UNIVERSITI TEKNOLOGI MARA

PHYTOCHEMICAL ANALYSIS OF *Etlingera coccinea* (BLUME) S. SAKAI & NAGAM FROM RANAU AND TAMBUNAN, SABAH USING HPLC AND MASS SPECTROMETRY-BASED APPROACH

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MSc

May 2026

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Thesis submitted in fulfilment of the requirements for the degree of
Master of Science
(Chemistry)

Faculty of Applied Science

May 2026

CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 17th June 2025 to conduct the final examination of Nurfateen Helmy Abdellah binti Nazeer on her Master of Science thesis entitled "Phytochemical Analysis of *Etlingera coccinea* (Blume) S. Sakai & Nagam from Ranau and Tambunan, Sabah using HPLC and Mass Spectrometry-Based Approach" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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ABSTRACT

Secondary metabolites (phytochemicals) are important in plant beneficial capabilities, however *Etlingera coccinea*'s chemical composition is poorly known in comparison to other members of the *Etlingera* genus. This work fills this gap by examining the metabolite profiles of *E. coccinea* stalks obtained from two different locations in Sabah—Ranau and Tambunan—to determine whether geographical variation influences phytochemical composition. Crude extracts were obtained via maceration using 10% aqueous methanol, followed by partitioning with *n-hexane* and chloroform. Ranau samples provided more crudes and methanolic extracts than Tambunan samples. Phytochemical analysis revealed the presence of flavonoids, terpenoids, and tannins in all extracts. HPLC studies demonstrated similar maximum absorption wavelengths of 200-210 nm and ~240 nm. LC-MS profiling identified ten metabolites in the Ranau methanolic extract and eight in the chloroform extract, whereas the Tambunan samples contained eight and four, respectively. The GC-MS study identified fifteen metabolites in Ranau *n-hexane* extracts against nine in Tambunan. While both locations had similar phytochemical classes, variations in metabolite amounts and extract yields indicate that locale may alter *E. coccinea*'s chemical composition. This is the first comparable metabolomic study of the species, and it demonstrates its potential as a source of various bioactive chemicals for future pharmacological and structural research.

ACKNOWLEDGEMENT

Firstly, I wish to express my sincere gratitude to Allah for granting me the opportunity to pursue my Master's in Science and for enabling me to complete this challenging journey successfully.

I am deeply grateful to my supervisor, ChM. Dr. Julenah Ag Nuddin, and my co-supervisor, Assoc. Prof. ChM. Dr. Fatimah binti Salim, for their guidance and support throughout my studies. My thanks also go to the lab assistants from UiTM and UMS for providing facilities, knowledge and assistance.

Special appreciation goes to my partner, Faizul, and my brothers, Ameer and Danial, for their help with plant sampling and unwavering support. I am also thankful to my siblings—Faeqah, Fahmee, Faez, and Adam—for their encouragement and understanding throughout this journey. I am grateful to myself for remaining persistent and trusting the journey, even during its most challenging moments.

Finally, this thesis is dedicated to my parents, Nazeer and [redacted], whose vision, determination, and constant support have made this achievement possible. This accomplishment is a tribute to both of you. Alhamdulillah.

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LIST OF SYMBOLS

Symbols

%	Percentage
‘	Minute
“	Second
µL	Micro Liter
µm	Micro-meter
C	Celsius
C_5H_8	2-methyl-1,3-butadiene
Ca	Calcium
cm	Centimeter
Co	Cobalt
Cr	Chromium
E	East (longitude)
$FeCl_3$	Ferric (III) Chloride
g	Gram
H_2O	Water
H_2SO_4	Sulfuric Acid
K	Potassium
k/min	Rate Constant per Minute
kg	Kilogram
m	Meter
m/z	Mass-to-Charge Ratio
min	Minute
mL	Mililiter
mL/min	Mililter per Minute
mm	Milimeter
Mn	Manganese
N	North (latitude)
Ni	Nickel
nm	Nanometer

º	Degree
p	Para-
P	Phosphorus
pH	Hydrogen Ion Concentration
Rf	Retention Factor
-th	Ordinal Number Indicator
α	Alpha-
β	Beta-
δ	Delta-

LIST OF ABBREVIATIONS

Abbreviations

ABTS	2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ACN	Acetonitrile
aq	Aqueous
AR	Analytical Reagent
BADH	Betaine Aldehyde Dehydrogenase
DAD	Diode-Array Detector
DMS	Degree, Minute, Second
DPPH	2,2-Diphenyl-1-picrylhydrazyl
ECSC-R	*Etlingera coccinea* Stalk Chloroform - Ranau
ECSC-T	*Etlingera coccinea* Stalk Chloroform - Tambunan
ECSH-R	*Etlingera coccinea* Stalk Hexane - Ranau
ECSH-T	*Etlingera coccinea* Stalk Hexane - Tambunan
ECSM-R	*Etlingera coccinea* Stalk Methanol - Ranau
ECSM-T	*Etlingera coccinea* Stalk Methanol - Tambunan
EI/CI	Electron Ionization/Chemical Ionization
ESI	Electrospray Ionisation
FRAP	Ferric Reducing Antioxidant Power
GC-MS	Gas Chromatography Mass-Spectrometry
HP-5ms	Hewlett-Packard (5%-phenyl)-methylpolysiloxane
HPLC	High-Performance Liquid Chromatography
JKM/MBS	Jabatan Ketua Menteri/Majlis Biodiversiti Sabah
KOMSAT	Kompleks Makmal Sains,dan Agroteknologi
LC-MS	Liquid Chromatography Mass-Spectrometry
MSD	Mass Selective Detector
NIST	National Institute of Standards and Technology
PIPS	Pusat Instrumentasi dan Perkhidmatan Sains
ppm	Parts per Million
QTOF	Quadrupole Time-of-Flight
RT	Retention Time

SaBC	Sabah Biodiversity Centre
SB-C18	Stable-Bond Carbon-18
TLC	Thin Layer Chromatography
UiTM	Universiti Teknologi MARA
UMS	Universiti Malaysia Sabah
UV	Ultra-Violet
UV-VIS	Ultra-Violet Visible
XL	Extra Large

CHAPTER 1
INTRODUCTION

1.1 Research Background

Plants engage in an infinite synthesis of natural compounds. Natural items have always been utilised for healthcare and illness prevention. They can be located within leaves, stems, shoots, rhizomes, or may also originate from fruits. Jabeen *et al.*, (2014) stated in their article that natural products represent a significant avenue for the discovery and investigation of novel pharmaceuticals. In recent years, the applications of traditional functional plants have gained popularity among researchers. The secondary metabolites, often known as phytochemicals, naturally occur in plants. Plant-derived biologically active chemicals exhibit efficacy in suppressing numerous diseases (Jabeen *et al.*, 2014). It was not until the mid-19th century that significant efforts were made to study therapeutic herbs. The researcher aimed to extract and purify the active principles in these medicines. The efforts are persistently sustained. The examination of the origins of novel and sanctioned pharmaceuticals for human illness management indicates that natural products persist in playing a significant role in the drug discovery and development process (Newman & Cragg, 2016).

