CHEMICAL PROFILING ON BIOACTIVE METABOLITES OF RHIZOME *Curcuma xanthorrhiza.*

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THIS DESSERTATION WAS WRITTEN TO FULFILL A PART OF THE REQUIREMENT TO OBTAIN A DEGREE IN BACHELOR OF SCIENCE WITH HONORS

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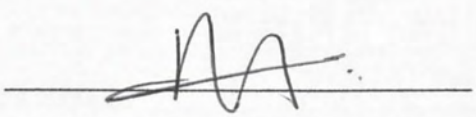

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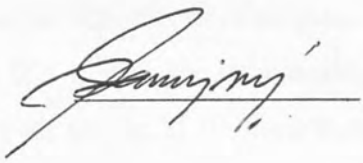
(DR. CHARLES S. VAIRAPPAN) 

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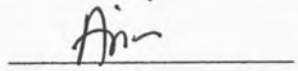
(PUAN NOR AZIZUN RUSDI) 

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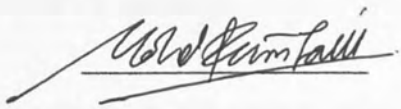
(ENCIK MOHD FAIRUS JALIL) 

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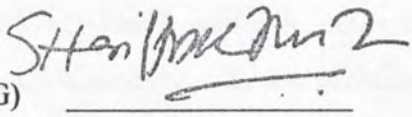
(ASSOCIATE PROFESSOR DR. SHARIFF A. K. OMANG) 

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ABSTRACT

Chemical profiling and characterization of bioactive metabolites of the rhizome *Curcuma xanthorrhiza* from Tawau was investigated. It involved bioassay (Antibacterial and Antioxidant) guided separation of bioactive metabolites the isolation, purification and characterization of the active metabolite. Its yellow essential oil was extracted *via* hydrodistillation and 30 constituents were identified with the aid of GCMS. The major constituent of the essential oil was Cycloisolongifolene (8, 9-dehydro-9-formyl) and it makes up 40.79% of the essential oil. Disc diffusion assay showed that essential oil had minimal antibacterial activity against *Clostridium cellobioparum*. Dark brown crude extract paste was extracted *via* Soxhlet extraction. A DPPH antioxidant test was carried out on the crude producing a high average scavenging activity of 92.03% and an average total antioxidant activity of 0.57 after 24 h. This implies the strong presence of antioxidant compounds within the crude extract. Isolated active compound was obtained as pale yellow paste (Compound A). Preliminary PTLC bioassay showed strong antibacterial properties with four out of five positive inhibitions. The bacteria that were positively inhibited are *Clostridium cellobioparum, Clostridium sordelli*, *Clostridium noryi* and *Vibrio alginolyticus.* Structure elucidation *via* ^{1}H-NMR, ^{13}C-NMR and FTIR showed that compound A consists of a benzene ring, 3 methyls and an exo-methylene and is most likely to be a phenolic compound with an alkyl branch.

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ABSTRAK

Penyelidikan ke atas profil kimia dan pencirian metabolit bioaktif rizom *Curcuma xanthorrhiza* dari Tawau telah dijalankan. Penyelidikan ini melibatkan pengasingan metabolit bioaktif melalui proses pemencilan, penulenan dan pencirian metabolit aktif dengan panduan bioassai (antibakteria dan antioksida) Minyak pati kuning telah diekstrak dengan mengunakan proses penyulingan hidro dan 30 komponen telah diidentifikasikan dengan pertolongan GCMS. Komponen terbesar minyak pati ini adalah Cycloisolongifolene (8, 9-dehydro-9-formyl) sebanyak 40.79% daripada minyak pati tersebut. Resapan cakera assai menunjukkan bahawa minyak pati ini mempunyai aktiviti antibakteria yang minima terhadap *Clostridium cellobioparum*. Pengekstrak Soxhlet telah digunakan untuk mengekstrak ekstrak kasar pekat yang berwarna coklat tua. DDPH antioksida telah diuji ke atas ekstrak kasar dan didapati bahawa ekstrak kasar mempunyai aktiviti "scavenging" purata yang tinggi iaitu sebanyak 92.03% dan purata aktiviti antioksida keseluruhan sebanyak 0.57 selepas 24 j. Ini mengimplikasikan bahawa terdapat kehadiran kuat komponen antioksida dalam ektrak kasar. Kompaun aktif yang telah diasingkan sebagai kompaun yang berwarna kuning pudar (Kompaun A). Penyaringan PTLC bioasay menunjukkan aktiviti antibakteria yang kuat dimana empat daripada lima bakteria menunjukkan aktiviti antibakteria yang positif Bakteria yang mengalami kesan antibakteria yang positif adalah *Clostridium cellobioparum, Clostridium sordelli*, *Clostridium noryi* and *Vibrio alginolyticus*. Elusidasi struktur melalui ^{1}H-NMR, ^{13}C-NMR dan FTIR menunjukkan bahawa kompaun A terdiri daripada rantai benzena, 3 metil dan exo-metilen dan dijangka adalah kompaun fenol dengan cabang alkil.

