yang berpotensi sebelum ujian yang lebih lanjut dilaksanakan, manakala analisis spektrofotometrik boleh digunakan sebagai pengukuran tambahan untuk memerhati corak kecenderungan mikrob apabila dirawat dengan agen antimikrob.

© COPYRIGHT UPM

ACKNOWLEDGEMENTS

It would not have been possible to complete this study without the endless help and support from people mentioned below. I am indebted to each of them and it is my honour to have this opportunity to remark my gratitude to these individuals.

I owe my deepest gratitude to the chairman of my supervisory committee, Assoc. Prof. Dr. Mohd. Nasir bin Mohd. Desa. You have been a tremendous supervisor throughout the period of this study, offering me your greatest opinion and sharing your unsurpassed knowledge wherever and whenever possible. This study and thesis would not have been possible without your guidance, persistent help and patience. Discussions with you have been insightful and invaluable. Your advice, suggestions and constructive criticism have been so helpful and I can't thank you enough for that. Thank you very much for your encouragement and supporting me all the way from the start till the end of this study.

I am grateful to my co-supervisor, Assoc. Prof. Dr. Zainul Amiruddin Zakaria, for his suggestive comments and advice all the while. I appreciate each feedback offered by you that have helped me so much in my study, for which I am truly grateful.

Special thanks to Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) and Ministry of Higher Education (MOHE) for their financial support and providing laboratory facilities. I would also like to offer my thanks to Institute of Bioscience, UPM for providing technical help in identifying the samples employed in this study.

My appreciation goes to the laboratory staff in the Applied Microbiology Laboratory, Mr. Sabri for his kindest help in providing the materials that I needed in this study. Thank you for your supportive and encouraging words every now and then, aside from the efforts that you have put in to ensure the punctuality of the arrival of the orders made so that my experiment could be done without much delay.

I would like to thank my fellow course mates for assisting me, sharing their knowledge and opinions. It has been a great pleasure working with all of you and I will always remember how we used to motivate each other to stay strong and ride this journey of learning together.

Last but not least, I would like to express a very special thanks to my family. Words cannot express how grateful I am to my father, mother and brother for all the sacrifices that you all have made. Neither of you have ever given up on me but instead provided me with endless moral support which have brought me to where I am now. The three of you are the reason that keeps me going, braving through these years despite all the ups and downs. Thank you for being a huge part of my life, showering me with endless love.

© COPYRIGHT UPM

I certify that a Thesis Examination Committee has met on 21 August 2014 to conduct the final examination of Chuah Ee Ley on her thesis entitled "Comparative Analyses of Antimicrobial Activities of *Bauhinia purpurea* L., *Dicranopteris linearis* (Burm.f.) Underw., *Melastoma malabathricum* L. and *Mutingia calabura* L. Methanolic Extracts" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Members of the Thesis Examination Committee were as follows:

Roslida binti Abd Hamid @ Abdul Razak, PhD
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Chairman)

Mohamad Aris bin Mohd Moklas, PhD
Senior Lecturer
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Zamberi bin Sekawi, PhD
Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Internal Examiner)

Vasudevan Mani, PhD
Associate Professor
Universiti Teknologi MARA
Malaysia
(External Examiner)

NORITAH OMAR, PhD
Associate Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 19 September 2014

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Mohd. Nasir bin Mohd. Desa, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Zainul Amiruddin Zakaria, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia
Date:

© COPYRIGHT UPM

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any other institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and Innovation) before thesis is published (in the modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules, or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/ fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2013 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software.

Signature: _____ Date: _____

Name and Matric No.: _____

© COPYRIGHT UPM

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to:

Signature: _____

Name of Chairman of Supervisory Committee: _____

Signature: _____

Name of Member of Supervisory Committee: _____

© COPYRIGHT UPM

TABLE OF CONTENTS

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF TABLES

© COPYRIGHT UPM

LIST OF FIGURES

© COPYRIGHT UPM

© COPYRIGHT UPM

© COPYRIGHT UPM

LIST OF ABBREVIATIONS

AAD	Antibiotic-associated diarrhoea
ATCC	American Type Culture Collection
BPME	*B. purpurea* methanolic extract
CFU	Colony-forming units
CFU/mL	Colony-forming unit per mililitre
CLSI	Clinical nad Laboratory Standards Institute
COAD	Chronic obstructive airways disease
DAEC	Diffusely adherent *E. coli*
DLME	*D. linearis* methanolic extract
DMSO	Dimethyl sulfoxide
EAEC	Enteroaggregative *E. coli*
EHEC	Enterohemorrhagic *E. coli*
EIEC	Enteroinvasive *E. coli*
EPEC	Enteropathogenic *E. coli*
ETEC	Enterotoxigenic *E. coli*
ExPEC	Extraintestinal pathogenic *E. coli*
IBS	Institute of Bioscience
MBC	Minimum bactericidal concentration
MCME	*M. calabura* methanolic extract
MDR	Multidrug resistance
MHA	Mueller-Hinton Agar
MHB	Mueller-Hinton Broth
MIC	Minimum inhibitory concentration
mg	milligram

© COPYRIGHT UPM

mg/disc	milligram per disc
mg/L	milligram per litre
mg/mL	milligram per millilitre
mm	millimeter
MMME	*M. malabathricum* methanolic extract
MNEC	Meningitis-associated *E. coli*
MRSA	Methicillin-resistant *S. aureus*
MRSA/VISA	Methicillin-resistant/Vancomycin-intermediate *S. aureus*
MSSA	Methicillin-sensitive *S. aureus*
nm	nanometer
NNIS	National Nosocomial Infections Surveillance
PBP2a	Penicillin-binding protein 2a
REMA	Resazurin microtiter assay
SPSS	Scientific Package of Social Science
UPEC	Uropathogenic *E. coli*
UPM	Universiti Putra Malaysia
UTIs	Urinary tract infections
VISA	Vancomycin-intermediate *S. aureus*
VRSA	Vancomycin-resistant *S. aureus*
v/v	volume per volume
w/v	weight per volume
ZOI	Zone of inhibition
°C	degree Celsius
µg	microgram
µL	microlitre
µm	micrometer

