PHYTOCHEMICAL AND BIOACTIVITY EVALUATION OF *Etlingera coccinea* (Blume) S. Sakai & Nagam COLLECTED FROM SIX DIFFERENT LOCATIONS IN SABAH

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THESIS SUBMITTED IN FULFILLMENT FOR THE DEGREE OF MASTER

INSTITUTE FOR TROPICAL AND BIOLOGY CONSERVATION
UNIVERSITI MALAYSIA SABAH
2011

UNIVERSITI MALAYSIA SABAH

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DEGREE : **MASTER OF SCIENCE (BIODIVERSITY ADVANCEMENT)**

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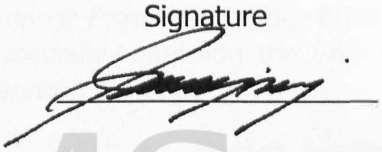

ACKNOWLEDGEMENT

First and foremost, thanks to God Almighty for the guidance and helping in giving me strength to complete this thesis.

I would like to thank my supervisor, Assoc. Prof. Dr. Charles S. Vairappan for his guidance and advice throughout this thesis study.

I would also like to take this opportunity to express my utmost gratitude to Assoc. Prof. Dr. Abdul Hamid Ahmad for the opportunity to do my masters degree in his department.

Appreciation is also to all the lecturers and staff of Institute for Tropical Biology and Conservation (ITBC), for their help and kindness in supporting this study, especially to Pn. Norazizun Rusdi for her valuable guidance in the antioxidant and phenolic data analysis, and not forgetting Assoc. Prof. Dr. Henry Bernard for the valuable lesson in statistical analysis.

I would like to express my sincere appreciation to Madam Selekoh Gudou of Kg. Kokob Baru, Ranau and Mr. Robert Francis (ITBC lecturer) for assisting me in sample collection. A word of thanks also goes to Mr. Viva Chan of Fisher Scientific, Kuala Lumpur for his support in my research experiments especially regarding the GCMS analysis. Not forgetting to NAGAO International for supporting my study.

Finally, I am forever indebted to my beloved husband, Salikin Hj. Durasit for his continuous support, encouragement and sacrifices.

Jacqueline Vincent
26 July 2011

ABSTRACT

PHYTOCHEMICAL AND BIOACTIVITY EVALUATION OF *Etlingera coccinea* (Blume) S. Sakai & Nagam COLLECTED FROM SIX DIFFERENT LOCATIONS IN SABAH

Etlingera coccinea (Blume) S. Sakai and Nagam is a member of *Zingiberaceae* family and it is found growing in many places throughout Southeast Asia. Although it has a pungent odour, it is regarded as a delicacy by the Kadasan Dusun people in Sabah, Malaysia. The inner stem and its florescence are eaten and the whole plant is regarded to have importance in traditional medicine. Due to its importance to the local community, this research was conducted to study its chemical conposition and biological potentials. Plant materials were collected from six different locations in Sabah; Ranau (EC1), Sandakan (EC2), Danum (EC3), Ulu Kimanis (EC4), Paitan (EC5) and Long Pasia (EC6). Yield of essential oil and crude extract of all samples were in the following range: 22.33% ~ 30.50%, of its fresh weight; 7.29% ~ 14.61%, of its dry weight. Both extracts were tested for antimicrobial, antioxidant activity and total phenolic content. It was evident that extracts with antimicrobial potential had no antioxidant activity, while extracts with high antioxidant and phenolic content exhibited weak antimicrobial activity. The essential oils of all specimens showed inhibition (10-67 %) against tested microbes except against *Bacillus* sp., *Salmonella typhi*, *Salmonella typhymurium* and *Listeria monocytogenes*. On the other hand, crude ethanol extracts only exhibited moderate and weak activity against *Clostridium cellobioparum*, *Pseudomonas aurelis*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aereus*, *Vibrio cholera*, and *Crytococcus neoformans*. The ethanol extract from EC1 showed high phenolic, free radical scavenging activity, ferric reducing antioxidant power and inhibition of linoleic acid peroxidation as compared to other extracts. Phenolic content and free radical scavenging activity were strongly correlated (r=0.863) and it might be the major contributor to the antioxidant activity of this species. This is the first report of the volatile chemical constituents of *E. coccinea* essential oil. In this investigation, inner stems of *E. coccinea* of six different populations were distilled and the essential oils were subjected to GC-MS analysis. A total of 21 types of volatile compounds were identified in the course of this investigation. The major volatile compounds in five specimens (EC1, EC2, EC4, EC5 and EC6) of *E. coccinea* essential oils were linalool and citronellyl propionate. As for specimen EC3, its major compounds were dodecanal and linalool. The crude ethanol extract of *E. coccinea* had been profiled by using thin layer chromatograph. It was found that TLC fingerprinting of all the compounds appear at the same R_f value and can be assumed that all the crude ethanol extracts of *E. coccinea* collected from six different populations possessed compounds of similar retention factor. However, further analysis is needed to find out the bioactive compounds responsible for the bioactive potential in this particular species.