Etlingera constitutes the most extensive genus within the Zingiberaceae family. It is typically utilised by indigenous populations. *Etlingera coccinea* is indigenous to Sumatra, Java, the Malay Peninsula, and Brunei. In Sabah, the Dusun and Murut indigenous peoples refer to it as 'tuhau.' In Sarawak, the Kelabits refer to it as 'tubu nanung,' whereas the Ibans call it 'tepus' in both Brunei and Sarawak. It is frequently employed as a conventional medicine for food poisoning, abdominal pain, and gastrointestinal issues. It is readily recognisable because to its potent odour, elongated red blossom with a yellow central border, and its proximity to the ground. The fruit is consumable and utilised by the locals in salads or soups. The central portion of the leafy shoot is utilised as a condiment in Borneo and Java. The young shoots are harvested for use as vegetables and for pickling purposes. The seed oil possesses the distinctive perfume of a foul bug.

Figure 1.1 *E. coccinea* Flower Source: (Naive *et al.*, 2018)

Medicinal plants include various natural compounds that necessitate sophisticated extraction methods to get active fractions or pure natural products through separation and purification (Zhang *et al.*, 2019). Chromatographic and spectral fingerprint analysis is essential for validating the quality of intricate herbal medicinal items (Karthika & Paulsamy, 2015). The integration of chromatography with mass spectrometry represents a significant technological advancement in the life sciences. This condensed analytical platform has been developed over the past decade to enhance dependability and sensitivity. Chromatography coupled with mass spectrometry has been a fundamental and widely employed analytical technique in metabolomics (Adebo *et al.*, 2021; Gröger *et al.*, 2020). Chromatographic analysis of plants has been widely utilised in the research community. Separation methods rely on the inherent physical or chemical differences of individual natural products (Zhang *et al.*, 2019).

Thin Layer Chromatography (TLC) serves as the initial phase in identifying the phytochemical constituents present in a sample. This technique is crucial in the initial stages of phytochemical research since it provides a straightforward, economical, reliable, reproducible, and rapid method for separating and identifying individual components in a complex mixture (Karthika & Paulsamy, 2015). Chemical profiling is primarily performed using High-Performance Liquid Chromatography (HPLC). This quantitative detection technique is recognised for its sensitivity, precision, and accuracy (Kurmudle *et al.*, 2013). HPLC is predominantly utilised in reverse phase chromatography, utilising combinations of water, acetonitrile, ethanol, and methanol (Kurmudle *et al.*, 2013; Wakte *et al.*, 2011).

Gas Chromatography Mass-Spectrometer (GC-MS) and Liquid Chromatography Mass-Spectrometer (LC-MS) techniques are typically employed concurrently to identify all altered metabolites during illness diagnosis (Zeki *et al.*, 2020). Zeki indicated in their study that the amalgamation of these methodologies enhances the accuracy and precision of identifying phenotype-related metabolites. This combinatorial technique involves the initial isolation of thousands of metabolites from biological samples using a chromatographic system that exploits their distinct chromatographic properties (Theodoridis *et al.*, 2011). The interactions between metabolites and the chromatographic system are dictated by the physicochemical properties of the metabolites and the nature of the stationary phase, which in turn affect the elution sequence in the chromatographic system. GC-MS is frequently employed to examine volatile chemical compounds, lipids, and derivatised substances (Zeki *et al.*, 2020).

All these secondary metabolites are found in either the volatile essential oil or the non-volatile fraction. GC-MS analysis is suitable for the examination of volatile chemicals, whereas LC-MS is designed for the detection of polar molecules; hence, these two techniques are mutually complementary (Kulyal *et al.*, 2021). GC-MS is recognised as one of the most efficient analytical platforms for metabolites. GC-MS is proficient in identifying thermally stable metabolites, encompassing both volatile and semi-volatile substances. This characteristic is essential as it enables the accurate identification and quantification of a wide array of metabolites that are difficult to analyse by alternative approaches (Adebo *et al.*, 2021). Consequently, the investigation of natural products will encompass extractions, identification, and quantification, as well as the development of novel approaches to enhance extraction yields (Oroian & Escriche, 2015).

1.2 Problem Statements

Etlingera genus plants are renowned for their varied phytochemical compounds and corresponding therapeutic attributes. Nonetheless, investigations into *E. coccinea* are scarce in comparison to the more thoroughly examined species like *E. elatior*. Current research on *E. coccinea* has predominantly concentrated on its essential oils (Jems *et al.*, 2021; Joseph & Godoong, 2023), with limited exploration of its crude

extracts and comprehensive secondary metabolite profile. This represents a serious deficiency, as crude extracts frequently encompass non-volatile bioactive metabolites—such as flavonoids, terpenoids, and phenolic compounds—that substantially enhance therapeutic potential but are not identified through essential oil analysis alone (Azmi *et al.*, 2024).

Most prior investigations on the *Etlingera* genus have concentrated on their biological and chemical activities, inter-generic and inter-specific comparisons, and chemical profiling. In recent years, research on *E. coccinea* has increased among scholars due to the promising advantageous compounds inside their genus. This affirmative methodology aligns with the characteristics of the renowned species within the *Etlingera* genus, specifically *E. elatior*, commonly referred to as 'kantan'.

Moreover, prior investigations of *E. coccinea* predominantly concentrate on essential oils rather than crude extracts despite the fact that the stalk of *E. coccinea* is traditionally consumed by indigenous communities (Jems *et al.*, 2021). The isolation and chemical characterisation were previously conducted using hexane extracts from the leaf. To clarify, *E. coccinea* were frequently ingested by indigenous peoples in the stalk portions. Nevertheless, there remains a paucity of studies concerning the metabolites of *E. coccinea* stalks. The stalks should be set away; the chemical profiling of this species requires more comprehensive research. Currently, there is inadequate scientific data regarding the metabolites uniquely found in the stalks of *E. coccinea*, and nearly no thorough phytochemical profiling has been performed on extracts with varying solvent polarity. This constraint hinders a comprehensive study of the species' phytochemical variety, especially regarding non-volatile secondary metabolites that may substantially enhance its pharmacological and therapeutic potential. The absence of data about crude extracts, which typically have a wider array of bioactive chemicals than essential oils, constitutes a significant deficiency in the existing literature.