© COPYRIGHT UPM

CHAPTER 1

INTRODUCTION

1.1 Introduction

The discovery of microorganisms by Robert Hooke and Antonie van Leeuwenhoek back in the 17th century (Gest, 2004) has since opened the window to the world of microbiology. Microorganisms have been present by means of commensalism to human beings if not causing diseases which were then controllable with the use of traditional medicines. Over thousands of years, human have been depending on the nature as a medicinal source. The importance of traditional medicines in treating human diseases and as preventive measures is inarguable back in our ancestry era and has since been passed down from one generation to another by means of theories and practices. For decades to centuries, natural products have been derived from different sources ranging from terrestrial plants, microorganisms, vertebrates and invertebrates to pelagic organisms in search of cure against diseases (Newman *et al.*, 1999).

The use of plants in sophisticated traditional medicines by all ethnics and cultures has been acknowledged (Baquar, 1995) and is gaining popularity globally. They have been used for primary health care of the poor in developing countries as well as in countries where conventional medicines is the predominant diagnostic tools. Despite their existence and long history of usage in folkloric medicinal practices as well as their medicinal significances, the lack of attention received by traditional medicines from modern researchers and drug developments is undeniable (Tadeg *et al.*, 2005). Limited effort has been put in to promote the importance of ethnomedicines in advanced countries which focus more on synthetic drug developments. Although there have been documentations of the use of folk medicines in treating various illness and infectious diseases, the birth of antibiotics and their capability in reducing infectious diseases cases have successfully overwritten the essentiality of traditional medicines.

However, the use of antibiotics as the first line of defence against the spread of diseases have been compromised these days due to the emergence of antibiotic resistant pathogenic strains. Such is an evolutionary process of microorganisms acquiring the ability to resist the lethal action of antibiotics (Ahmad and Dar, 2011). Incidence of multi-drug resistant strains have been increasingly documented in recent years. An example of this is the emergence of methicillin-resistant *Staphylococcus aureus* in the 1960s which has since caused the increase of nosocomial infections caused by this particular strain (Abramson and Sexton, 1999). The rise of the multi-drug resistant strains is due to the genetic mutations within the microbial populations over the years. Besides that, the misuse of antimicrobial agents in drug prescriptions, lack of quality in drug developments and non-obsequiousness of patients in drug administration have caused the emergence of antibiotic resistant strains which presence has resulted many complicacies in human health (Mwambete, 2009). Bacterial resistance to most of the available antibacterial agents has been reported (Tanaka *et al.*, 2006) and this has raised the concern of public health care worldwide. Pharmaceutical and biotechnology companies have since battling with time and

© COPYRIGHT UPM

intensify their efforts in discovering novel antibacterial agents which are mandatory in the attempt to overcome this serious matter.

Administration of synthetic antimicrobial agents has been reported to affect the natural microflora in the human body. Antibiotics are capable of reducing the popularity of intestinal microbiota which plays essential role in general gut health, failing of which may cause acute diseases and chronic health problems (Dethlefsen *et al.*, 2008). A study found that antibiotic-associated diarrhoea (AAD) is caused by altered functionality of gut microflora by antibiotics (Beaugerie and Petit, 2004). As such, synthetically derived drugs have been withdrawn from the market years after their commercialisations due to adverse side effects to human health (Choudhury *et al.*, 2011).

Since the use of synthetically derived antimicrobial agents have caused so much mishaps to mankind, attempts to go back to the nature in lieu of searching for natural product from plants sources capable of overcoming the spread of diseases caused by these infectious and dangerous strains has been done. Plants produce bioactive compounds, whereby most of which serve as their defence mechanisms against pesticides, herbivores and microorganisms, hence their potential as sources of antimicrobial agents (Cowan, 1999, Mithraja *et al.*, 2012). The remarkably vast diversity of plants with an estimation of 250,000 to 500,000 species on Earth (Borris, 1996) increase the possibility of finding novel antimicrobial agents to subdue the arising antimicrobial resistance problem.

The pharmacotherapeutic agents in some local plants in Malaysia have been previously reported to elicit antimicrobial activities by either one of the two most commonly used screening method in determining the antimicrobial susceptibility level, disc or agar well diffusion assay and the broth dilution assay. Whilst most studies focus on the antimicrobial activity of one species of plant against a few bacterial strains or vice versa, or utilized single assay to determine the antimicrobial activity level, this study attempts to evaluate the antimicrobial activities of four species of plants (*Bauhinia purpurea*, *Dicranopteris linearis*, *Melastoma malabathricum* and *Muntingia calabura*) by means of comparing their activity levels using three antimicrobial assays (the disc diffusion assay, broth microdilution assay and colorimetric resazurin microtiter assay (REMA)) against four American Type Culture Collection (ATCC®) strains (*Escherichia coli* ATCC® 25922™, *Pseudomonas aeruginosa* ATCC® 27853™, *Staphylococcus aureus* ATCC® 25923™ and *Staphylococcus aureus* ATCC® 700699™).

A research by Zakaria *et al*. (2010) has found that methanolic extract elicited highest antimicrobial activity compared to other extracts extracted with other solvents, namely aqueous and chloroform. A separate study by Yao *et al.* (2004) also proved that methanol is capable of drawing out bioactive compounds from the plants at significantly higher level compared to water, chloroform and ethyl acetate. Another study reported that most of the antibacterial activity were portrayed by methanolic extracts of plants in the respective study (Rabe and Staden, 1997). Hence, methanol will be employed in the plant extraction process in this study and the outcome among the different plant extracts subjected to the various antimicrobial assays will be compared.