ABSTRAK

Etlingera coccinea (Blume) S. Sakai & Nagam ialah ahli dalam keluarga Zingiberaceae dan ia didapati tumbuh di kebanyakkan tempat di Asia Tenggara. Walaupun ia mempunyai bau yang tajam, ia merupakan makanan bagi masyarakan Kadasan Dusun di Sabah. Bahagian dalam batang dan florescence dimakan dan keseluruhan bahagian tumbuhan dianggap mempunyai kepentingan dalam perubatan tradisional. Ekoran kepentingannya kepada masyarakat tempatan, kami telah mengkaji kandungan kimia semulajadi yang terkandung dan potensi kandungan bioaktifnya. Sampel tumbuhan dikumpul daripada enam lokasi yang berbeza di Sabah; Ranau (EC1), Sandakan (EC2), Danum (EC3), Ulu Kimanis (EC4), Paitan (EC5) and Long Pasia (EC6). Hasil minyak pati dan ekstrak mentah bagi semua sampel,masing-masing adalah seperti dalam siri berikut: 22.33% ~ 30.50%, daripada berat basah; 7.29% ~ 14.61%, daripada berat kering. Kedua-dua ekstrak telah dikaji berdasarkan aktiviti antimikrob, antioksida, dan kandungan sebatian fenolik. Dapat dilihat bahawa ekstrak dengan potensi antimikrob tidak mempunyai aktiviti antioksida dan kandungan sebatian fenolik dan begitu juga sebaliknya. Minyak pati bagi spesis ini didapati menunjukkan perencatan petumbuhan mikroorganisma (10-67 %) kecuali terhadap Bacillus sp., Salmonella typhi, Salmonella typhymurium and Listeria monocytogenes. Manakala, ekstrak etanol pula hanya merencat beberapa bakteria seperti Clostridium cellobioparum, Pseudomonas aurelis, Proteus mirabilis, Escherichia coli, Salmonella enteritidis, Staphylococcus aereus, Vibrio cholera, dan Crytococcus neoformans.. Ekstrak etanol bagi EC1 didapati menunjukkan kandungan fenolik, aktiviti bebas radikal, kekuatan antioksida penurunan ferik dan perencatan peroksidaan asid linolik yang tertinggi berbanding dengan ekstrak etanol yang lain. Kandungan fenolik dan aktiviti bebas radikal berkorelasi tinggi (r=0.863) dan ia mungkin penyumbang terbesar kepada aktiviti antioksidan bagi spesis ini. Komponen mudah meruap bagi E. coccinea dikaji buat pertama kalinya. Bahagian dalam batang bagi E. coccinea daripada enam populasi yang berbeza disuling and minyak pati yang terhasil dianalisis menggunakan GC-MS. Sejumlah 21 jenis sebatian meruap telah dikenalpasti dalam kajian ini. Komponen utama minyak pati dalam lima spesimen (EC1, EC2, EC4, EC5 and EC6) E. coccinea merupakan linalool dan citronellyl propionate. Manakala, bagi spesimen EC3 pula komponen utamanya adalah dodecanal dan linalool. Ekstrak etanol bagi E. coccinea telah diprofil menggunakan kromatografi lapisan nipis. Didapati bahawa kesemua sebatian kelihatan berada pada nilai Rf yang sama dan ini dapat dianggapkan bahawa kesemua sebatian bagi ekstrak E. coccinea yang dikumpul dari enam populasi yang berbeza mempunyai faktor penahanan yang sama. Walaubagaimanapun, analisis yang lebih terperinci perlu dilakukan bagi mengenalpasti sebatian-sebatian bioaktif yang mempunyai potensi bioaktif bagi spesis ini.

