PHYTOCHEMICALS, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *Mikania micrantha* Kunth AND *Chromolaena odorata* (L.) R.M.King & H.Rob.

By

NAFIZAH BINTI HASSAN

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

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

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Chairman : Associate Professor Ts Rasmina Halis, PhD
Faculty : Forestry and Environment

Mikania micrantha (M. micrantha) and *Cromolaena odorta (C. odorata)* are an invasive plant that has become a weed pest, grows aggressively, spreads, and displaces other plants in agriculture corps and generally consumed as traditional medicine purposes by local practitioners. This study was conducted with the aim to determine the physical and botany characteristics of both plants, to identify phytochemical in both species and to assess antimicrobial and antioxidant capacity (DPPH, ABTS and FRAP) in hot water, methanolic and petroleum ether extract of *M. micrantha* and *C. odorata* leaf and stem. The physical characteristics of both species such as shape, type, arrangement, margin, base, apex, and vein of leaf, and stem were determined based on dendrology characterization and identification. Phytochemical of methanolic and petroleum ether extract obtained from leaf and stem of *M. micrantha* and *C. odorata* was analyzed using Gas Chromatography – Mass Spectrometry (GC-MS) for the identification of biochemical components present. Phytochemical analysis of *M. micrantha* extract by GC-MS revealed the presence of various compounds such as phenol, fatty acid, sesquiterpenes, diterpenes, alkane hydrocarbon and others which have diverse use. All extract from *M. micrantha* and *C. odorata* species were evaluated for their antimicrobial activity through disc diffusion method against four microbes (*Bacillus subtilis, Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli)* and one fungus *(Candida albicans)*. *M. Micrantha* extract demonstrated minimal inhibition of targeted microbial activity, and some displayed no inhibition at all. As for antifungal activity, *C. albicans* were resistant to all extract and there was no activity detected. For *C. odorata* extract, the result displayed no inhibition to slight inhibition activity towards *B. subtilis and S. aureus.* Meanwhile, all extract did not show any inhibition zone towards *P. aeruginosa* and *E. coli.* The screening of antifungal activity towards *C. albicans* showed that only hot water extract of *C. odorata* stems have the reaction while the others were inactive. The measurement of

Total Phenolic Content (TPC) of hot water, methanolic and petroleum ether extract of *M. micrantha* and *C.odorata* leaves and stems was achieved using folin-ciocalteu reagent reveals that hot water extract for *M. micrantha* leaves and stems had the TPC value 335.5 mg GAE/g and 323.14 mg GAE/g respectively which was found slightly higher ($p<0.05$) when compared to standard gallic acid reference. On top of that, all TPC value obtained was above average. The DPPH capacity of each extract 5 mg/ml was tested and it is found that hot water extract for *C. odorata* leaves and methanolic extract for *M. micrantha* stems have slightly higher scavenging capacity (91.85 µg/ml and 91.46 µg/ml) respectively at $p<0.05$ as compared to Trolox while ABTS radical were obtained to be higher in hot water extract for both *C. odorata* leaves (96.88%) and *M. micrantha* leaves (96.27%). For FRAP activity hot water and methanol extract for both *M. mirantha and C. odorata* have slightly higher ($p<0.05$) antioxidant value while the petroleum ether extract for both *M. mirantha and C. odorata* shows slightly lower value. The results obtained in this study clearly indicate that hot water and methanolic extract for *M. micrantha* and *C. odorata* leaves has a significant potential use as natural antioxidant agent and further research should be explored on the utilization of these resource to cure oxidative damage – related disease.

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

FITOKIMIA, ANTIMIKROB DAN ANTIOKSIDAN bagi *Mikania micrantha* Kunth DAN *Chromolaena odorata* (L.) R.M.King & H.Rob.