Geographical and environmental factors, such as altitude, temperature, soil composition, and microclimatic conditions, are acknowledged as critical determinants of secondary metabolite production in various plant species. Altitude significantly influences metabolite accumulation by prompting physiological and biochemical changes in plants (Wu & Xiao, 2024). Notwithstanding this proven link, *E. coccinea* has yet to undergo comprehensive evaluation to ascertain whether locality-dependent variation affects its phytochemical composition. The lack of comparison studies

analysing samples from various geographical regions restricts comprehension of the impact of environmental circumstances on metabolite diversity and abundance in this species.

Therefore, performing an extensive chemical profiling of *E. coccinea* stalk extracts with solvents of differing polarities is anticipated to provide further understanding of the presence and distribution of secondary metabolites, especially those of greater polarity that are not easily identified through essential oil analysis. This approach facilitates a more comprehensive characterisation of phytochemical elements and enhances the understanding of the species' chemical complexity.

This study entails the identification and comparison of the chemical profiles of *E. coccinea* stalks sourced from two separate locations in Sabah: Ranau and Tambunan. The Ranau region is geographically bordered by Kota Marudu to the north, Kota Belud to the northeast, Tuaran to the west, Tambunan to the southwest, Keningau to the south, Tongod to the southeast, and Beluran to the east. It is distinguished by its hilly topography, elevated altitude, and comparatively cool climate, and is renowned as a favoured tourist destination for its picturesque scenery. The Tambunan region, situated southwest of Ranau, is primarily defined by lowland agricultural zones, including vast rice fields, and encounters distinct environmental and meteorological circumstances.

The differing geographical and climatic attributes of these two regions offer an appropriate basis for analysing potential discrepancies in metabolite composition and extract yields. Thus, the comparison of plant samples from Ranau and Tambunan facilitates the evaluation of how environmental variables may affect the chemical profiles and species-specific yields of *E. coccinea*. This comparison method not only improves comprehension of the species' phytochemical diversity but also facilitates future research into its bioactive potential and ecological adaptability.

1.3 Significance of study

Research on medicinal and aromatic plants has garnered heightened interest in recent decades owing to their prospective applications in healthcare, pharmaceuticals, cosmetics, and the food industry. Plant-derived secondary metabolites are acknowledged as significant sources of bioactive chemicals, providing alternatives to manmade pharmaceuticals that may exhibit adverse side effects. Aromatic plants play

a crucial role in the fragrance, cosmetic, and flavouring industries, underscoring the necessity of comprehending their chemical makeup and bioactive potential.

The *Etlingera* genus is recognised for its fragrant properties and varied phytochemical components. Nevertheless, scientific research has predominantly concentrated on specific species, notably *E. elatior*, whereas other species, such as *E. coccinea*, have been insufficiently examined. Current research on *E. coccinea* has predominantly focused on essential oil composition, resulting in little data regarding the chemical profiles of crude extracts and non-volatile secondary metabolites, particularly from traditionally utilised plant components like the stalk.

This study is important since it offers detailed phytochemical profiling of *E. coccinea* stalk extracts utilising solvents of varying polarity, thereby offering novel data on secondary metabolites that may have therapeutic significance. This research provides insights into the impact of geographical and environmental factors on extract yield and metabolite diversity by comparing samples obtained from two separate localities: Ranau and Tambunan.

The results of this study provide a chemical "fingerprint" for *E. coccinea* stalks from various locales, establishing baseline reference data for subsequent research. This knowledge is essential for future pharmacological research, quality assurance of botanical products, chemotaxonomic classification, and the sustainable use of *E. coccinea* as a potential source of natural bioactive chemicals. This research enhances the comprehension of phytochemical diversity within the *Etlingera* genus and advocates for the ongoing investigation of underutilised medicinal plant species.

1.4 Research Aim and objectives

The aim of this study is to determine the chemical profile of *E. coccinea* species which differs by their locations and method of extraction. The objectives of this study are:

1. To extract and determine percentage yields of *E. coccinea* sampled from Ranau and Tambunan areas extracts using cold maceration.
2. To profile the methanolic and chloroform plant extracts through phytochemical screening, TLC and HPLC.
3. To characterize the secondary metabolites of *n-hexane* fractions using GC-MS and

methanol and chloroform using LC-MS.

1.5 Scope of study

The scope of this study encompasses the specific parameters and boundaries within which the research is conducted, detailing the aspects that will be examined and the limitations that may affect the findings. This study examines a specific species within the *Etlingera* genus, specifically *E. coccinea*, which belongs to the Zingiberaceae family. This study focusses on the analysis of secondary metabolites in the stalks of this species, emphasising the variations due to geographical differences. The study focusses exclusively on phytochemical and metabolite profiling, lacking comparisons with other *Etlingera* species and detailed biological or pharmacological activity assays.

Plant samples were obtained from two separate locations in Sabah, Malaysia: Poring Hot Spring in Ranau and the Sub-station of Crocker Range Park in Tambunan. The selected locations exhibit contrasting environmental and geographical characteristics that may impact secondary metabolite biosynthesis. This study investigates the degree to which locality-related factors influence variations in the chemical composition and extract yields of *E. coccinea*.

Systematic chromatographic and spectrometric analyses were performed to identify and profile secondary metabolites in extracts with differing solvent polarities. HPLC, LC-MS and GC-MS was utilised to enhance the detection and characterisation of metabolites. The analytical work focusses on qualitative and comparative profiling, rather than on the complete structural elucidation of individual compounds.

This study aims to identify similarities and differences in metabolite composition between samples from two locations, thereby offering insights into geographical influences on phytochemical diversity. The findings provide baseline data for subsequent research on the bioactivity, pharmacological potential, and chemotaxonomic relevance of *E. coccinea*.

CHAPTER 2
LITERATURE REVIEW

2.1 Zingiberaceae Family

This chapter provides an exhaustive assessment of the literature pertaining to the botanical, morphological, phytochemical, and analytical dimensions of the Zingiberaceae family, focussing specifically on the genus *Etlingera* and the species *E. coccinea*. The chapter commences with a comprehensive taxonomic and morphological characterisation to ascertain the biological identity of the species being examined. This is succeeded by a comprehensive examination of previously known phytochemical constituents at both the species and genus levels, the analytical methodologies utilised for their identification, and the established medicinal significance of these compounds. This review identifies research gaps and offers a compelling scientific justification for the current investigation.

Species of Zingiberaceae are located in tropical and subtropical regions, predominantly in Asia (Maimulyanti & Prihadi, 2015). Zingiberaceae is commonly known as the ginger family. *Zingiber officinale*, a widely utilised spice, is commonly known as ginger. Specifically, the genus *Zingiber* and the family Zingiberaceae (Anthony *et al.*, 2013). The term *Zingiber* is derived from the Sanskrit word 'sringavera', meaning 'deer antler', referencing the distinctive horn-like shape of the rhizomes (Anthony *et al.*, 2013). It is a flowering plant with around 13,000 species categorised into 52 genera, predominantly found in tropical forests (Nurjannah *et al.*, 2023).The predominant species of Zingiberaceae are rhizomatous or perennial herbs, characterised by their growth from subterranean stems called rhizomes (Ewon & Bhagya, 2019).