© COPYRIGHT UPM

The antimicrobial activities of crude methanolic leaf extracts instead of isolated pure compounds will be evaluated to look at the synergistic effect of the constituents combined. It was investigated that single bioactive compound is capable of changing its properties when other compounds are present (Barnabas and Nagarajan, 1988).

Many studies have shown that methanolic plant extracts exhibited higher antimicrobial activities compared to aqueous plant extracts (Doughari, 2006, Zakaria *et al.*, 2007b, Zakaria *et al.*, 2010b). Hence, this study will be focusing on methanol extraction to yield plant extracts with more bioactive compounds.

1.2 Problem Statement

Most of the previous antimicrobial activity studies focused on one single bacterial strain against one or more plant extracts, or a single plant extract against one or more bacterial strains. Besides that, most of the previous studies employed only one or two antimicrobial assays, which are either the disc diffusion and broth dilution or broth dilution and colorimetric assay.

This study aims to evaluate the antimicrobial activities of four plant extracts against four bacterial strains in a single study, unlike previous study which only looked at the antimicrobial activity of a plant extract at one time. On top of that, all three antimicrobial assays commonly used in antimicrobial activity studies will be employed in this study to enhance the accuracy of the results as most of the previous studies only used one antimicrobial assay per study. Four instead of one ATCC® strains is employed in this study to look at the susceptibility pattern of the microorganisms comparatively when tested against the plant extracts and this will directly determine which of the four plant extracts has the highest efficacy against the bacterial strains. With similar methodology settings, the results obtained in this study can be compared between one another to determine the plant extract which elicit the greatest antimicrobial activity.

1.3 Objectives

The objectives of this study are as listed:

1.3.1 General Objective

To compare the antimicrobial activities of methanolic leaf extracts of *B. purpurea*, *D. linearis*, *M. malabathricum* and *M. calabura* against *S. aureus* ATCC® 25923™, *S. aureus* ATCC® 700699™, *E. coli* ATCC® 25922™ and *P. aeruginosa* ATCC® 27853™ through different antimicrobial assays.

1.3.2 Specific Objectives

1. To screen for antimicrobial activities of methanolic leaf extracts against the ATCC® strains using the disc diffusion assay.
2. To determine the minimum inhibitory concentration (MIC) values and minimum bactericidal concentration (MBC) values using broth microdilution assay and REMA.

© COPYRIGHT UPM

3. To compare the association of broth microdilution assay and REMA in determining the MIC and MBC values.

1.4 Research Hypothesis

Four methanolic leaf extracts of *B. purpurea*, *D. linearis*, *M. malabathricum* and *M. calabura* possess antimicrobial activities.

© COPYRIGHT UPM

REFERENCES

Abramson, M. A., and D. J. Sexton. 1999. Nosocomial methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* primary bacteremia: At what cost? Infection Control and Hospital Epidemiology 20:408-411.

Ahmad, S., and M. I. Dar. 2011. Antibiotics and preventing their misuse. Physicians Academy 5:24-28.

Akiyama, H., K. Fujii, O. Yamasaki, T. Oono, and K. Iwatsuki. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. Journal of Antimicrobial Chemotherapy 48:487-491.

Ali, N. A. A., W. D. Julich, C. Kusnick, and U. Lindequist. 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology 74:173-179.

Alnajar, Z. A. A., M. A. Abdulla, H. M. Ali, M. A. Alshawsh, and A. H. A. Hadi. 2012. Acute toxicity evaluation, antibacterial, antioxidant and immunomodulatory effects of *Melastoma malabathricum*. Molecules 17:3547-3559.

Alwash, M. S., N. Ibrahim, and W. Y. Ahmad. 2013a. Bio-guided study on *Melastoma malabthricum* Linn leaves and elucidation of its biological activities. American Journal of Applied Sciences 10:767-778.

Alwash, M. S., N. Ibrahim, and W. Y. Ahmad. 2013. Identification and mode of action of antibacterial components from *Melastoma malabathricum* Linn leaves. American Journal of Infectious Diseases 9:46-58.

Ananth, K. V., M. Asad, N. P. Kumar, S. M. B. Asdaq, and G. S. Rao. 2010. Evaluation of wound healing potential of *Bauhinia purpurea* leaf extracts in rats. Indian Journal of Pharmaceutical Sciences 72:122-127.

Annegowda, H. V., M. N. Mordi, S. Ramanathan, M. R. Hamdan, and S. M. Mansor. 2012. Effect of extraction techniques on phenolic content, antioxidant and antimicrobial activity of *Bauhinia purpurea*: HPTLC determination of antioxidants. Food Analytical Methods 5:226-233.

Balamurugan, K., G. Sakthidevi, and V. R. Mohan. 2013. *In vitro* antioxidant activity of *Melastoma malabathricum* L. leaf (Melastomataceae). The Global Journal of Pharmaceutical Research 2:1676-1687.

Bangham, A. D., and R. W. Horne. 1962. Action of saponin on biological cell membranes. Nature Reviews Microbiology 196:952-953.

Baquar, S. R. 1995. The Role of Traditional Medicine in Rural Environment p. 141-142. *In* S. Issaq (ed.), Traditional Medicine in Africa. East Africa Educational Publishers Ltd., Nairobi.

© COPYRIGHT UPM

Barnabas, C. G. G., and S. Nagarajan. 1988. Antimicrobial activity of flavonoids of some medicinal plants Fitoterapia 59:508-510.

Beaugerie, L., and J. Petit. 2004. Antibiotic-associated diarrhoea. Best Practice & Research Clinical Gastroenterology 18:337-352.