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LIST OF ABREVIATION

TLC	Thin Layer Chromatography
DPPH	Diphenylpicryl-hydrazil
FRAP	Ferric Reducing Antioxidant Power
FTC	Ferric Thiocyanate
TPC	Total Phenolic Compound
AADisc	Antibiotic Assay Disc
AEAC	Ascorbic acid equivalent antioxidant capacity
HPLC	High Performance Liquid Chromatography
R_f	Retention factor
GC-MS	Gas Chromatography - Mass Spectrometer
UV	Ultra Violet
RT	Retention time
RI	Retention index
EC	*Etlingera coccinea*
mm	mili meter
mL	mili litre
mg	mili gram
g	gram
µg	micro gram
AA	Ascorbic acid
%	Percentage
BHT	Butylated hydroxyltoulene
AU	Absorbance unit
MeOH	Methanol
GAE	Gallic acid equivalent
HE	Hexane : Ethyl acetate
CM	Chloroform : Methanol

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Since time memorial, plants have long been a source of therapeutic agents used by man (Fowler, 2006). Furthermore, it is a tremendous source for the discovery of new products of medicinal values for drug development (Vanisree *et al.*, 2004). In recent years, there has been a worldwide trend towards the use of the natural phytochemicals present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Deiana *et al.*, 1999; Lee and Shibamoto, 2000; Velioglu *et al.*, 1998; Wang and Jiao, 2000). It can be seen that plant secondary metabolites have been used for centuries in many cases and only more recently discovered in other instance. The interest in plant secondary metabolites has risen dramatically in recent years due to their diverse effects; include antioxidant, anti-viral, antibacterial, and anticancer effects (Makkar *et al.*, 2007).

Zingiberaceae species are among the most prolific plants in the tropical rainforest (Ruslay *et al.*, 2007). Previously, many studies had been conducted on some members of the *Zingiberaceae* such as ginger (*Zingiber officinale* Roscoe) (Alfaro *et al.*, 2003; Zaeoung *et al.*, 2005; Chan *et al.,* 2007), turmeric (*Curcuma longa* Linn.) (Jayaprakasha *et al.*, 2002; Zaeoung *et al.*, 2005; Chan *et al.*, 2007), *Boesenbergia* (*Bosenbergia pandurata* (Roxb.) Schltr) (Zaeoung *et al.*, 2005) and *Alpinia* (*Alpinia galangal* (Linn) Stunz) (Zaeoung *et al.*, 2005; Chan *et al.*, 2007; Mayachiew and Devahastin, 2007). Plants of *Zingiberaceae* are well known for its biological activities like antitumor, antioxidant, antimicrobial, and anti-inflammatory (Negi *et al.*, 1999; Habsah *et al.*, 2005; Chandrana *et al.*, 2005; Habsah *et al.*, 2005; Jirawan *et al.*, 2006; Natta *et al.*, 2008).