Oleh

NAFIZAH BINTI HASSAN

Mac 2021

Pengerusi : Professor Madya Ts Rasmina Halis, PhD
Fakulti : Perhutanan dan Alam Sekitar

Mikania micrantha (M. micrantha) dan *Cromolaena odorta (C. odorata)* adalah tumbuhan invasif yang telah menjadi rumpai perosak, tumbuh secara agresif, menyebarkan, dan menggantikan tanaman lain di ladang pertanian dan umumnya digunakan sebagai tujuan perubatan tradisional oleh pengamal perubatan tempatan. Kajian ini dilakukan dengan tujuan untuk menentukan ciri-ciri fizikal dan botani kedua-dua tumbuhan, untuk mengenal pasti fitokimia pada kedua-dua spesies dan untuk menilai kapasiti antimikrob dan antioksidan (DPPH, ABTS dan FRAP) dalam ekstrak air panas, metanol dan petroleum eter bagi daun dan batang *M. micrantha* dan *C. odorata*. Ciri-ciri fizikal kedua-dua spesies seperti bentuk, jenis, susunan, margin, dasar, puncak, urat daun, dan batang ditentukan berdasarkan ciri dan identifikasi dendrologi. Fitokimia ekstrak metanol dan petroleum eter yang diperoleh dari daun dan batang *M. micrantha* dan *C. odorata* dianalisis menggunakan *Gas Chromatography - Mass Spectrometry* (GC-MS) untuk mengenal pasti komponen fitokimia yang ada. Analisis fitokimia ekstrak *M. micrantha* oleh GC-MS mendedahkan adanya pelbagai sebatian seperti fenol, asid lemak, sesquiterpenes, diterpenes, hidrokarbon alkana dan lain-lain yang mempunyai pelbagai kegunaan. Semua ekstrak dari spesies *M. micrantha* dan *C. odorata* dinilai untuk aktiviti antimikrob melalui kaedah penyebaran cakera terhadap empat mikrob *(Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli)* dan satu kulat *(Candida albicans).* Ekstrak *M. Micrantha* menunjukkan perencatan minimum terhadap aktiviti mikrob yang disasarkan dan terdapat mikrob yang tidak menunjukkan perencatan. Bagi aktiviti antikulat, *C. albicans* tahan terhadap semua ekstrak dan tiada aktiviti yang dikesan. Untuk ekstrak *C. odorata*, hasilnya menunjukkan tidak terdapat aktiviti perencatan terhadap *B. subtilis* dan *S. aureus*. Sementara itu, semua ekstrak tidak menunjukkan sebarang zon perencatan terhadap *P. aeruginosa* dan *E. coli*. Pemeriksaan aktiviti antikulat terhadap *C. albicans* menunjukkan bahawa hanya ekstrak air panas batang *C. odorata* yang mempunyai reaksi sementara yang lain adalah tidak aktif. Pengukuran total kandungan fenolik (TPC) ekstrak air panas, metanol dan

petroleum eter bagi daun dan batang *M. micrantha* dan *C.odorata* dicapai dengan menggunakan reagen *folin-ciocalteu* yang menunjukkan bahawa ekstrak air panas untuk daun dan batang *M. micrantha* mempunyai nilai TPC 335.5 mg GAE/g dan 323.14 mg GAE/g masing-masing yang didapati lebih tinggi sedikit ($p<0.05$) jika dibandingkan dengan rujukan standard asid gallic. Selain itu, semua nilai TPC yang diperoleh adalah melebihi purata. Kapasiti DPPH setiap ekstrak 5 mg/ml diuji dan didapati bahawa ekstrak air panas untuk daun *C. odorata* dan ekstrak metanol untuk batang *M. micrantha* mempunyai kapasiti antioksidan lebih tinggi sedikit pada $p<0.05$ (91.85 µg / ml dan 91.46 µg / ml) masing-masing berbanding dengan Trolox sementara ABTS radikal didapati lebih tinggi sedikit dalam ekstrak air panas untuk kedua-dua daun *C. odorata* (96.88%) dan daun *M. micrantha* (96.27%). Aktiviti FRAP menunjukkan bahawa terdapat sedikit ($p<0.05$) sifat antioksidan bagi ekstrak air panas dan metanol untuk *M. mirantha* dan *C. odorata* sementara nilai antioksidan yang lebih rendah sedikit dikesan dalam ekstrak petroleum eter untuk *M. mirantha* dan *C. odorata*. Hasil yang diperoleh dalam kajian ini menunjukkan dengan jelas bahawa ekstrak air panas dan metanol untuk daun *M. micrantha* dan *C. odorata* mempunyai potensi penggunaan sebagai agen antioksidan semula jadi dan penyelidikan lebih lanjut harus diterokai mengenai penggunaan sumber ini untuk menyembuhkan penyakit yang berkait dengan kerosakan oksidatif.