The Zingiberaceae family comprises perennial, rhizomatous herbs distinguished by aromatic underground rhizomes that serve as both storage organs and means of vegetative propagation (Ewon & Bhagya, 2019). Their leaves are arranged in a separated manner, with covering leaf bases that create pseudostems, a characteristic trait of numerous taxa. The leaf blades are generally simple, lanceolate to oblong, and exhibit significant variation in length and width among species. The inflorescences of Zingiberaceae exhibit significant variation, manifesting terminally on leafy branches,

laterally, or directly from the rhizome. Vividly hued bracts frequently encase emerging buds and enhance the aesthetic allure of numerous species. The flowers exhibit zygomorphy, are bisexual, and generally possess a well-developed labellum derived from fused staminodes, a feature crucial for pollinator attraction. The blooms have one fertile stamen, with the other stamens transformed into petaloid structures, resulting in a distinctive floral morphology. These characteristics collectively signify the evolutionary adaptations of the family and facilitate the taxonomic distinction of genera.

Species of Zingiberaceae demonstrate significant ecological relationships with particular environmental circumstances. Gobilik and Mashitah assert that stream banks and alluvial terraces in Sabah have the highest species richness, with reports of up to 60 species in these environments(Gobilik & Mashitah, 2005). Plateau forests exhibit moderate biodiversity, but ridge tops and stony soils harbour fewer species (Anthony *et al.*, 2013). Soil type significantly influences species distribution: limestone substrates foster the highest diversity, while ultramafic and podsolic soils, typically abundant in heavy metals, sustain fewer species.

Primary forests harbour over 40 documented species, while secondary or damaged forests accommodate approximately half that quantity (Gobilik & Mashitah, 2005). Highland species exhibit enhanced tolerance to disturbance owing to colder temperatures and elevated humidity, which alleviate heat stress and desiccation—frequent limiting factors for lowland gingers (Anthony *et al.*, 2013).

Limestone soils are the most prevalent, comprising over 20 distinct types; however, Sabah exhibits relatively fewer distributed outcrops in comparison to other regions of Borneo (Anthony *et al.*, 2013). Podzolic soils in heath woods and ultramafic soils rich in hazardous metals such as nickel exhibited the lowest species diversity, with fewer than five species identified at this stage. Sandstone, clay, and sandy soils sustained an average of 10 to 15 species. Their findings indicated that primary forests contained over 40 prominent ginger species, whereas secondary or damaged forests had approximately half that number (Anthony *et al.*, 2013; Gobilik & Mashitah, 2005). Furthermore, it was found that the cold, overcast, and humid conditions at elevated altitudes enhanced the resilience of highland species to forest disturbances. The study found that the opening of the forest canopy did not lead to the desiccation and mortality of gingers due to increased daytime temperatures, a phenomenon typically observed in lowland forests (Anthony *et al.*, 2013; Gobilik & Mashitah, 2005).

The Zingiberaceae family is recognised for generating a variety of secondary metabolites, such as essential oils, phenolics, flavonoids, terpenoids, and diarylheptanoids. These chemicals underpin their conventional therapeutic applications and are the foundation for their scent, flavour, and pharmacological properties. Volatile compounds including monoterpenes and sesquiterpenes are typically examined with GC-MS, whilst LC-MS and HPLC are often employed to identify non-volatile compounds such as flavonoids and phenolic acids. The phytochemical properties are crucial for taxonomic identification and underscore the ethnobotanical importance of the family.

The Zingiberaceae family encompasses numerous genera. The majority were reported to possess known metabolites that confer benefits to consumers. The rhizomes of *Zingiber*, a genus within the Zingiberaceae family, are discussed first. *Zingiber* species are widely utilised in traditional therapeutic practices (M. Sharifi-Rad *et al.*, 2017; Yit & Zainal-Abidin, 2024). *Zingiber officinale*, commonly known as ginger, is a notable species within the *Zingiber* genus, characterised by its abundance of phenolic and terpene phytochemicals (Feng *et al.*, 2011; M. Sharifi-Rad *et al.*, 2017; Yit & Zainal-Abidin, 2024). The primary pungent phenolic compounds in fresh ginger rhizomes are gingerols, specifically 4-, 6-, 8-, 10-, and 12-gingerol (Ghasemzadeh *et al.*, 2018). Gingerol-related chemical compounds exhibit thermal instability and are prone to dehydration (Yit & Zainal-Abidin, 2024).

Consequently, they can be converted into the corresponding shogaol forms that impart the spicy flavour to dried ginger (Semwal *et al.*, 2015). In addition to the previously described major components, gingerenone A, quercetin, 6-dehydrogingerdione, and zingerone represent other phenolic phytochemicals found in ginger. Conversely, the predominant components of ginger essential oils include *Zingiber*ene, β-bisabolene, β-sesquiphellandrene, and *α*-farnesene (Mao *et al.*, 2019). All of these compounds exhibit antimicrobial properties (Akullo *et al.*, 2022; Babaeekhou & Ghane, 2021; Gunasena *et al.*, 2022). Secondly, examine the species within the *Curcuma* genus. The primary biologically active compounds in *Curcuma* rhizomes consist of non-volatile curcuminoids, volatile sesquiterpenoids, and monoterpenoids. Curcumin, demethoxycurcumin, and bisdemethoxycurcumin represent three distinct curcuminoids. Demethoxycurcumin and bisdemethoxycurcumin are derivatives of curcumin. The yellow hue of turmeric rhizomes is attributed to three

chemical compounds classified as polyphenols (Huang *et al.*, 2020). Essential oils are primarily composed of monoterpenoids and sesquiterpenoids. *C. longa*, commonly referred to as turmeric, exhibits a wide range of pharmacological activities. This encompasses anticancer and anti-inflammatory properties due to its high concentration of curcumin (Burapan *et al.*, 2020; J. Sharifi-Rad *et al.*, 2020).

The subsequent focus is on the species within the *Alpinia* genus. The phytochemical analysis of *Alpinia* identified the presence of diarylheptanoids, terpenes such as sesquiterpenoids and monoterpenes, as well as flavonoids within the genus. Substances exhibiting anti-inflammatory, hepatoprotective, antioxidant, and anticancer properties have been identified (Cheng *et al.*, 2021). This genus comprises five distinct subcategories of diarylheptanoids, characterised by structural diversity among phenolic compounds. Types include linear, dimeric, cyclic, chalcone, flavanone, and entirely novel forms (Yit & Zainal-Abidin, 2024). Linear and chalcone or flavanone diarylheptanoids are commonly found in *Alpinia* species. Research on this genus has predominantly centred on *Alpinia galanga* (L.) Willd due to its higher concentration of bioactive compounds relative to other species within the genus. The compounds include *α*-fenchyl acetate, β-bisabolene, β-pinene, *α*-bergamotene, 1,8-cineole, 1'-acetoxychavicol acetate, and β-farnesene (24). The essential oil extracted from both dried and fresh *A. galanga* demonstrates antimicrobial activity against a range of bacteria, yeast, fungi, and parasitic organisms (Subramanian & Nishan, 2015).