Betts, J., C. Murphy, S. Kelly, and S. Haswell. 2012. Minimum inhibitory and bactericidal concentrations of theaflavin and synergistic combinations with epicatechin and quercetin against clinical isolates of *Stenotrophomonas maltophilia*. Journal of Microbiology, Biotechnology and Food Sciences 1:1250-1258.

Bhardwaj, S., and S. K. Gakhar. 2005. Ethnomedicinal plants used by the tribals of Mizoram to cure cuts & wounds. Indian Journal of Traditional Knowledge 4:75-80.

Boonphong, S., P. Puangsombat, A. Baramee, C. Mahidol, S. Ruchirawat, and P. Kittakoop. 2007. Bioactive compounds from *Bauhinia purpurea* possessing antimalarial, antimycobacterial, antifungal, anti-inflammatory, and cytotoxic activities. Journal of Natural Products 70:795-801.

Borchardt, J. R., D. L. Wyse, C. C. Sheaffer, K. L. Kauppi, R. G. Fulcher, N. J. Ehlke, D. D. Biesboer, and R. F. Bey. 2008. Antimicrobial activity of native and naturalized plant of Minnesota and Wisconsin. Journal of Medicinal Plants Research 2:98-110.

Borra, R. C., M. A. Lotufo, S. M. Gagioti, F. d. M. Barros, and P. M. Andrade. 2009. A simple method to measure cell viability in proliferation and cytotoxicity assays. Brazilian Oral Research 23:255-262.

Borris, R. P. 1996. Natural products research: Perspectives from a major pharmaceutical company. Journal of Ethnopharmacology 51:29-38.

Broekema, N. M., T. T. Van, T. A. Monson, S. A. Marshall, and D. M. Warshauer. 2009. Comparison of cefoxitin and oxacillin disk diffusion methods for detection of *mecA*-mediated resistance in *Staphylococcus aureus* in a large-scale study. Journal of Clinical Microbiology 47:217-219.

Carmeli, Y., N. Troillet, G. M. Eliopoulos, and M. H. Samore. 1999. Emergence of antibiotic-resistant *Pseudomonas aeruginosa*: Comparison of risk associated with different antipseudomonal agents. Antimicrobial Agents and Chemotherapy 43:1379-1382.

Chambers, H. F. 1988. Methicillin-resistant staphylococci. Clinical Microbiology Reviews 1:173-186.

© COPYRIGHT UPM

Chang, S., D. M. Sievert, J. C. Hageman, M. L. Boulton, F. C. Tenover, F. P. Downes, S. Shah, J. T. Rudrik, G. R. Pupp, W. J. Brown, D. Cardo, and S. K. Fridkin. 2003. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. The New England Journal of Medicine 348:1342-1347.

Chao, S., G. Young, C. Oberg, and K. Nakaoka. 2008. Inhibition of methicillin-resistant *Staphylococcus aureus* (MRSA) by essential oils. Flavour and Fragrance Journal 23:444-449.

Chen, J., H. Lee, C. Duh, and I. Chen. 2005. Cytotoxic chalcones and flavonoids from the leaves of *Muntingia calabura*. Planta Medica 71:970-973.

Chew, Y. L., E. W. L. Chan, P. L. Tan, Y. Y. Lim, J. Stanslas, and J. K. Goh. 2011. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia BMC Complementary and Alternative Medicine 11:1-10.

Chopda, M. Z., and R. T. Mahajan. 2009. Wound healing plants of Jalgaon district of Maharashtra state, India. Ethnobotanical Leaflets 13:1-32.

Choudhury, M. D., D. Nath, and A. D. Talukdar. 2011. Antimicrobial activity of *Melastoma malabathricum* L. Assam University Journal of Science & Technology: Biological and Environmental Sciences 7:76-78.

Chung, K. T., S. E. S. Jr, W. F. Lin, and C. I. Wei. 1993. Growth inhibition of selected food-borne bacteria by tannic acid, propyl gallate and related compounds. Letters in Applied Microbiology 17:29-32.

Cobben, N. A. M., M. Drent, M. Jonkers, E. F. M. Wouters, M. Vaneechoutte, and E. E. Stobberingh. 1996. Outbreak of severe *Pseudomonas aeruginosa* respiratory infections due to contaminated nebulizers. Journal of Hospital Infection 33:63-70.

Cockerill, F. R., M. A. Wikler, J. Alder, M. N. Dudley, G. M. Eliopoulos, M. J. Ferraro, D. J. Hardy, D. W. Hecht, J. A. Hindler, J. B. Patel, M. Powell, J. M. Swenson, R. B. Thomson, M. M. Traczewski, J. D. Turnidge, M. P. Weinstein, and B. L. Zimmer. 2012. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-second Informational Supplement. Clinical and Laboratory Standards Institute (CLSI).

Costerton, J. W., P. S. Stewart, and E. P. Greenberg. 1999. Bacterial biofilms: A common cause of persistent infections. Science 284:1318-1322.

Cowan, M. M. 1999. Plant products as antimicrobial agents. Clinical Microbiology Reviews 12:564-582.

Cushnie, T. P. T., and A. J. Lamb. 2005. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents 26:343-356.

© COPYRIGHT UPM

Dash, M., P. Misra, and S. Routaray. 2013. Bacteriological profile and antibiogram of aerobic burn wound isolates in a tertiary care hospital, Odisha, India. International Journal of Medicine and Medical Sciences 3:460-463.

Derus, A. R. M. 1998. Pengenalan dan Penggunaan Herba Ubatan, vol. 75. Multiple Triple Vision, Kuala Lumpur.

Dethlefsen, L., S. Huse, M. L. Sogin, and D. A. Relman. 2008. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLOS Biology 6:2383-2400.

Devehat, F. L., A. Bakhtiar, C. Bezivin, M. Amoros, and J. Boustie. 2002. Antiviral and cytotoxic activities of some Indonesian plants. Fitoterapia 73:400-405.

