EVALUATION AND COMPARISON ON CHEMICAL COMPONENTS, ANTIOXIDANT, ANTIMICROBIAL, AND ANTIDIABETES ACTIVITIES FROM SELECTED TROPICAL PLANTS

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by

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When I was in my final semester of Bachelor's degree, doing a final year project, I saw one of my classmates taking some flowers to her laboratory. I was curious and I asked her why because we have never studied botany in our degree. She told me they are flowers of Cannonball trees and she shared with me about her project which is natural products from plants. The project is very interesting and it caught my eyes. Then, I have decided I would like to take this project if I ever wanted to embark on a Ph.D. program.

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LIST OF ABBREVIATIONS

ABTS	2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
ATCC	American Type Culture Collection
BUT	Butanol
CFU	Colony forming unit
DMSO	Dimethyl sulfoxide
DPPH	Diphenylpicryl-hydrazyl
EA	Ethyl acetate
FRAP	Ferric reducing antioxidant potential
GAE	Gallic acid equivalent
HEX	Hexane
MeOH	Methanol
MIC	Minimum Inhibitory Concentration
mM	Milimolar
NA	Nutrient agar
NB	Nutrient broth
PDA	Potato dextrose agar
PDB	Potato dextrose broth
Rf	Relative frequency
SD	Standard deviation
TPC	Total phenolic content
TFC	Total flavonoid content
UPLC	Ultra Performance Liquid Chromatography
UV	Ultraviolet

PENILAIAN DAN PERBANDINGAN KOMPONEN KIMIA, ANTIOKSIDAN, ANTIMIKROB DAN ANTIDIABETES DARI TUMBUHAN TROPIKA TERPILIH

ABSTRAK

Rengas (*Gluta torquata*), Damar Minyak (*Agathis borneensis*), Kelat Merah (*Eugenia chlorantha*), Cina Maki (*Leptospermum flavescens)* dan Podo Bukit (*Podocarpus neriifolius*) adalah spesies pokok yang tersebar luas yang boleh didapati di hutan tropika Malaysia. Sampel pokok ini telah dipilih dalam kajian ini kerana kekurangan bukti saintifik dan pengenalan fitokimia. Kajian ini adalah untuk menyiasat jumlah kandungan fenolik (TPC) dan jumlah kandungan flavonoid (TFC) daripada 19 ekstrak metanol daripada 4 bahagian tumbuhan (daun, kulit kayu, kayu teras dan kayu gubal) sebagai antioksidan untuk perencatan α-amilase dan α-glucosidase dan antimikrobial. Untuk *Gluta torquata* hanya daun, kulit kayu dan kayu gubal dipilih. Sifat-sifat antioksidan ekstrak metanol telah dianalisis dengan menggunakan asai penyingkiran radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH), potensi antioksida mengurangkan ferik (FRAP), penyingkiran radikal bebas 2'-azinobis (2-etilbenzothiazolin-6-sulfonik asid) (ABTS), dan penglekat logam. Menggunakan hasil daripada kajian ini, semua ekstrak daun yang menghasilkan lebih banyak ekstrak mengandungi kandungan fenolik yang lebih tinggi dalam peratusan daripada bahagian-bahagian lain tumbuhan seperti kulit kayu, kayu teras dan kayu gubal. Jumlah kandungan fenolik (TPC) mempunyai korelasi positif dengan asai DPPH, FRAP dan ABTS. Menariknya, kedua-dua DPPH dan FRAP asai lebih berkait rapat berbanding dengan asai antioksidan lain. Kayu gubal *Gluta torquata*

menghasilkan jumlah kandungan tertinggi flavonoid dengan nilai 39.6 (0.1) μg GAE / mg ekstrak, kedua tertinggi jumlah kandungan fenolik dengan nilai 79 (2) μg GAE / mg ekstrak dan menunjukkan aktiviti antioksidan yang tertinggi. Kayu gubal *Gluta torquata* dipilih untuk proses fraksinasi. Dengan menggunakan Kromatografi Cecair Prestasi Ultra (UPLC), kayu gubal *Gluta torquata* telah dikesan mengandungi sebatian fenolik seperti asid protokatekuik yang merupakan penyumbang kepada aktiviti antioksidan yang baik. Aktiviti perencatan α-amylase and α-glucosidase untuk semua ekstrak sampel telah diuji untuk mengenali keupayaan sampel dalam melambatkan pencernaan karbohidrat yang membantu untuk mengawal hiperglisemia setelah makan. Kayu gubal *Gluta torquata* menunjukkan perencatan α-amylase and α-glucosidase tertinggi masing masing dengan nilai EC_{50} 122 (1) mg / mL dan 6.5 (0.1) mg / mL. Kayu gubal *Gluta torquata* dipilih untuk proses fraksinasi dan keputusan analisis UPLC menunjukkan kandungan asid kafeik, sulfuretin, kuersetin dan kaempferol. Aktiviti-aktiviti yang telah disumbangkan oleh sebatian fenolik tetapi tidak bergantung kepada dos kandungan sebatian fenolik. Dalam kajian ini, bakteria lebih mudah terdedah kepada semua sampel berbanding dengan kulat. Daun *Gluta torquata* dikesan mengandungi kuersetin manakala daun *Leptospermum flavescens* dikesan mengandungi kuersetin dan kaempferol yang menyumbang kepada aktiviti antimikrob.