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LIST OF ABBREVIATION

TLC	Thin Layer Chromatography
FTIR	Fourier Transform Infrared Spectroscopy
PTLC	Preparative Thin Layer Chromatography
NMR	Nuclear Magnetic Resonance
$CDCl_3$	Deuterated Chloroform
GC	Gas Chromatography
MS	Mass Spectrometry
TMS	Timethyl silasine
UV	Ultra Violet
R_f	Mobility relative to front
°C	Degree Celcius
m	Meter
cm	Centimetre
µm	Micrometer
nm	Nanometer
mg	Miligram
g	Gram
L	Litre
mL	Mililitre
µL	Microlitre
h	Hour
min	Minutes

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CHAPTER 1

INTRODUCTION

1.1 Overview

The terrestrial ecosystem takes up about 30% of the earth. It was found that terrestrial ecosystem has species richness higher than that compared to the marine ecosystem due to the wider diversification of habitat and climate. Terrestrial ecosystem had undergone adaptation and speciation. Besides this, terrestrial plants have formed a symbiotic coevolution with insects thus increasing the biodiversity. Therefore, on the species level there is more natural product diversity on terrestrial land compared to the ocean. Bioactive compounds have gained much notice among organic chemists and ecological biochemists (Pietra, 2002)

In this 21st Century, there is a global demand on medicinal plants from the terrestrial ecosystem from four main users which are the housewives involved in home

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care, pharmaceutical industries, traditional health practitioners and traditional health system. These medicinal plants are also very important to those from developing or third world countries as most of them rely on raw materials from plants for their healthcare. These people are unable to pay for the medicine from the pharmaceutical companies. However, there has to be guidelines on sustainable usage of the plants so that it will not easily face extinction due to over exploitation (Lambert, 1997). One example of a medicinal family of plants that is used by people is the family Zingiberaceae.

1.2 The Ginger Family Zingiberaceae

1.2.1 Description and Characteristics

Zingiberaceae is also called the ginger family and the name Zingiber probably came from the combination of the Arabic word *zanjabil* and the sanskript word *singabera.* These two words were combined to be the classical Greek word called *zingiberi* and lastly developed into the Latin word *zingiber* (Larsen *et al.*, 1999).

The research and studies on the Zingiberaceae family had been done for quite some time by many scientists and herbal medicine practitioners in the areas where it was found. Among the scientist and researchers of this century who have contributed to this research and documentation of the ginger family are like Ridley, Holttum, Y. K. Kam, B. C. Stone, S. N. Lim, H. Ibrahim, K. Larsen and Burkill. Zingiberaceae are part of the order Zingiberales. The Zingiberaceae family is one of the largest family in the order. The

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Zingiberales order is divided into two parts. The first part is families with five stamens and families with one stamen. Zingiberaceae belongs to the family of one stamen. Zingiberaceae is part of the more advanced group where non-functional stamens have been developed as petaloids staminodes (Larsen *et al.*, 1999).

There are roughly 1200 species within the family of Zingiberaceae. About 1000 species occur in tropical Asia including the Malesian region. This Malesian region covers countries that include Malaysia, Indonesia, Brunei, Singapore, the Philippines and Papua New Guinea. The Malesian region alone has 24 genera and 600 species. Many parts within these countries like the island of Borneo and Sumatera are still unexplored yet for the ginger family. Zingiberaceae comes from the order of Zingiberales. The order Zingiberales is actually an isolated group within the monocotyledons. Zingiberaceae family is a family which has only one stamen and it's one of the largest family in the order Zingiberales. This family is primarily found in the tropical Asian region (Larsen *et al.*, 1999).