Doughari, J. H. 2006. Antimicrobial activity of *Tamarindus indica* Linn. Tropical Journal of Pharmaceutical Research 5:597-603.

Duffy, C. F., and R. F. Power. 2001. Antioxidant and antimicrobial properties of some Chinese plant extracts. International Journal of Antimicrobial Agents 17:527-529.

Epand, R. F., P. B. Savage, and R. M. Epand. 2007. Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (ceragenins). Biochimica et Biophysica Acta (BBA) - Biomembranes 1768:2500-2509.

Fluit, A. C., C. L. C. Wielders, J. Verhoef, and F. J. Schmitz. 2001. Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 24 university hospitals participating in the European SENTRY study. Journal of Clinical Microbiology 39:3727-3732.

Gautam, M. K., M. Gangwar, G. Nath, C. V. Rao, and R. K. Goel. 2012. *In-vitro* antibacterial activity on human pathogens and total phenolic, flavonoid contents of *Murraya paniculata* Linn. leaves. Asian Pacific Journal of Tropical Biomedicine 2:S1660-S1663.

Gest, H. 2004. The discovery of microorganisms by Robert Hooke and Antoni van Leeuwenhoek, fellows of the Royal Society. Notes and Records of the Royal Society 58:187-201.

Glauert, A. M., J. T. Dingle, and J. A. Lucy. 1962. Action of saponin on biological cell membranes. Nature Reviews Microbiology 196:953-955.

Gonzalez, R. J., and J. B. Tarloff. 2001. Evaluation of hepatic subcellular fractions for Alamar blue and MTT reductase activity. Toxicology in Vitro 15:257-259.

Grierson, D. S., and A. J. Afolayan. 1999. Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. Journal of Ethnopharmacology 66:103-106.

© COPYRIGHT UPM

Grosvenor, P. W., P. K. Gothard, N. C. McWilliam, A. Supriono, and D. O. Gray. 1995. Medicinal plants from Riau Province, Sumatra, Indonesia. Part 1: Uses. Journal of Ethnopharmacology 45:75-95.

Hossan, M. S., A. Hanif, B. Agarwala, M. S. Sarwar, M. Karim, M. Taufiq-Ur-Rahman, R. Jahan, and M. Rahmatullah. 2010. Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. Ethnobotany Research & Applications 8:61-74.

Hossan, M. S., A. Hanif, M. Khan, S. Bari, R. Jahan, and M. Rahmatullah. 2009. Ethnobotanical survey of the Tripura tribe of Bangladesh. American-Eurasian Journal of Sustainable Agriculture 3:253-261.

Hussain, F., M. A. Abdulla, S. M. Noor, S. Ismail, and H. M. Ali. 2008. Gastroprotective effect of *Melastoma malabathricum* aqueous leaf extract against ethanol-induced gastric ulcer in rats. American Journal of Biochemistry and Biotechnology 4:438-441.

Jatwa, R., and A. Kar. 2009. Amelioration of metformin-induced hypothyroidism by *Withania somnifera* and *Bauhinia purpurea* extracts in Type 2 diabetic mice. Phytotherapy Research 23:1140-1145.

Jofrry, S. M., N. J. Yob, M. S. Rofiee, M. M. R. M. M. Affandi, Z. Suhaili, F. Othman, M. A. Abdah, M. N. M. Desa, and Z. A. Zakaria. 2011. *Melastoma malabathricum* (L.) Smith ethnomedicinal uses, chemical constituents and pharmacological properties: A review. Evidence-Based Complementary and Alternative Medicine 2012:1-67.

Johnny, L., U. K. Yusuf, and R. Nulit. 2011. Antifungal activity of selected plant leaves crude extracts against a pepper anthracnose fungus, *Colletotrichum capsici* (Sydow) butler and bisby (Ascomycota: Phyllachorales). African Journal of Biotechnology 10:4157-4165.

Johnny, L., U. K. Yusuf, and R. Nulit. 2010. The effect of herbal plant extracts on the growth and sporulation of *Colletotrichum gloeosporioides*. Journal of Applied Biosciences 34:2218-2224.

Jones, G. A., T. A. McAllister, A. D. Muir, and K.-J. Cheng. 1994. Effects of sainfoin (*Onobrychis viciifolia* Scop.) condensed tannins on growth and proteolysis by four strains of ruminal bacteria. Applied and Environmental Microbiology 60:1374-1378.

Jorgensen, J. H., and M. J. Ferraro. 2009. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Diseases 49:1749-1755.

Jorgensen, J. H., and J. D. Turnidge. 2007. Antibacterial susceptibility tests: Dilution and disk diffusion methods. Manual of Clinical Microbiology 11:52-72.

© COPYRIGHT UPM

Kalemba, D., and A. Kunicka. 2003. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry 10:813-829.

Kamisan, F. H., F. Yahya, N. A. Ismail, S. S. Din, S. S. Mamat, Z. Zabidi, W. N. W. Zainulddin, N. Mohtarrudin, H. Husain, Z. Ahmad, and Z. A. Zakaria. 2013. Hepatoprotective activity of methanol extract of *Melastoma malabathricum* leaf in rats. Journal of Acupuncture and Meridian Studies 6:52-55.

Kaneda, N., J. M. Pezzuto, D. D. Soejarto, A. D. Kinghorn, and N. R. Farnsworth. 1991. Plant anticancer agents, XLVIII. New cytotoxic flavonoids from *Muntingia calabura* roots. Journal of Natural Products 54:196-206.

Kaper, J. B., J. P. Nataro, and H. L. T. Mobley. 2004. Pathogenic *Escherichia coli*. Nature Reviews Microbiology 2:123-140.

Kelmanson, J. E., A. K. Jager, and J. v. Staden. 2000. Zulu medicinal plants with antibacterial activity. Journal of Ethnopharmacology 69:241-246.