The species listed is classified within the *Amomum* genus. Previous studies indicate its efficacy in treating stomach disorders, oral infections, lung disease, malaria, cancer, and inflammatory conditions (Cai *et al.*, 2021; Van, 2021; Yit & Zainal-Abidin, 2024). The *Amomum* genus has been examined for its secondary metabolites, including flavonoids, terpenoids, and diarylheptanoids. Reports indicate that as many as 160 compounds have been identified, and their biological actions have been reviewed (Cai *et al.*, 2021; Yit & Zainal-Abidin, 2024). The phytochemical profiles of *Amomum* volatile oils indicate that the predominant compounds include limonene, 1,8-cineole, camphor, *α*-pinene, caryophyllene, santolina triene, bornyl acetate, β-elemene, delta-3-carene, allo-aromadendrene, farnesyl acetate, methyl chavicol, D-camphor, β-pinene, and camphene. *Amomum subulatum Roxb*., commonly known as black cardamom or the 'Queen of Spices', is a significant economic crop in the Himalayan region. It has also been utilised for its therapeutic properties in the management of various respiratory

ailments (Yit & Zainal-Abidin, 2024). The species of the *Kaempferia* genus follows next in line.

The *Kaempferia* genus has demonstrated efficacy in addressing specific symptoms, including wound infections, cough, viral illnesses, and digestive issues (Pham *et al.*, 2020). Species within this genus display identical secondary metabolites to those found in other genera of Zingiberaceae. Secondary metabolites include phenolic compounds, flavonoids, diterpenoids, and volatile oils. Isopimarane-type diterpenoids represent a significant group within this genus that has been studied for their anti-inflammatory properties, whereas steroids constitute a minor category of natural compounds in *Kaempferia* species (Elshamy *et al.*, 2019). The primary secondary metabolites found in essential oils derived from this plant are phenylpropanoids and cinnamates, followed by monoterpenes. *Kaempferia galanga L.*, the representative species of this genus, exhibits pharmacological and therapeutic properties due to the presence of trans-ethyl cinnamates and p-methoxycinnamate in its extracted essential oils (Munda *et al.*, 2018). The Zingiberaceae family is extensively studied through its genera. Many chemical compounds have been identified across various genera. Although the structures may have differed, the primary class remains consistent.

The botanical diversity, ecological adaptability, and extensive phytochemical profiles of the Zingiberaceae family underscore its importance as a source of biologically active secondary metabolites. Although many genera within the family have been thoroughly examined, phytochemical research is unevenly distributed, with certain genera receiving relatively little focus. The genus *Etlingera* is a taxonomically distinct and ecologically significant group within Zingiberaceae, characterised by unique morphological features and increasing evidence of its chemical and biological importance. A focused examination of the genus *Etlingera* is necessary to enhance understanding of its phytochemical characteristics and to contextualise the species-specific investigations presented in the following sections.

2.2 The Genus *Etlingera*

Etlingera is a highly unique and species-rich genus within the Zingiberaceae, encompassing over 100 species found across Southeast Asia and the Pacific. These

plants generally inhabit lowland dipterocarp woods, peat swamp environments, and riverine areas, where they create substantial clusters originating from extensive rhizome networks. The genus *Etlingera* was given its name after Andreas Ernst Etlinger, a German botanist. The inflorescence of all species is distinct from the leafy stalk, which can be 1-8 meters tall. The peduncle's length varies greatly, as does the lip. This is the family's largest genus in Borneo, with 42 taxa identified thus far. The flowers are mainly attracted by birds, notably spider hunters (Anthony *et al.*, 2013).

Etlingera belongs to the Indo-Pacific region genus with approximately 100 species ranging from sea level to 2500 meters. From the west to the east, the plant is native to India (which includes the Nicobar Islands), Bangladesh, Burma, China, Laos, Cambodia, Vietnam, Thailand, Malaysia, Singapore, Indonesia, the Philippines, Brunei, Papua New Guinea, Australia, and a few other islands in the Pacific region (Poulsen, 2006). Species in the genus *Etlingera* exhibit a unique morphology that differentiates them from other family members. They have substantial, perennial, and highly fragrant rhizomes that generate vigorous clusters of vegetative shoots. The pseudostems, composed of closely wrapped leaf bases, are generally tall—often attaining or surpassing three meters—and bear wide, pleated leaves placed distichously along the stem. A distinguishing feature of *Etlingera* is its geophilous inflorescence, which arises directly from the rhizome at ground level instead of from the leafy stalk. These inflorescences consist of vividly hued bracts, typically red, pink, or orange, which safeguard the growing floral buds. The tubular flowers feature an extended floral tube, a conspicuous labellum, and nectar-secreting appendages that facilitate pollination by insects and birds. Subsequent to fertilisation, the species yield fleshy, fragrant fruits that are globular to ellipsoidal in form and contain many seeds. These characteristics collectively render *Etlingera* one of the most recognised genera within the Zingiberaceae family.

Members of this genus possess considerable cultural and economic significance. Numerous species are utilised as vegetables or employed as flavouring agents in ancient culinary practices. Rhizomes and leaves are utilised in traditional treatments for fever, inflammation, gastrointestinal issues, and postpartum recovery. The genus is extensively cultivated as an ornamental for its vivid inflorescences and remarkable structure.

Phytochemical investigations of *Etlingera* species have identified an extensive

number of volatile and non-volatile compounds. GC-MS analyses commonly identify monoterpenes (e.g., α-pinene, 1,8-cineole), sesquiterpenes (e.g., caryophyllene, humulene), aldehydes, esters, aromatic compounds, and long-chain fatty acids. LC-MS analyses additionally characterise flavonoid glycosides, diarylheptanoids, and phenolic acids. The phytochemical characteristics support the genus's historical applications and demonstrate its extensive metabolic diversity.

Among species in the *Etlingera* genus, *E. elatior* has been the subject of extensive research in recent years. Consequently, their fingerprinting is frequently compared and serves as a reference point within their genus and family. Maimulyanti and Prihadi identified several metabolites in *E. elatior* flowers during their research(Maimulyanti & Prihadi, 2015). The compounds identified were 1-dodecanol, dodecanal, and 17-pentatriacontene. In 2017, Nagappan investigated the volatile compounds in *E. elatior*. Nine metabolites were successfully identified. The compounds were categorised into major and minor constituents. The primary components comprised borneol, 10-dodecanol, lauryl aldehyde, aromadendrene oxide, and elemicin. The minor volatile constituents included camphor, 5-decen-1-ol, caryophyllene, and β-caryophyllene oxide (Nagappan *et al.*, 2017).

