PHYTOCHEMICAL STUDY SELECTED *CINNAMOMUM* SPP. (LAURACEAE) IN SABAH, MALAYSIA

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INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH
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IJAZAH: **MASTER OF SCIENCE (BIODIVERSITY AND BIOSYSTEMATICS)**

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Tarikh: 7 September 2018

DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, excerpts, equations, summarise and references, which have been duly acknowledged.

19 July 2018

Siti Joanni Binti Matlan
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CERTIFICATION

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2. CO- SUPERVISOR

Mr. Julius Kulip _____

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ABSTRACT

Cinnamomum Schaeffer is an aromatic, and woody plant species. This genus was famous because of "Cinnamon", with distinguished economic significance as herbs and medicinal plant. There are few studies and limited information on *Cinnamomum* in Sabah, especially in phytochemical and taxonomical study, which indicate only little we know about this genus. Thus, the main justification for this study is to increase our understanding on this plant group. As well to updates and support a continuous efforts on phytochemical and taxonomical documentation of *Cinnamomum* spp. With objectives, To extract crudes and interpret phytochemical profiles. To extract essential oil and identify the volatile chemical constituents of selected *Cinnamomum* in Sabah. and to study and provide "Species Identification Key" for *Cinnamomum* spp. (Lauraceae) in Sabah. The methods used in this study comprise, chemotaxonomy assessment, Lab work, HPTLC, H-NMR and GC-MS. Not only that literature reviews and herbarium studies, fields sampling, taxonomical appraisal. As Result, *Cinnamomum* profiles for sample S.1, S.2, S.3, and S.4 perceived significant consistency and similarities in chromatographic bands of HPTLC results visualized under UV 366 nm, White RT and Molybdo derivatives and equivalent spectrum peaks developed between 7.0δ-8.0δ dan 3.0δ-5.0δ, which possibly represents aromatic, amides, esters, phenol and alkanes compounds based on Proton NMR Chemical Shift comparison for four *C. burmanni* samples collected from Sabah. The essential oil analysis via GC-MS have revealed 16 compounds above 3% for *C. iners* , with a major compounds alpha-Cadinene with 20.52%, follow by 10- epi-cis-Dracunculifoliol 15.98%, Sesquisabinene at 14.90%, Lavandulol 11.20%, whereas 16 compounds above 3% also for *C. burmanni* samples, and major identified compounds were Cinnamyl formate with 91.48%, follow by Hex-1-en-3-yl acetate 41.95%, and Selina-4(15),7(11)-diene for 10.70 %, Myrcenol 9.85% Nopol 9.33%. Not only that, 20 species of *Cinnamomum* were recognized in Sabah includes three possible new recorded species. Apart from that map distribution and identification key were provided for the use and future reference of *Cinnamomum* species in Sabah.