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Rasmina Halis, PhD
Associate Professor Ts
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Chairman)

Norhaizan Mohd Esa, PhD
Associate Professor
Faculty of Medicine and Health Science
Universiti Putra Malaysia
(Member)

Ummi Hani Abdullah, PhD
Senior Lecturer
Faculty of Forestry and Environment
Universiti Putra Malaysia
(Member)

_____
ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 14 October 2021

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Name of Chairman of Supervisory Committee: Assoc. Prof. Ts Dr. Rasmina Halis

Signature: _____
Name of Member of Supervisory Committee: Assoc. Prof. Dr. Norhaizan Mohd Esa

Signature: _____
Name of Member of Supervisory Committee: Dr. Ummi Hani Abdullah

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abs	Absorbance
ABTS	2,2-Azinobis (3-ethyl-benzothiazoline-6-sulfunic acid)
AEAC	Ascorbic Acid Equivalent Antioxidant Capacity
ANOVA	Analysis of variance
DPPH	2,2-diphenyl-1-picrylhydrazyl
Fe2+	Iron
FRAP	Ferric Reducing Antioxidant Power
GC	Gas Chromatography
GC-MS	Gas chromatography mass spectrometry
HPLC	High Performance Liquid Chromatography
Hrs	Hours
$K_2S_2O_8$	Potassium persulfate
Min	Minutes
MS	mass spectrometry
$NaCO_3$	Sodium carbonate
NIST	National Institute Standard and Technology
nm	Nanometre
psi	pounds per square inch
RIs	Retention indices
SE	Soxhlet extraction
Sec	Seconds
SEC	Size-Exclusion Chromatography
SFC	Supercritical Fluid Chromatography
SPSS	Statistical Package for the Social Sciences

TIC	Total ion count
TLC	Thin-Layer Chromatography
TPC	Total phenolic content
TPTZ	(2,4,6-tri(2-pyridyl)- 1,3,5-triazine)
UV	Ultra-violet

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

For generations, local herbal medicine practitioners in Malaysia have inherited the natural product as a remedy. The natural product is an organic compound formed by living organisms, including livestock and plants. The research of medicinal or herbal plants usually focuses on natural products that have been traditionally recognised and used in the treatment of diseases for thousands of years, as they are recognised in many medicinal values by elderly people. Various plants have been used as a substance for treating various diseases and more than 1200 plants has been reported with medicinal properties (Hunter, 2011) including invasive plant.

Invasive organism defined as an organism that is not indigenous to the ecological system under consideration and whose arrival contributes or is likely to trigger disruption to the environment, economy or people's health (Esham, 2012). This includes microorganism, fungi, animal and plant. A plant that has become a weed pest, spreads, grows and takes over other plants violently, is considered an invasive plant. They tend to show up in disturbed areas, and the most violent can disrupt established ecosystems, such as young plantations and agricultural crops. (Cock et al., 2000). Generally, invasive plants are undesirable because they are difficult to manage, may escape cultivation and can occupy entire regions. These invasive plants also can affect soil quality through the releases of allelopathy chemical from plant (Weidenhamer et al., 2010). An aggressive plant that is liberated from its environmental, pest, and disease limits may become an invader of other ecosystems (Pérez-Amador et al., 2010). There is increasing evidence that allelopathy may play a key role in the successful of plant invaders (Murrell et al., 2011).

Allelopathy refers to chemical interaction between plants, whereby directly or indirectly produced secondary compounds inhibit the growth and health of other organisms (Inderjit & del Moral, 1997; Murrell et al., 2011). They can cause interference and result in losses for the cultivation of plants to absorb nutrients and water from the soil and reception of light for photosynthesis. The allelopathic effects are likely to contribute to the success of several major plant invaders, such as *Mikania micrantha (M. micrantha)* and *Cromolaena odorata* (*C. odorata)* in the Malaysian agricultural plantation area (RISDA, 2011).

1.2 Problem Statement and Justification

M. micrantha and *C. odorata* has been classified by the International Union for Conservation of Nature as 100 of the world's worst invasive alien species (Lowe et al., 2000). They are belonging to *Asteraceae* family (Zhang et al., 2016) that is the largest family of flowering plants.