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ABSTRACT

Rengas (*Gluta torquata*), Damar Minyak (*Agathis borneensis*), Kelat Merah (*Eugenia chlorantha*), Cina Maki (*Leptospermum flavescens*), and Podo Bukit (*Podocarpus neriifolius*) are widespread distributed plant species which can be found in Malaysia tropical forests. The plant species were selected in this study due to their lack of scientific evidence and phytochemical identification. This study is to investigate the total phenolic (TPC) and total flavonoid contents (TFC) of the 19 methanolic crude extracts from 4 parts of plants (leaf, bark, heartwood and sapwood) as antioxidants for α-amylase and α-glucosidase inhibitory and antimicrobial. For *Gluta torquata*, only leaf, bark and sapwood of were chosen. The antioxidant properties were analyzed by the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, the ferric reducing antioxidant power (FRAP), 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid *(ABTS*) radical scavenging, and metal chelating assays. Using the results obtained from this study, all leaf extracts which yield more extracts contain higher phenolic compounds in percentages than other parts of plants such as bark, heartwood and sapwood. The total phenolic content (TPC) have positive correlations with DPPH, FRAP and ABTS assays. Interestingly, both DPPH and FRAP assays are correlated strongly compare with other antioxidant assays. *Gluta torquata* sapwood yielded the highest total flavonoid content which was 39.6 (0.1) µg QE/ mg extract, the highest of total phenolic content which was 79 (2) µg

GAE/ mg extract and exhibited outstanding antioxidant activities. *Gluta torquata* sapwood was chosen for further fractionation. By using the Ultra Performance Liquid Chromatography (UPLC), *Gluta torquata* sapwood was detected to contain phenolic compounds such as protocatechuic acid which was contributor to the good antioxidant activity. The inhibitory effects of all samples on α-amylase and α-glucosidase were tested *in vitro* to investigate the ability in delaying the breakdowns of carbohydrates which helps to control postprandial hyperglycaemia. *Gluta torquata* sapwood showed excellent α-amylase and α-glucosidase enzyme inhibitions with EC_{50} values of 121 (1) µg/mL and 6.5 (0.1) µg/mL, respectively. *Gluta torquata* sapwood was further fractionated and the UPLC analysis identified the presence of caffeic acid, sulfuretin, quercetin and kaempferol. The activities were contributed by these phenolic compounds but not dose-dependent on the content of phenolic compounds. In this study, bacteria were more susceptible to all the samples compared to fungi. *Gluta torquata* leaf was detected to contain quercetin while *Leptospermum flavescens* leaf was detected to contain quercetin and kaempferol which contributed to the antimicrobial activities.

Chapter 1 General introduction

There are many species of Malaysian plants are being left without knowing their functions and compositions, for example, Rengas (*Gluta torquata*), Damar Minyak (*Agathis borneensis*), Kelat Merah (*Eugenia chlorantha*), Cina Maki (*Leptospermum flavescens*) and Podo Bukit (*Podocarpus neriifolius*). They are highly available because they are widespread species of tropical plants.

There are incomplete studies on antioxidant, antimicrobial and antidiabetic activities of these widespread species of tropical plants as mentioned before. Furthermore, each part of plants has different properties because the chemical compositions of each part are different. In this study, the five species of Malaysia tropical plant are separated into 4 different parts to be investigated on their antioxidant, antimicrobial and antidiabetic activities except for *Gluta torquata* where only 3 parts are chosen.

Plants have been a great source of bioactive compounds. Investigations in the past proven that bioactive compounds from plants have been a potential source of natural antioxidant, antifungal and other biological activities. They are present in the form of extractives in trees and soluble in organic solvents (Bernhoft, 2010). These compounds are also known as secondary metabolites (Lee *et al*., 2013). The most widespread class of secondary metabolites are phenolic compounds. Their redox potential enables them to act excellently in different mechanisms of antioxidant, antifungal and antidiabetes (Pereira *et al*., 2009). Phenolic compounds can be further divided into two groups which are phenolic acids and flavonoids. The evaluations of