ABSTRAK

KAJIAN PHYTOKIMIA BAGI SPESIES TERPILIH CINNAMOMUM SPP. (LAURACEAE) DI SABAH, MALAYSIA

Cinnamomum Schaeffer ialah genus tumbuhan herba, berkayu dan aromatik. Ia terkenal dengan nama "Kayu Manis" dan terbukti mempunyai kepentingan nilai ekonomi juga terkenal sebagai salah satu genus yang bermasalah. Kajian mengenai Cinnamomum tidak banyak di Sabah, Malaysia dan informasi berkaitan spesies ini adalah terbatas, lebih-lebih lagi dalam kajian fitokimia dan taksonomi, yang mana ini menunjukkan pengetahuan kita mengenai tumbuhan ini sangat sedikit. Oleh itu justifikasi utama kajian ini adalah untuk memperolehi dan meningkatkan pengetahuan kita terhadap tumbuhan ini, dan juga untuk mengemas kini dan meneruskan usaha dalam mendokumentasi taksonomi dan fitokimia Medang Tiga Urat di Sabah. Dengan objektif utama, untuk mengekstrak pati daun dan mentafsir hasil profil kimia untuk dan mengekstrak pati minyak dan mengenalpasti sebatian kimia terdapat pada daun spesies yang Cinnamomum yang dipilih di Sabah, dan juga untuk mengenali tumbuhan ini dan membina "Kekunci pengecaman spesies" untuk Cinnamomum di Sabah. Metodologi kajian ini merangkumi penilaian kemotaxonom, kerja makmal, analisa HPTLC, H-NMR dan GC-MS, kajian ulasan kesusastera, kajian spesimen-spesimen di herbarium, kerja lapangan, serta penilaian sistematik morfologi. Hasil kajian, profil kimia HPTLC menunjukkan kesaksamaan pada band kromatografi dilihat pada UV 366 nm, RT Putih dan derivatif Molybdo. Sama seperti corak spektrum dilihat pada 7.0δ-8.0δ, 3.0δ-5.0δ yang mungkin mewakili aromatic sebatian kimia, amide, ester dan alkane, seperti dalam rujukan "Proton NMR Chemical Shift" bagi spesies C. burmanni. Manakala hasil bagi analisa pati minyak mendapati 16 sebatian kimia diatas 3 peratus dikenalpasti untuk C.iners. Alpha-Cadinene 20.52%, 10- epi-cis-Dracunculifoliol 15.98%, Sesquisabinene 14.90%, dan Lavandulol 11.20% adalah sebatian kimia utama. 16 sebatian kimia diatas 3 peratus juga dikenalpasti untuk C.burmanni, dengan komposisi utama Cinnamyl formate with 91.48%, Hex-1-en-3-yl acetate 41.95%, Selina-4(15),7(11)-diene 10.70 %, Myrcenol 9.85% dan Nopol 9.33%. Bukan itu saja 20 spesies Cinnamomum direkodkan dalam kajian ini, termasuk kebarangkalian tiga spesies yang baru di rekodkan untuk Sabah. Peta distribusi dan kekunci pengenalan spesies juga disediakan untuk kegunaan dan rujukan Cinnamomum di Sabah pada masa akan datang.

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LIST OF SYMBOLS AND ABBREVIATIONS

CBD	Convention of Biological Diversity
cm	Centimetre
diam	Diameter
EO	Essential Oil
GC-MS	Gas Chromatograph- Mass Spectrum
HPTLC	High Performance Thin Layer Chromatography
m	Meter
mm	Millimetre
NMR	Nuclear Magnetic Resonances
OTS's	Operational Taxonomic Units
ppm	Part per million
RI	Retention Index
RT	Retention Time
sp.	species (one)
spp.	species (many)
TLC	Thin Layer Chromatography
UNEP	United Nation Environment Program

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CHAPTER 1

INTRODUCTION

Plants are very important to human survival since ancient time. They provide important uncountable natural services, nutrition, and medicine as well shelters for human and all living organisms. Nowadays, botany, ethnobotany and chemotaxonomy are getting more popular when many scientific investigations were conducted in order to satisfy the curiosity and the desire to understand the plants in the surroundings, as well to meet an increase demand in biomedicine and pharmaceutical industries (Kulip, 2014; Vairappan *et al.*, 2012). Interaction between human and plants will be forever mandate, together with development and advancement of technology.

1.1 The Scope of Phytochemistry and Chemotaxonomy

Phytochemistry, plant biochemistry and organic chemistry are all synonyms and can be define as the study of chemicals in plants. It is concerned with the massive variety natural substances and its biological function that are accumulated in plants. These technique have been established and widely inrole practically to all branches of plant sciences, namely plant physiology, pathology, plant systematics and palaeobotany. The employment of phytochemistry in Plant systematics is one of most rapid in the present time, it is basically the hybrid discipline of chemical and taxonomy which is currently known as chemotaxonomy or biomedical systematic (Harborne, 1980).

Research has revealed the potential of some species *Cinnamomum* essential oils as natural bio-control agents, it was stated that the oils of *C. camphora, C. glaucescens* and *C. tamala* displayed toxicity towards *Aspergillus niger*, were insecticidal against fire ants and fruit flies (Satyal, Mallik, Gautam, Vogler, & Setzer, 2013). medicinal values and chemicals compound found in some group of plants (Stace, 1989). To date, wild plants are still actively studied as new sources of drugs especially in the diverse and understudy tropical forest (Vairappan *et al.*, 2014). The genus *Cinnamomum* preserve many uncover potentials in phytochemistry area for us to revealed.

Besides biomedical and natural by product, phytochemistry has been employed as part of biosystematics study, it is believed that some plant own their signature profile patterns and chemical composition which possibly can be used as taxonomic characters in identification (Hegnauer, 1986; R. Singh, 2016; Vairappan, Nagappan, & Kulip, 2014). Study reported that four chemical classes (phenylpropanoids, phenylethanol derivatives, flavonoids and phenolic acids) were determined to evaluate and distinguish the chemotaxonomy of *Rhodiola* samples, which could be use effectively for species identification in *Rhodiola* (Liu *et al.*, 2013). Thus, phytochemical for selected *Cinnamomum* species in Sabah was investigated for chemotaxonomy outlook.