In Sabah, a large number of ginger species are cultivated and used as source of food, medicine and ornamentals by the indigenous people (Halijah, 1990). Gingers such as *Etlingera elatior* (Jack) R. M. Smith, *Etlingera coccinea* (Blume) S. Sakai and Nagam, *Zingiber officilnale* (Roscoe), *Achasma megalocheilos* (Griff) and *Hornstedtia*

sp. (Noweg *et al.*, 2003) are consumed as food ingredients, applied in traditional medicine, and for many other difference purposes. For instance, the whole plant of *Curcuma* sp. is used as poison antidotes, while the rhizome of *Zingiber* sp. is used to treat runny nose (Kulip *et al.*, 2005). It was also mentioned by Larsen (1998) that *Z. offinale* is the best-known ginger species used in traditional medicine, where it is effective in treating vertigo and motion sickness and was found to contain at least 10 antiviral compounds (Swerdlow, 2000).

Plants of *Etlingera* have various traditional and commercial uses and have been applied widely by the indigenous people in Sabah as condiment, pickle, and as traditional medicinal. For instance, the heart of young shoots, flower buds, and fruits of *E. elatior*, *Etlingera rubrolutea* (Baker) C.K.Lim, and *Etlingera littoralis* (J. Koenig ex Retz.) Giseke, are consumed by the indigenous communities as condiment, eaten raw or cooked (Noweg *et al.*, 2003). The species under this genus are known to have importance use in traditional medicine. *E. coccinea*, a well-known ginger species in Sabah, has been extensively used by the indigenous people as traditional herbal medicines to remediate various illnesses such as dizziness, and haemorrhoids (Barnabas, 1997).

1.2 Problem Statement

In accordance to these numerous claims reported on gingers pertaining to their benefits and uses, there is a continuous increase in the study of phytochemical in gingers. Although many species have been investigated, there are still a wide variety of gingers that are still not studied. The present investigation was initiated based on local traditional knowledge in Sabah. *E. coccinea* or commonly known as "tuhau", has been widely used in various traditional remedies. Hence, with the increase in diseases and resistance microbes, it is an exciting area of research to delve upon.

This study was conducted to investigate the bioactivity, as well as the chemical constituents of essential oil of *E. coccinea*. Though this species is surrounded with various claims, there are no scientific evidences to support these claims. The underlying mechanisms involved in its physiological effects are lacking

and scientific approval is required. Thru this research it will prove whether this plant possesses any bioactivity as claimed by the indigenous people of Sabah.

1.3 Significance of the Study

The results of this study will contribute towards the growing database of knowledge on ginger and help to advocate the safe and effective use in traditional medicine. It is believed that the screening of this plant will also add to the ever increasing scientific database of medicinal plants, not only in Sabah or Malaysia but also globally. The uses of plants for treatment in rural area are largely due to the inability of the common people to access modern medicine or the use of registered pharmaceutical products.

Furthermore, this study will contribute to knowledge in pharmaceutical, cosmetic, the food industry, traditional medicine practices and the indigenous people of Sabah. This study will give brief information on the bioactive potential, i.e. antimicrobial activity, antioxidant activity and total phenolic content, and also the chemical constituents of the essential oil extract of six different populations. This data will validate the use of *E. coccinea* extracts as natural antimicrobial and antioxidant agents and qualify tuhau extracts as potent natural product source.

1.4 Objectives

Based on the above mentioned rationale and need, the investigation to determine the chemistry and biological potential of *E. coccinea* was carried out. The primary objectives in this study could be stipulated as follows:

1. To extract and determine the quantity of essential oil and crude extracts of the *Etlingera coccinea* collected from six population in Sabah
2. To determine the chemical composition of its essential oil using Gas Chromatography-Mass Spectrometer (GC-MS) and to profile the chemical composition of its crude extract by using Thin Layer Chromatograpy (TLC)
3. To determine the bioactive potentials of essential oil and its crude extracts including;
    a. Antimicrobial activity
    b. Antioxidant activity, and
    c. Total Phenolic Content