M. micrantha known as *selaput tunggul* is considered as a weed in Malaysia where it can intrude the growth and productivity of several crops such as rubber, oil palm and cocoa plantation and it is reported every year, about 8-10 million dollars has been invested to monitor its growth (Sankaran, 2008). This plant grows abundantly along the roadside, swampy forests, bushes of moist places, forest borders, idle plantation field, and even along streams and rivers in its habitat, making the plant readily available for traditional treatment (Saha et al., 2015).

C. odorata locally known as kapal terbang (Jumaat et al., 2017a) or other name include Siam Weed, Christmas Bush, Devil Weed, Camfhur Grass and Common Floss Flower (Nanadini *et al*, 2014). It is a vertical plant or shrub, in the open and thick scrambling bushes up to 10 m high between trees, it can stand up to 2.5 m tall, the leaves are thinly hairy, occur in pairs and are pointed ovate to triangular. The colour of leave are range from light to medium green in addition when it being crushed, they clearly have a distinctive smell (Luwum, 2002).

Despite these limitations, both *M. micrantha* and *C. odorata* are commonly known and have been used as folk medicine for their medicinal properties. (Bhardwaj & Gakhar, 2005; Vital and Rivera, 2009). However, there is still minimal research of *M. micrantha* and *C. odorata* phytochemicals as scientific indication to prove their conventional uses. This study provides information on plant chemicals or bioactive compound in *M. micrantha* and *C. odorata* extracted using hot water, methanol and petroleum ether which can provide baseline information for future research.

Although both *M. micrantha* and *C. odorata* are classified as invasive plants, they are commonly used by local medical practitioner as remedy to cure new wound and cut (Ishak et al., 2016). They have been using these plants from generation to generation, but there are still many young people who do not recognize this plant due to the identification of physical and botanical aspect of *M. micrantha and C. odorata* through scientific approach rather scanty.

Scientists and clinicians around the world have recently been concerned about the increasing resistance of microorganisms to available antimicrobial agents. It is noted that the treatment of pathogenic viruses, bacteria, fungi and protozoa with current medications is more difficult (Orhan et al., 2010). Antimicrobial drugs produced from natural resources such as plant should be developed to resolve the disadvantages of existing antimicrobial agents and to acquire more efficient drugs.

Natural products are a significant source of chemical diversity and have provided essential medicinal products for many bacterial diseases. Plant- derived medicines produced a variety of phytochemical constituents known as therapeutic properties (Vukovic et al., 2007).

Other than that, results indicate that antioxidants with free radical scavenging properties of plant origin can have significant therapeutic effect in free radical induced diseases such as diabetes, cancer, neurodegenerative disease, cardiovascular disease, ageing, and gastrointestinal diseases. (Dev et al., 2015). Several synthetic antioxidants have shown toxic and/or mutagenic effects, whereas plant-based medications have less side effects than synthetic drugs (Tapsell et al., 2006).

Therefore, this research was done to investigate the phytochemicals of *M. micrantha* and *C. odorata* on their antimicrobial and antioxidant activities.

1.3 Significance of The Study

The goal of this research is to have a better knowledge of how invasive species are used. Furthermore, the study could be important to the future researchers, community and medical practitioner.

The idea presented could be used as a reference data in conducting new research or in testing the validity of the related findings. Other than that, the community can use this finding to identify the species and use the species instead of treat them as an invader and the outcome of the study will facilitate medical practitioner to formulate efficient strategies and worthwhile approaches to make the use of these species.

1.4 Objectives

The main objective is to examine the phytochemical component, antimicrobial and antioxidant activity of leaves and stem extracts from *M. micranta* and *C. odorata*.

Specific objectives:

1. To determine the physical and botany characteristics of *M. micrantha* and *C. odorata* on its leaf and stem parts.
2. To identify the phytochemical constituents in *M. micrantha* and *C. odorata* leaves and stems extracts derived with different solvents using GCMS.
3. To evaluate the antimicrobial and antioxidant activity of *M. micrantha* and *C. odorata.*

1.5 Scope of the study

This study was designed to understand the botany aspect and the medicinal aspect of Malaysia invasive species, *M. micrantha* and *C. odorata* based on only leaf and stem part that found in Meru Klang. Therefore, the extraction process has been done to extract both species using polar and non-polar solvent: hot water, methanol and petroleum ether. Only methanolic and petroleum ether extract were analyzed using GC-MS. Antimicrobial study includes only bacteria and fungi that available in institute of biotechnology UPM.

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