In 2018, Sukandar conducted research on the essential oils of *E. elatior*. GC-MS analysis identified five primary components present in the essential oils. The identified components include 1-dodecanol, dodecanal, trans-cryophyllene, cyclododecane, and dodecyl ester(Sukandar *et al.*, 2018). Compound 1-dodecanol and dodecanal were previously identified in the essential oils of *E. elatior* flower in the studies conducted by (Maimulyanti & Prihadi, 2015). Subsequently, the investigations of the hexane extracts of *E. sessilanthera* leaves by Daniel-Jambun *et al.*, (2017), a member of the *Etlingera* genus, were conducted. An 8(17),12-labdadiene-15,16-dial, an aldehyde compound, was successfully isolated from the extracts. The essential oils of *E. sayapensis* have been studied in relation to its stem, leaves, and stolon parts. The components identified included monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbons (Mahdavi *et al.*, 2017).

Another species within the *Etlingera* genus is *E. brevilabrum*. Vairappan *et al.*, (2012) identified ten volatile compounds in the essential oils of *E. brevilabrum*. The identified volatile compounds included *α*-fenchol, borneol, *α*-terpineol, delta-selinene, methyl isoeugenol, thujospene, β-farnesene, caryophyllene, β-bisaboelene, lauryl

acetate, 1-tetradecanol, and tetradecanol acetate. Additionally, it was reported to contain sesquiterpene hydrocarbons and oxygenated monoterpenes. Another report by Mahdavi *et al.*, (2013) discusses the essential oils of *E. brevilabrum*. This instance pertained to its rhizome, stems, and leaf components. The research identified that the predominant compounds in rhizome oil included eucalyptol, β-pinene, caryophyllene oxide, and *α*-thujene. In the stems, the oils comprised limonene, β-pinene, and caryophyllene oxide. Finally, the leaf oils contained β-pinene, α-thujene, and o-cymene. Mahdavi *et al.*, (2015) investigated the essential oils of *E. brevilabrum*. The essential oils were extracted from various parts of *E. brevilabrum*, including the leaves, stem, stolon, and rhizome. Several compounds were identified in each section. The leaves contained *α*-thujene, p-cymen-7-ol, and β-pinene. In the stem, delta-3-carene, *α*-thujene, and limonene are present. In the stolon, the compounds identified include β-pinene, p-cymen-7-ol, and exo-fenchol. Finally, the rhizome contains perilla aldehyde, bornyl acetate, and verbenyl acetate.

Mahdavi *et al.*, (2018) examined the chemical composition of smoke liquids derived from fresh and dried leaves of *Etlingera brevilabrum*, subjected to thermal treatment under varying atmospheric conditions. This study involved heating plant materials at temperatures between 400 and 600 °C in air or nitrogen environments, enabling an evaluation of the impact of processing conditions on the formation of chemical constituents in smoke-derived extracts. The analysis indicated that the smoke liquids' chemical composition was primarily characterised by phenolic compounds, fatty acids, and aromatic hydrocarbons, with significant variation influenced by the condition of the plant material, the surrounding atmosphere, and the applied temperature. Under various experimental conditions, phenolic compounds, including phenol, methoxyphenols, catechol derivatives, and cresols, were consistently identified as the primary constituents of the smoke liquids. These compounds are frequently linked to the thermal degradation of lignocellulosic plant material, characterised by their pronounced aromatic properties and potential biological activity. Alongside phenolic compounds, long-chain fatty acids, especially palmitic acid, were commonly identified, indicating the thermal degradation of lipid components in the leaf tissues. Aromatic hydrocarbons and substituted phenols were present, indicating the occurrence of complex secondary reactions during high-temperature processing.

The research indicated that elevated temperatures typically led to higher

concentrations of phenolic compounds, with numerous smoke liquid samples exhibiting total phenol levels exceeding 10%. Smoke liquids obtained from fresh leaves at high temperatures exhibited notably elevated concentrations of mono-(2-ethylhexyl) phthalate, recognised as one of the predominant constituents across various conditions. Conversely, smoke liquids generated at reduced temperatures or from desiccated materials demonstrated increased chemical diversity, indicating variations in moisture levels and thermal decomposition processes. The findings of Mahdavi *et al.*, (2018) demonstrate that the chemical profile of smoke liquids from *E. brevilabrum* is significantly affected by three primary factors: the physical state of the plant material (fresh or dried), the atmospheric conditions during heating, and the applied temperature. This research emphasises the sensitivity of *Etlingera* species to processing conditions and illustrates how thermal treatment can substantially modify their chemical composition. While the formation of smoke liquid is not directly associated with traditional medicinal applications, the findings offer significant insights into the diversity and reactivity of secondary metabolites in *Etlingera* species, reinforcing the classification of this genus as a chemically diverse group within the Zingiberaceae family.

The most recent update on *E. brevilabrum* occurred in 2021. A preliminary survey and chemical profiling conducted by Mus *et al.* revealed that the essential oils derived from the leaves, stem, and rhizome of this species contain 35 distinct chemical constituents. The identification relied on GC-MS analysis. The stem contains the highest number of chemical constituents, totalling 25, whereas both the leaves and rhizomes each comprise 18 chemical compounds. The stems and leaves of *E. brevilabrum* were primarily composed of monoterpenes, with eucalyptol identified as the most abundant compound. The rhizomes of *E. brevilabrum* have been reported to contain caryophyllene, a sesquiterpene, as the most abundant compound present. In addition to monoterpenes and sesquiterpenes, another significant volatile compound identified was phenylpropanoid methyleugenol, found in the stems and leaves of *E. brevilabrum*. Based on the studies by Nagappan *et al.*, (2017) concerning the *Etlingera* genus, several constituents have been identified. The studied volatile compounds of *E. pyramidosphaera* and *E. megalocheilos* included both major and minor constituents. The primary constituents of both *Etlingera* species included borneol, 1-dodecanol, lauryl aldehyde, aromadendrene oxide, and elemicin. Their minor constituents included

camphor, caryophyllene, β-caryophyllene oxide, and delta-selinene.