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to *Zingiberaceae*

Zingiberaceae is one of the biggest kingdoms in plant kingdom (Sirirugsa, 1999) and predominantly distributed throughout the tropics particularly in Southeast Asia (Habsah, 2000). The ginger family (*Zingiberaceae*) consists of more than 1200 species; about 1000 species occur in tropical Asia and Borneo alone harbours more than 200 species (Poulsen, 2006; Larsen *et al.*, 1999). Plants in the *Zingiberaceae* family can be classified into distinct genera that are represented by several species. The genera also show the affinity as groups recognised as tribe. *Zingiberaceae* can be divided into 4 tribes; *Hedychieae*, *Zingibereae*, *Alpinieae* and *Globbeae*. Each species under each tribe is easily distinguished by its physical appearance.

Zingiberaceae, are rhizomatous herbs with secretory cells that produce aromatic oils (Gobilik and Mashitah, 2005) and their stems usually grow horizontally along or underground, produce roots and leaves that are arranged distichously, and the basal part may form a stem-like structure (Poulsen, 2006). However, according to Poulsen (2006), though gingers are herbaceous plants, some species can reach up to 8 m tall. Furthermore, the bases of this shoots becomes very stout and do not appear very herb-like. On the other hand, they are not woody in an anatomical sense.

Gingers are generally abundant in lowland to hill forests, notably between 200 m and 500 m above sea level, and it is less profused in higher altitudes and rather scarce on very high mountain (Larsen *et al.*, 1999). *Zingiberaceae* species grow naturally in damp, shaded parts of the low-land or on hill slopes, as scattered plants or thickets. Most members of the family are easily recognized by the characteristic aromatic leaves and flesh rhizome when both of them are crushed and also by the elliptic to elliptic-oblong leaves arranged in two ranks on the leaf-shoot (Habsah, 2000).

In Sabah, gingers are distributed all over the state. It is reported to be present in large amount in Crocker Range, where about half of the gingers in Sabah are found with 12 genera and 45 species were recorded in previous study by Takano *et al.* (2004). The existence of gingers are also reported at several other places such as Tawau Hill (Halijah, 1999), and Trus Madi Range (Gobilik and Mashitah, 2005).

2.2 Genus *Etlingera*

Etlingera is a genus form *Alpineae* tribe, and it is a genus that can produce colourful inflorescence, flowers and fruits (Larsen *et al.,* 1999). All species under this genus have the inflorescence separated from the 2-8 m tall leafy shoot; however, the length of the peduncle and the lip is widely varied. It is an Indo-Pacific genus of more than 100 species growing from sea level to 2,500 m altitude (Poulsen, 2006).

This genus is distributed from India to the Pacific Islands with centres of species richness assumed in Borneo with 40 species of *Etlingera* had been recognized in the present account, where 80% are found in Sarawak, 70% in Sabah and 65% in Kalimantan (Poulsen, 2006). In Sabah, itself, it can be found in Papar district, Kimanis to Keningau, Penampang district, Crocker Range, and between Donggongon and Tambunan (Takano *et al.*, 2002). According to Gobilik and Mashitah (2005), the genus *Etlingera* had the highest number of species (five) at Trus Madi Range compared to other genus. Some species of *Etlingera* for instance, rapidly inhabit disturbed secondary forests or newly opened areas and subsequently spread like weeds. Some of these species however are useful indicators of disturbed area (Larsen *et al.*, 1999). Figure 2.1 depicted the distribution of *Etlingera* species all over Sabah, Malaysia.

The species under this genus are commonly used by the people all over Borneo for many different purposes. The most common use is as food by extracting the inner sheaths of the leafy shoot and consume them raw (sometimes mixed with chilli), cooked as a vegetable or as a condiment (similar to the use of onion) (Poulsen, 2006). Besides that, several species under this genus have been used as medicine to treat various illnesses. For instance, the decoction of the fruits of *E. elatior* has been used to treat earache and the leaves are used to clean wounds