Lai, H. Y., Y. Y. Lim, and S. P. Tan. 2009. Antioxidative, tyrosinase inhibiting and antibacterial activities of leaf extracts from medicinal ferns. Bioscience, Biotechnology and Biochemistry 73:1362-1366.

Lakshmi, B. V. S., N. Neelima, N. Kasthuri, V. Umarani, and M. Sudhakar. 2009. Protective effect of *Bauhinia purpurea* on gentamicin-induced nephrotoxicity in rats. Indian Journal of Pharmaceutical Sciences 71:551-554.

Leboffe, M. J. 2011. Medical, environmental, and food microbiology. *In* B. E. Pierce (ed.), A Photographic Atlas for the Microbiology Laboratory, vol. 4. Morton Publishing, Colorado.

Lemeshko, V. V., V. Haridas, J. C. Q. Perez, and J. U. Gutterman. 2006. Avicins, natural anticancer saponins, permeabilize mitochondrial membranes. Archives of Biochemistry and Biophysics 454:114-122.

Leon, L. d., B. Beltran, and L. Moujir. 2005. Antimicrobial activity of 6-oxophenolic triterpenoids. Mode of action against *Bacillus subtilis*. Planta Medica 71:313-319.

Lim, T. K. 2012. *Muntingia calabura*. Edible Medicinal and Non Medicinal Plants 3:486-492.

Lin, F., J. Chen, and C. Shih. 2005. Antinociceptive and anti-inflammatory activity of the water-soluble extracts from leaves of *Muntingia calabura*. The Chinese Pharmaceutical Journal 57:81-88.

Livermore, D. M. 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: Our worst nightmare? Clinical Infectious Diseases 34:634-640.

© COPYRIGHT UPM

Mamat, S. S., F. H. Kamisan, W. N. W. Zainulddin, N. A. Ismail, F. Yahya, S. S. Din, Z. Zabidi, N. Mohtarrudin, A. K. Arifah, and Z. A. Zakaria. 2012. Effect of methanol extract of *Dicranopteris linearis* leaves against paracetamol- and carbon tetrachloride (CCl4)-induced liver toxicity in rats. Journal of Medicinal Plants Research 7:1305-1309.

Mehltreter, K. 2010. Problem ferns: their impact and management, p. 269. *In* L. R. Walker and J. M. Sharpe (ed.), Fern Ecology. Cambridge University Press, New York.

Mithraja, M. J., J. M. Antonisamy, M. Mahesh, Z. M. Paul, and S. Jeeva. 2012. Chemical diversity analysis on some selected medicinally important pteridophytes of Western Ghats, India. Asian Pacific Journal of Tropical Biomedicine 2:S34-S39.

Mwambete, K. D. 2009. The *in vitro* antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*: A Tanzania medicinal plant. African Health Sciences 9:34-39.

Nataro, J. P., and J. B. Kaper. 1998. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews 11:142-201.

Negi, B. S., B. P. Dave, and Y. K. Agarwal. 2012. Evaluation of antimicrobial activity of *Bauhinia purpurea* leaves under in vitro conditions. Indian Journal of Microbiology 52:360-365.

Nemoto, K., K. Hirota, T. Ono, K. Murakami, K. Murakami, D. Nagao, and Y. Miyake. 2000. Effect of Varidase (streptokinase) on biofilm formed by *Staphylococcus aureus*. Chemotherapy 46:111-115.

Newman, D. J., G. M. Cragg, and K. M. Snader. 1999. The influence of natural products upon drug discovery. Natural Product Reports 17:215-234.

Nostro, A., M. P. Germano, V. D. Angelo, A. Marino, and M. A. Cannatelli. 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology 30:379-384.

Nshimo, C. M., J. M. Pezzuto, A. D. Kinghorn, and N. R. Farnsworth. 1993. Cytotoxic constituents of *Muntingia calabura* leaves and stems collected in Thailand. Pharmaceutical Biology 31:77-81.

Nuresti, S., S. H. Baek, and A. Asari. 2003. Chemical components of *Melastoma malabathricum*. ACGC Chemical Research Communications 16:28-33.

Omar, S. N. C., J. O. Abdullah, K. A. Khairoji, C. C. Sieo, and M. Hamid. 2012. Potential of *Melastoma malabathricum* Linn. flower and fruit extracts as antimicrobial infusions. American Journal of Plant Sciences 3:1127-1134.

Ong, H. C. 2006. Tapak kuda, p. 240, Tanaman Hiasan: Khasiat Makanan & Ubatan, vol. 2. Utusan Publications, Kuala Lumpur.

© COPYRIGHT UPM

Ong, H. C., and M. Nordiana. 1999. Malay ethno-medico botany in Machang, Kelantan, Malaysia. Fitoterapia 70:502-513.

Ong, H. C., and J. Norzalina. 1999. Malay herbal medicine in Gemencheh, Negri Sembilan, Malaysia. Fitoterapia 70:10-14.

Panda, S., and A. Kar. 1999. *Withania somnifera* and *Bauhinia purpurea* in the regulation of circulating thyroid hormone concentrations in female mice. Journal of Ethnopharmacology 67:233-239.

Pettit, G. R., A. Numata, C. Iwamoto, Y. Usami, T. Yamada, H. Ohishi, and G. M. Cragg. 2006. Antineoplastic agents. 551. Isolation and structures of bauhiniastatins 1-4 from *Bauhinia purpurea*. Journal of Natural Products 69:323-327.

Poole, K. 2001. Multidrug efflux pumps and antimicrobial resistance in *Pseudomonas aeruginosa* and related organisms. Journal of Molecular Microbiology and Biotechnology 3:255-264.