The Zingiberaceae family can be identified by their typical herbaceous habit. Their leaves have parallel veins from a midrib and each flower has a single fertile stamen (Whistler, 2000). Gingers possess sympodial growth where a new shoot is produced from the rhizome each season. There are also alternate leaves in opposite ranks and paddle shaped. Normally, the petioles are also very long. There is a ligule on either side of the base of the petiole clasps the stalk. The three sepals and three petals are fused and small (Llamas, 2003).

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1.2.2 Usage of Zingiberaceae

Zingiberaceae has many usages and has been used since the time of the ancient Greeks. The genera of *Alpinia, Amomum, Curcuma, Zingiber*, *Boesenbergia, Elettaria, Elettariopsis,* and *Etlingera* are the main gingers that are used. There are 20 or more ginger species that have been grown for their usages as spices, condiments, flavours, fresh vegetables, medicine, ornamentals and cut flowers. Species like *Elettaria cardamomum,* and *Zingiber officinale* are used as spices. Meanwhile the aromatic flowers that are used are like *Hedychium coronarium* and *H. flavescens* which are used for perfume purposes. Several other species within this family are used as ornamentals for their beautiful flowers (Whistler, 2000).

There are three species within the Zingiberaceae family that has important commercial value. This three are the *Zingiber officinale* Rose, *Curcuma domestica* and *Elletaria cardomomum* (Larsen *et al.*, 1999). *Z. officinale* is one of the oldest herb spices from the Zingiberaceae family and was a very profitable trade during the past between the East and the Western world. This ginger is still very commonly used by people as an ingredient in food, bakeries, confectionaries, beverages and traditional medicine. The old rhizomes of this plant are used as flavouring while the young ginger is eaten raw or pickled. It's also used in the production of ginger beer and gingerbread or biscuit. *Elettaria cardamomum* is indigenous to southern India and Sri Lanka. The fruits and seeds of this ginger plant is taken and dried before cardamom is obtained. It grows in mountainous area. The other commercially valuable species is the *Curcuma domestica* or

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also known as turmeric. This turmeric is used domestically in many households as a spice used in curries. It is also a food flavoring and in ancient times it's also utilized as a dye. The broad leaves of *Curcuma domestica* are sold in the markets where it's cultivated and used as a fish wrapper before steaming or baking. Normally, it's imported together with *Elettaria cardamomum* (Larsen *et al.*, 1999).

1.2.3 Bioactive Metabolites

Most of those within the Zingiberaceae family will be aromatic in all or most parts or at least one parts of the plant. Most of the members are also rich in terpenoid and flavonoids. The documentation of alcohols and phenolics is not done well. Alkaloids also have been found in the Zingiberaceae family (Larsen *et al.*, 1999). There is presence of saponins in several of the genera within the Zingiberaceae family which among them are *Curcuma, Globba , Hedychium, Zingiber* and *Alpinia* (Merh *et al.*,1986).

Experienced in the field and knowledgeable people that studies the Zingiberaceae family can identify the plants just by smelling the crushed leaves or the rhizomes in the non flowering specimens. Gingers are rich in essential oils that are full of many types of chemical compounds. For example, three species which are *Zingiber spectabile, Z. officinale* and *Alpinia galangal* shows a total of 34 compounds with 18 compounds, 20 compounds and 31 compounds each respectively. The common compounds that were recorded are α-pinene, β-pinene, limonene, and β-elemene. The similar compounds from

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this three species are like P-cymene, camphor, 1,8–cineol, citral-a, linalool, β-caryophyllene, β-bisabolene, and α-humulene (Larsen *et al.*, 1999).

1.3 *Curcuma* Genus.

Curcuma comes from the family of Zingiberaceae. There are many types of *Curcuma* including the *Curcuma xanthorrhiza, Curcuma domestica, Curcuma aeruginosa,* and *Curcuma auriantica* (Mat-salleh & Latiff, 2002). *Curcuma* has been known as a spice and has many healing properties. The name of the *Curcuma* genus originates from the Arabic word called *Kurkum* (Larsen *et al.*, 1999).