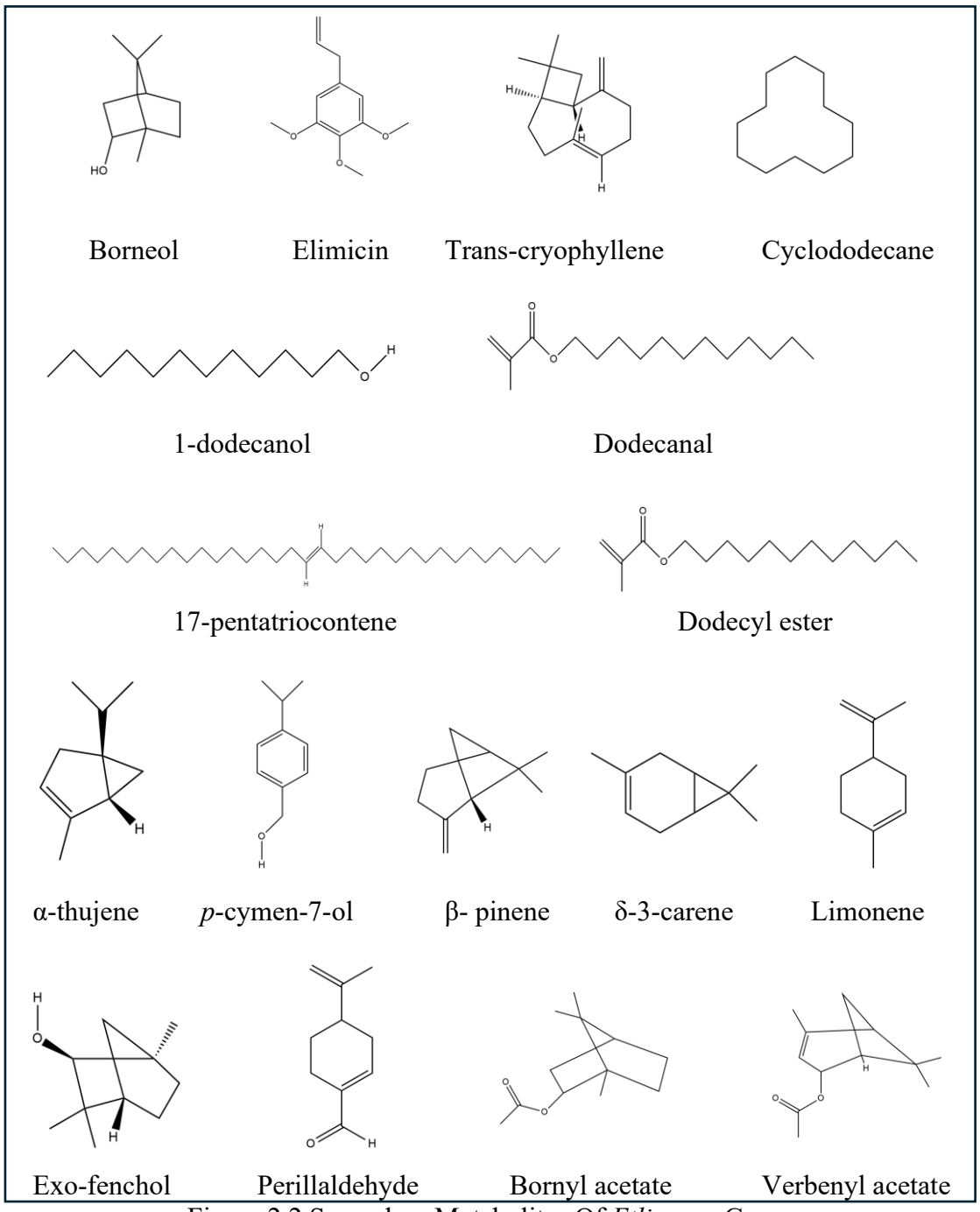


Figure 2.2 Secondary Metabolites Of *Etlingera* Genus

Recent untargeted metabolite profiling investigations have further illustrated significant chemotaxonomic consistency within the Zingiberaceae family, especially among plants of the genus *Etlingera*. A comparative analysis utilising untargeted GC-MS across various Zingiberaceae species indicated that monoterpenes and

sesquiterpenes are the predominant classes of volatile compounds among closely related taxa, exhibiting significant overlap in chemical profiles at the genus level (Wen *et al.*, 2023). These findings endorse the utilisation of genus-level phytochemical data to elucidate the chemical composition of inadequately researched species like *E. coccinea*, particularly in the absence of species-specific data. The identified chemotaxonomic patterns underscore the significance of previously documented volatile compounds in *Etlingera* species and validate the extrapolation from genus-level research to inform focused phytochemical studies.

2.3 *Etlingera coccinea*

E. coccinea is an obscure species within the genus, however it exhibits numerous ecological and physical characteristics akin to its relatives. It generally resides in shady, humid lowland forests in Malaysia and Borneo. *E. coccinea* is a species that grows as a big cluster of spaced-out leafy stems to 3-8m tall, with inflorescences developing at ground level, usually with groups of a minimum of 5 flowers, each with a long yellow lip and an unrolled, crimson edge. Spiderhunters pollinate the flowers. When young, the fruits are pink, but when ripe, they change to cream and become woody (Anthony *et al.*, 2013).

E. coccinea possesses morphological traits typical of its genus, alongside unique features that facilitate its distinction from closely similar species. It generates dense clusters originating from robust, fragrant rhizomes that stretch horizontally beneath the soil. The pseudostems typically measure between 1.5 and 3.0 meters in height and feature broad, elliptic-lanceolate leaves that taper gracefully at the apex. The inflorescences emerge immediately from the plant's base and consist of densely organised bracts exhibiting vivid shades of red to orange, creating compact conical formations. The flowers are vivid red and tubular, featuring a gently curved labellum that boosts both their aesthetic allure and functional significance in pollinator interactions. Subsequent to anthesis, the plant produces ellipsoid fruits that encompass numerous scented seeds. The physical characteristics distinguish *E. coccinea* from its closely related species, *E. elatior* and E. littoralis, especially regarding inflorescence structure, bract shape, and colour intensity.

Research on *E. coccinea* is very few; nonetheless, existing studies indicate a

phytochemical composition mostly characterised by volatile compounds, such as monoterpenes, sesquiterpenes, aldehydes, and fatty acids. Identified chemicals from GC-MS tests comprise β-caryophyllene, α-humulene, decanal, and dodecanal. LC-MS analyses reveal the existence of non-volatile metabolites, including phenolic acids, flavonoids, and other semi-polar compounds, which are indicative of the Zingiberaceae family.