1.2 *Cinnamomum* Schaeffer (Lauraceae)

Recent revision of *Cinnamomum* in Borneo (Wuu-Kuang, 2011), reported twenty six species of *Cinnamomum* recognised in Borneo which generally covered area Kalimantan, Brunei, Sabah and Sarawak. Seventeen species were recognised as endemic to Borneo. The *Cinnamomum* was said to be an Asiatic genus with two sections, first section is Camphora with leaves characteristic mostly penninerved and alternate arrangement and the other section is '*Cinnamomum*' or 'Malabathrum' with leaves characters mostly triplinerved and opposite arrangement (Lorea Hernández, 1996; Wuu-Kuang, 2011).

With 450 species in the world, only a few of *Cinnamomum* species were known as international spices. A major ingredient in Indian plates, widely use among bakers, with cinnamon rolls or pretzel is the common signature dish for bread lovers. Apart from that, the wood were utilised in making furniture and sculptures. It was also value as timber and trade to neighbouring country, by the name of camphor wood or *"medang"* along with other species in Lauraceae (Kochummen, 1989). The camphor wood is classify as a light-weight to medium-weight hardwood and are good for manufacturing of plywood, cabinets as well in construction. The novelty of the wood as it has own special fragrant and this natural fragrant believes to give additional protection to the wood from insect attack (Pereira & Hastie, 2014). There are many other uses of this *Cinnamomum* species, it has been utilise as herbs in traditional knowledge, almost all the species has this function as treatment for many general occasion and sickness for example chest pain, headache and fever (Wuu-Kuang,

2011). This shows that this taxon is valuable and preserves a lot of potential based on its ethnobotanical study that can be developed (Kulip, 1999).

Despite of poor delineate and taxonomic description on the genera, this create certain confusion within the species levels. Even though, this plant group was among the commonest family that can be found in natural vegetation plots, but there so many holes in taxonomic and basic understanding of this genus (Kostermans, 1956, 1965; Werff & Richter, 1996). One criteria that might be a reason for this family was known as a complicated taxa, was because this family has a small flower and specimens with flowering and fruiting material was scare in collection (Werff, 2001).

1.3 Research Justification

Moreover, Sabah and generally Malaysia have numbers of studies conducted for *Cinnamomum* species which covers certain curiosity in chemical constituents, molecular and bio-product but yet there still many gaps in between especially in plant chemotaxonomy and diversity study for this genus due to lack of intention and awareness especially for Sabah *Cinnamomum* species which has not fully understand. Therefore with curiosity to learn, it is certain that this genus should be addressed and highlighted in Sabah as one interesting and important genus to be investigated.

These studies will focus to study, accumulate and disseminate information and knowledge on this potential genus, *Cinnamomum* spp. (Lauraceae) specifically for Sabah and with attentive interest, i) To observed and consider the chemical diversity through phytochemical profiles as chemotaxonomy markers, and ii) To study the genus generally based on taxonomical approaches and provide some additional information to present understanding of *Cinnamomum* species in Sabah.

1.2 Objectives of Study

In this biodiversity and biosystematics study, there are four research objectives as follow:

1. To extract crudes and interpret phytochemical profiles of selected *Cinnamomum* species collected in Sabah.
2. To extract essential oil and identify the volatile chemical constituents of selected *Cinnamomum* species leaves that were utilised traditionally by the local people In Sabah.
3. To study and provide "Species Identification Key" for *Cinnamomum* spp. (Lauraceae) in Sabah.

CHAPTER 2

LITERATURE REVIEWS

Human actions have caused exploitation to fragile plant communities and ecosystems; it has been phenomenon currently occurred at high speed, this happening in tropical rain forest worldwide due to their destruction from forest conversion. What not realised was the disappearance and extinction of countless number of plant species, many of which were never identified, much less studied for their potential economic utility, could be we had loss the plants that are important as medicines for serious virus and diseases we face today due to our greed actions. The loss of ecosystem not only a great loss to our community, besides the loss of biomes was irreplaceable, possibly theres many plant, animal and insect lost and extinct without knowing them. Therefore biodiversity, ecological and systematic studies was the great counteract actions for this alarming situation, to understand our resources especially plants, to promote conservation and create or disseminate sustainable livelihood and environment to all livings.