One of the oldest recorded usages of *Curcuma* is in China where Marco Polo first discovered it in China. It has been used as a medicine over the years. In 1783, turmeric was introduced to Jamaica. In Jamaica, turmeric is grown as a crop (Tainter & Grenis, 2001). Turmeric's orange – yellow root powder which turned brown after being exposed to alkaline chemicals was discovered by chemists in the 1870s. Then turmeric paper was invented where thin strips of tissue was soaked in turmeric and dried. Laboratories around the world used this turmeric paper as a test for alkalinity during the late 19th century. Nowadays, the function of turmeric paper has been replaced by litmus paper (Castleman, 2001)

Curcuma genus has several characteristics that identifies itself. There normally are flowers or inflorescence that forms between the leaves or on a separate shoot with a short

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scape. The flower has broad bracts attached to each other to form closed pouches where small partial inflorescences develop. Green or coloured bracts and the inflorescence is generally crowned with a rosette of coloured sterile coma. Normally the flowers have a different colour compared to the bracts. There is also an overlap of the upper corolla lobe and staminodes where a hooded structure is formed. The stamen of the *Curcuma* is found under this hood and is short with an anther that has two curved spurs at its base and a short crest at its apex. The *Curcuma* genus is a large genus (Larsen *et al.*, 1999).

1.4 Research Objectives

The objective of this research is to investigate the inherently available secondary metabolites in terms of their:

i. Chemical Diversity, and

ii. Biological Activities (Antibacterial and Antioxidant)

Chemical Diversity investigation will involve the extraction and identification of essential oil and the extraction of crude extract and their TLC fingerprinting. Meanwhile, biological activities investigation will involve the Bioassay Guided Isolation of bioactive metabolites (Antibacteria and antioxidant) and the isolation, purification and characterization of the active metabolites.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Curcuma xanthorrhiza Roxb. is a tumeric plant which has rhizomes that are dark purple in colour at the inner side. *Curcuma xanthorrhiza* has many local names including *Temu Lawak* and *Temu Raya.* The leaves are dark green in colour with purple in the centre that is spiky, lateral and hairy and the base is striped green. The flower of the *Curcuma xanthorrhiza* is white creamish in colour (Mat-Salleh & Latiff, 2002). This *Curcuma xanthorrhiza* can reach up to 2 m in height.

The inflorescence is formed separately from the leafy shoot. The flowering spike with the stalk is very colourful and is about 22 cm - 45 cm tall. The foliage leaves are at the end of the inflorescence. The base of the spike has pale green bracts while the coma is purple in colour. These bracts and coma arranges itself as pouches where one to six yellow flowers will grow. The characteristics of the flowers includes having yellow lips

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with a dark yellow median stripe, surrounded by three pink petals and at the throat of the flower is a single stamen with two anther spurs (Larsen *et al.*, 1999).

This herb is believed to come from South East Asia and is known as a medicinal tonic for women that have just given birth. The rhizome when pounded can be applied on acne as a medicine (Mat-Salleh & Latiff, 2002).

Isolated compounds belong to many groups. One of them is sesquiterpenoids which is the largest group that naturally occurs in terpenoids of the world. Sesquiterpenoids ($C_{15}H_{24}$) have a range of smell from strong fragrance to odorless. The other groups include phenolics and monoterpenes. These three groups when combined together is an effective healing product where it cures a disease at the DNA level. Each group has a role in bringing this effective healing out. Phenolics function as a cleanser for the receptor sites. Monoterpenes act as a restoration and activator for the right cellular information. Meanwhile, sesquiterpenes deletes wrong information in the DNA memory and keeps potential unruly phenols or ketones under control and focused on its task until completion (Stewart, 2005). *Curcuma xanthorrhiza* isolated compounds consist of a mixture of groups that includes all three of these groups especially sesquiterpenoids.

2.2 Biological activity of *Curcuma xanthorrhiza*

Curcuma xanthorrhiza is known as a herbal medicine that can treat various sorts of illness and also as a preventive measure for other illnesses. Traditionally it has been used to cure

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