Prasanna, S. K., and C. S. Shastry. 2012. Evaluation of hepatoprotective activity of *Bauhinia purpurea* Linn. Advance Research in Pharmaceuticals and Biologicals 2:275-278.

Rabe, T., and J. v. Staden. 1997. Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56:81-87.

Raja, D. P., V. S. Manickam, A. J. d. Britto, S. Gopalakrishnan, T. Ushioda, M. Satoh, A. Tanimura, H. Fuchino, and N. Tanaka. 1995. Chemical and chemotaxonomical studies on *Dicranopteris* species. Chemical & Pharmaceutical Bulletin 43:1800-1803.

Rajenderan, M. T. 2010. Ethno medicinal uses and antimicrobial properties of *Melastoma malabathricum*. SEGi Review 3:34-44.

Rios, J. L., M. C. Recio, and A. Villar. 1988. Screening methods for natural products with antimicrobial activity: A review of the literature. Journal of Ethnopharmacology 23:127-149.

Russo, T. A., and J. R. Johnson. 2000. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. The Journal of Infectious Diseases 181:753-754.

Salatino, A., C. T. T. Blatt, D. Y. A. C. D. Santos, and A. M. S. F. Vaz. 1999. Foliar flavonoids of nine species of *Bauhinia*. Brazilian Journal of Botany 22:17-20.

Salawu, S. O., A. O. Ogundare, B. B. Ola-Salawu, and A. A. Akindahunsi. 2011. Antimicrobial activities of phenolic containing extracts of some tropical vegetables. African Journal of Pharmacy and Pharmacology 5:486-492.

© COPYRIGHT UPM

Scalbert, A. 1991. Antimicrobial properties of tannins. Phytochemistry 30:3875-3883.

Shihabudeen, H. M. S., D. H. Priscilla, and K. Thirumurugan. 2010. Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharma Sciences and Research 1:430-434.

Sincock, J. L., and G. E. Swedberg. 1969. Rediscovery of the nesting grounds of Newell's Manx Shearwater (*Puffinus puffinus newelli*), with initial observations. The Condor 71:69-71.

Singh, H. B. 2003. Economically viable pteridophytes of India, p. 427. *In* B. K. Nayar, S. Chandra, and M. Srivastava (ed.), Pteridology in the New Millennium: NBRI Golden Jubilee Volume in Honour of Professor B. K. Nayar. Kluwer Academic Publishers, Netherlands.

Singh, M., R. Govindarajan, A. K. S. Rawat, and P. B. Khare. 2008. Antimicrobial flavonoid rutin from *Pteris vittata* L. against pathogenic gastrointetinal microflora. American Fern Journal 98:98-103.

Sirat, H. M., D. Susanti, F. Ahmad, H. Takayama, and M. Kitajima. 2010. Amides, triterpene and flavonoids from the leaves of *Melastoma malabathricum* L. Journal of Natural Medicines 64:492-495.

Studemeister, A. E., and J. P. Quinn. 1988. Selective imipenem resistance in *Pseudomonas aeruginosa* associated with diminished outer membrane permeability. Antimicrobial Agents and Chemotherapy 32:1267-1268.

Su, B., E. J. Park, J. S. Vigo, J. G. Graham, F. Cabieses, H. H. S. Fong, J. M. Pezzuto, and A. D. Kinghorn. 2003. Activity-guided isolation of the chemical constituents of *Muntingia calabura* using a quinone reductase induction assay. Phytochemistry 63:335-341.

Sufian, A. S., K. Ramasamy, N. Ahmat, Z. A. Zakaria, and M. I. M. Yusof. 2013. Isolation and identification of antibacterial and cytotoxic compounds from the leaves of *Muntingia calabura* L. Journal of Ethnopharmacology 146:198-204.

Susanti, D., H. M. Sirat, F. Ahmad, and R. M. Ali. 2008. Bioactive constituents from the leaves of *Melastoma malabathricum* L. Jurnal Ilmiah Farmasi 5:1-8.

Susanti, D., H. M. Sirat, F. Ahmad, R. M. Ali, N. Aimi, and M. Kitajima. 2007. Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. Food Chemistry 103:710-716.

Tadeg, H., E. Mohammed, K. Asres, and T. Gebre-Mariam. 2005. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology 100:168-175.

© COPYRIGHT UPM

Tanaka, J. C. A., C. C. d. Silva, A. J. B. d. Oliveira, C. V. Nakamura, and B. P. D. Filho. 2006. Antibacterial activity of indole alkaloids from *Aspidosperma ramiflorum*. Brazilian Journal of Medical and Biological Research 39:387-391.

Vega-Avila, E., and K. Pugsley. 2011. An overview of colorimetric assay methods used to assess survival or proliferation of mammalian cells. Proceedings of the Western Pharmacology Society 54:10-14.

Vlietinck, A. J., L. V. Hoof, J. Totte, A. Lasure, D. V. Berghe, P. C. Rwangabo, and J. Mvukiyumwami. 1995. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. Journal of Ethnopharmacology 46:31-47.

Wiegand, I., K. Hilpert, and R. E. W. Hancock. 2008. Agar and broth dilutin methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols 3:163-175.

Xiao, J., X. Jiang, and X. Chen. 2005. Antibacterial, anti-inflammatory and diuretic effect of flavonoids from *Marchantia convoluta*. African Journal of Traditional, Complementary and Alternative Medicines 1:244-252.

Yadav, S., and B. K. Bhadoria. 2005. Two dimeric flavonoids from *Bauhinia purpurea*. Indian Journal of Chemistry 44B:2604-2607.

Yadava, R. N., and P. Tripathi. 2000. A novel flavone glycoside from the stem of *Bauhinia purpurea*. Fitoterapia 71:88-90.