2.1 Application of Phytochemistry and Chemotaxonomy

The practice of phytochemistry was inspired by man in the primeval centuries where they practice selecting plants as edible food or poisonous, and when herbalist start to sought and discover drugs in plants surely base on chemicals compound found in some group of plant that have medicinal values (Stace, 1989). To date, wild plants are still actively studied as new sources of drugs especially in the diverse and understudy tropical forest (Vairappan *et al.*, 2014).

Phytochemical screening important and comprehensive to be study since the results of phytochemical experiment was various upon many factor and techniques which depends on specific intentions (Tiwar *et al.*, 2011). Plant screening and taxonomic research was relevance and beneficial, synchronised with development strategy to address the needs of end users, general public in a strategic manner. Same to manufacturer and branding company taxonomic information enhance market and

improve the relevance and impact of research in natural products development (Victor *et al.*, 2015).

The conception of chemotaxonomy has been explained in the historical century, based on (De Candolle, 1816; R. Singh, 2016) *1) Plant taxonomy will be the most useful guide to man in his search for new industrial and medicinal plants, and 2) Chemical characteristics of plants will be most valuable to plant taxonomy in the future*. These statements believed to inspires the modern taxonomy and natural product chemistry scientist until today.

2.1.1 Tools and Techniques in Chemotaxonomy

Theres many tools and techniques in Phytochemisty, some was basic important techniques example, TLC and HPTLC, while advance tool and methods for example NMR, and GC-MS

a. Thin Layer Chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC)

Chromatography known as important primary techniques in the separation and purification of plant chemicals compounds. In the earlier development of chromatography they are three basic techniques namely, paper chromatography (PC), thin layer chromatography (TLC) and gas liquid chromatography (GLC) (Harborne, 1976, 1980). Which are the foundations to advancement of techniques subjected to chromatographic fractionation that we have this day TLC, HPLC, HPLC, LC-MS and GC-MS.

Chromatography is one of the fast emerging and useful tools by which the quality screening control and fingerprint of herbs can be maintained and less complicated with quick results. With this technique, identification of numerous and variety of chemical markers of the herbal constituent can be easily done and it also helps to identify the same herbs in combination and provide additional information for taxa or plant classification (Kasar, Gogia, Shah, Vetriselvan, & Anand, 2013).

The consistent of TLC or HPTLC profile of the phytochemical can be applied for the similarity or dissimilarity in observation and practice to find out the presence or

absence of the certain phytochemicals. Appropriate TLC/HPTLC has excellent resolution and, therefore, permits simultaneous identification of a wide range of substances in a single run. Identification can be established by comparison of a sample with a reference on a same plate and reference guide. The advantages of TLC/HPTLC, not only the entire sample crudes can be observed but also several numbers of samples can be easily compared at the same time (Kasar *et al.*, 2013; Srivastava, 2011).

The prevailing value of HPTLC fingerprints is the auto standardize and better chromatographic results, which can be further expanded by multiple detections base via automated visualization and the use of derivatization. With great practises broad spectrum of constituent can thus be detected and described without the need to know the chemical nature of each zone of the chromatogram. Apart from that, fingerprint chromatograms with a visible pattern of bands provide fundamental data. Ideally, to achieve as much indicators and information for each taxa, many suitable methods should be developed and tested for fingerprinting different sets or groups of plants (Arunachalam, Iniyavan, & Parimelazhagan, 2013; Kasar *et al.*, 2013; Reich, 2013)

b. Proton Nuclear Magnetic Resonance (1H-NMR)

In 1946, Foundation model of NMR was co-discovered by Purcell, Pound and Torrey of Harvard University and Bloch, Hansen and Packard of Stanford University. The first discovery came about when they noticed that magnetic nuclei, such as 1H and 31P (read: proton and Phosphorus 31) were able to absorb radio frequency energy when placed in a magnetic field of a strength that was specific to the nucleus. During the absorption process, the nuclei begin to resonate and different atoms within molecule also resonated at different frequencies, observation of Proton shift, Carbon shift and others allowed a detailed analysis of the structure of a molecule (Kaseman & Ganesh Iyer, 2003). Therefore, it is believed that this proton-NMR can be valuable to chemotaxonomy point of view if it can produce consistency spectrums profiles for set of samples or group of plants.

Most practitioners of this field use NMR to determined and elucidate structure of small molecules. Nuclear Magnetic Resonance (NMR) is a nucleic/nuclear profile