Yahya, F., N. A. Ismail, F. H. Kamisan, S. S. Mamat, S. S. Din, W. N. W. Zainulddin, Z. Zabidi, N. Mohtarrudin, Z. Suhaili, and Z. A. Zakaria. 2012. Effect of aqueous extract of *Melastoma malabathricum* leaves against paracetamol- and CCI4-induced liver toxicity in rats. African Journal of Pharmacy and Pharmacology 6:2682-2685.

Yao, L., Y. Jiang, N. Datta, R. Singanusong, X. Liu, J. Duan, K. Raymont, A. Lisle, and Y. Xu. 2004. HPLC analyses of flavanols and phenolic acids in the fresh young shoots of tea (*Camellia sinensis*) grown in Australia. Food Chemistry 84:253-263.

Yoshida, T., F. Nakata, K. Hosotani, A. Nitta, and T. Okudat. 1992. Dimeric hydrolysable tannins from *Melastoma malabathricum*. Phytochemistry 31:2829-2833.

Zakaria, Z. A. 2007a. Free radical scavenging activity of some plants available in Malaysia. Iranian Journal of Pharmacology & Therapeutics 6:87-91.

Zakaria, Z. A., A. M. Desa, K. Ramasamy, N. Ahmat, A. S. Mohamad, D. A. Israf, and M. R. Sulaiman. 2010. Lack of antimicrobial activities of *Dicranopteris linearis* extracts and fractions. African Journal of Microbiology Research 4:71-75.

© COPYRIGHT UPM

Zakaria, Z. A., C. A. Fatimah, A. M. M. Jais, H. Zaiton, E. F. P. Henie, M. R. Sulaiman, M. N. Somchit, M. Thenamutha, and D. Kasthuri. 2006b. The *in vitro* antibacterial activity of *Muntingia calabura* extracts. International Journal of Pharmacology 2:439-442.

Zakaria, Z. A., Z. D. F. A. Ghani, R. N. S. R. M. Nor, H. K. Gopalan, M. R. Sulaiman, and F. C. Abdullah. 2006. Antinociceptive and anti-inflammatory activities of *Dicranopteris linearis* leaves chloroform extract in experimental animals. Journal of the Pharmaceutical Society of Japan 126:1197-1203.

Zakaria, Z. A., Z. D. F. A. Ghani, R. N. S. R. M. Nor, H. K. Gopalan, M. R. Sulaiman, A. M. M. Jais, M. N. Somchit, A. A. Kader, and J. Ripin. 2008. Antinociceptive, anti-inflammatory, and antipyretic properties of an aqueous extract of *Dicranopteris linearis* leaves in experimental animal models. Journal of Natural Medicines 62:179-187.

Zakaria, Z. A., N. A. M. N. Hazalin, S. N. H. M. Zaid, M. A. Ghani, M. H. Hassan, H. K. Gopalan, and M. R. Sulaiman. 2007c. Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models. Journal of Natural Medicines 61:443-448.

Zakaria, Z. A., E. E. A. Hisam, M. S. Rofiee, M. Norhafizah, M. N. Somchit, L. K. Teh, and M. Z. Salleh. 2011a. *In vivo* antiulcer activity of the aqueous extract of *Bauhinia purpurea* leaf. Journal of Ethnopharmacology 137:1047-1054.

Zakaria, Z. A., A. M. M. Jais, M. Mastura, S. H. M. Jusoh, A. M. Mohamed, N. S. M. Jamil, M. S. Rofiee, and M. R. Sulaiman. 2007b. *In vitro* antistaphylococcal activity of the extracts of several neglected plants in Malaysia. International Journal of Pharmacology 3:428-431.

Zakaria, Z. A., Y. W. Loo, N. I. A. Rahman, A. H. A. Ayub, M. R. Sulaiman, and H. K. Gopalan. 2007. Antinociceptive, anti-inflammatory and antipyretic properties of the aqueous extract of *Bauhinia purpurea* leaves in experimental animals. Medical Principles and Practice 16:443-449.

Zakaria, Z. A., A. M. Mohamed, N. S. M. Jamil, M. S. Rofiee, M. N. Somchit, A. Zuraini, A. K. Arifah, and M. R. Sulaiman. 2011b. *In vitro* cytotoxic and antioxidant properties of the aqueous, chloroform and methanol extracts of *Dicranopteris linearis* leaves. African Journal of Biotechnology 10:273-282.

Zakaria, Z. A., R. N. S. R. M. Nor, G. H. Kumar, Z. D. F. A. Ghani, M. R. Sulaiman, G. R. Devi, A. M. M. Jais, M. N. Somchit, and C. A. Fatimah. 2006a. Antinociceptive, anti-inflammatory and antipyretic properties of *Melastoma malabathricum* leaves aqueous extract in experimental animals. Canadian Journal of Physiology and Pharmacology 84:1291-1299.

Zakaria, Z. A., M. S. Rofiee, L. K. Teh, M. Z. Salleh, M. R. Sulaiman, and M. N. Somchit. 2011. *Bauhinia purpurea* leaves extracts exhibited *in vitro*

© COPYRIGHT UPM

antiproliferative and antioxidant activities. African Journal of Biotechnology 10:65-74.

Zakaria, Z. A., A. S. Sufian, K. Ramasamy, N. Ahmat, M. R. Sulaiman, A. K. Arifah, A. Zuraini, and M. N. Somchit. 2010b. *In vitro* antimicrobial activity of *Muntingia calabura* extracts and fractions. African Journal of Microbiology Research 4:304-308.

Zakaria, Z. A., H. Zaiton, E. F. P. Henie, A. M. M. Jais, D. Kasthuri, M. Thenamutha, F. W. Othman, R. Nazaratulmawarina, and C. A. Fatimah. 2010a. The *in vitro* antibacterial activity of *Corchorus olitorius* and *Muntingia calabura* extracts. Journal of Pharmacology and Toxicology 5:480-486.

© COPYRIGHT UPM