Etlingera genus possessed medicinal benefits (A. & Mohammad, 2019). The previous study from (Tachai & Nuntawong, 2016) regarding on *Etlingera*, they inhibit significant several biological activities such anti-inflammatory, anti-asthmatic, anti-cancer, anti-carcinogenic, anti-mutagenic, anti-microbial, anti-pyretic and antioxidant activities. As for *E. coccinea*, they were used as a traditional medicines by the indigenous people in Borneo and as well as the infusion to remove body odor (Vairappan *et al.*, 2012). Previous studies on *E. coccinea* primarily emphasised their volatile compounds. Consequently, the extraction primarily focusses on the essential oils from various parts of the plants. The *E. coccinea* emitted a strong pungent odour that attracted both insects and humans as consumers. Early research by Vairappan *et al.*, (2012) identified nine compounds through GC-MS analysis. The compounds identified include 3-thujanone, borneol, camphor, cedr-9-ene, L-calamenene, caryophyllene oxide, *α*-bisabolol, *α*-epi-muurolol, and cycloartanyl acetate. Borneol was the most dominant compound, followed by L-calamenene. The studies indicated that *E. coccinea* predominantly contains sesquiterpene hydrocarbons, followed by oxygenated diterpenes, oxygenated monoterpenes, and oxygenated sesquiterpenes. Nagappan *et al.*, (2017) conducted further investigations on the volatile compounds of the *Etlingera* genus. Seven volatile compounds were identified in the research. Five major compounds were identified: borneol, 1-dodecanol, lauryl aldehyde, aromandendrene oxide, and elemicin. The minor volatile compounds identified were camphor and 5-decen-1-ol. Subsequently, Daniel-Jambun *et al.*, (2017) investigated the hexane crude extracts of *E. coccinea* leaves. The report details the successful isolation of trans-2-dodecenal. This is the first isolated compound of *E. coccinea*, to the best of my knowledge.

The 2021 findings on the chemical compounds of *E. coccinea* indicate that an investigation of the essential oils from its leaves, stems, and rhizomes was conducted by (Jems *et al.*, 2021). The GC-MS analysis identified 85 compounds across all

examined parts of *E. coccinea*. Monoterpenes were the most abundant, comprising 18 compounds in *E. coccinea*. This was followed by alkanes and alkenes, which included twelve compounds, and sesquiterpenes with eight compounds. Aldehydes were represented by seven compounds, while ketones, fatty acid derivatives, and esters each contained four compounds. Finally, amines and norterpenes were each represented by one compound.

Recent investigations, including previous GC-MS studies, have enhanced the phytochemical understanding of *E. coccinea*. A study analysing essential oils derived from various plant sections, including as leaves, stems, and rhizomes, indicated that sesquiterpenes and aliphatic aldehydes are the most volatile components in all tissues. Compounds like β-caryophyllene, α-humulene, decanal, and dodecanal were consistently identified, while differences in relative abundance were noted across different plant tissues, indicating tissue-specific metabolite distribution (Jems *et al.*, 2021). These results further confirm the prevalence of volatile terpenoids and aldehydes in *E. coccinea* and underscore the efficacy of GC-MS for thorough volatile analysis.

In addition to volatile chemicals, new phytochemical screening investigations have identified a wider array of secondary metabolites in *E. coccinea*. Initial qualitative investigations of leaf and rhizome extracts revealed the presence of flavonoids, glycosides, saponins, tannins, steroids, and proteins. Assays for antioxidant activity, such as DPPH, ABTS, and FRAP, shown significant radical scavenging activity, especially in leaf extracts, suggesting the existence of bioactive non-volatile compounds (Joseph & Godoong, 2023). While these findings stem from screening-level investigations, they offer significant preliminary evidence for the presence of many non-volatile metabolites in *E. coccinea* and underscore the necessity for sophisticated chromatographic methods, such as LC-MS, for comprehensive compound identification.

Phytochemical studies of *Etlingera* species, particularly *E. coccinea*, have primarily utilised chromatographic and spectrometric methods for the qualitative identification of compounds. Gas chromatography–mass spectrometry (GC-MS) is the predominant analytical instrument for profiling volatile and semi-volatile compounds, including monoterpenes, sesquiterpenes, aldehydes, and fatty acid derivatives. GC-MS facilitates the separation of compounds according to their volatility and polarity, subsequently enabling mass spectral fragmentation for compound identification by

comparison with recognised spectral libraries. Prior research on *E. coccinea* has effectively employed GC-MS to identify significant volatile components such as β-caryophyllene, α-humulene, decanal, and dodecanal, underscoring the appropriateness of this method for aroma-active compounds (Daniel-Jambun *et al.*, 2017; Jems *et al.*, 2021; Shahid-Ud-Daula *et al.*, 2015).

Conversely, LC-MS is frequently utilised for the investigation of non-volatile and thermally unstable metabolites, such as flavonoid glycosides, phenolic acids, and various polyphenolic substances. While studies utilising LC-MS specifically for *E. coccinea* are scarce, investigations at the genus level within *Etlingera* and related Zingiberaceae taxa have shown that LC-MS is effective for identifying semi-polar secondary metabolites that are not amenable to GC-MS analysis (Ghasemzadeh *et al.*, 2015; Resna *et al.*, 2021). Compound identification by LC-MS is often accomplished through precise mass measurement, fragmentation patterns, and comparison with reference databases or previously documented compounds.

HPLC, frequently combined with UV–Visible detection, has been utilised in phytochemical investigations of *Etlingera* species for compound separation and semi-quantitative assessment. Ultraviolet detection within the 200–400 nm spectrum is especially pertinent for phenolic and flavonoid chemicals owing to their distinctive chromophoric properties (Lachumy *et al.*, 2010). Despite the limited availability of quantitative HPLC data for *E. coccinea*, its utilisation at the genus level underscores its significance as a supplementary analytical method (Ghasemzadeh *et al.*, 2015).

The integrated application of GC-MS and LC-MS offers a thorough qualitative profiling approach, facilitating the characterisation of both volatile and non-volatile components. This comprehensive analytical method directly confronts the shortcomings recognised in earlier research and constitutes the foundation of the methodology employed in the current investigation.

Species of the genus *Etlingera* have been linked to traditional medicinal applications, encompassing treatments for inflammation, microbial infections, digestive disorders, and postpartum recovery. The therapeutic applications are associated with bioactive secondary metabolites, including terpenoids, phenolic acids, and flavonoids (Lachumy *et al.*, 2010; Nagappan *et al.*, 2017). Sesquiterpenes, including β-caryophyllene and α-humulene, identified in *E. coccinea*, demonstrate anti-inflammatory and antimicrobial properties, underscoring the medicinal significance of

the genus (Daniel-Jambun *et al.*, 2017; Jems *et al.*, 2021).

Aldehydes like decanal and dodecanal, found in *E. coccinea*, have demonstrated antimicrobial properties, thereby reinforcing the application of *Etlingera* species in traditional medicinal practices for infections (Shahid-Ud-Daula *et al.*, 2015). Non-volatile compounds, such as flavonoid glycosides and phenolic acids identified at the genus level, are well-documented for their antioxidant, anti-inflammatory, and cytoprotective properties (Ghasemzadeh *et al.*, 2015; Resna *et al.*, 2021).

Research on related species, especially *E. elatior*, has shown notable antioxidant and antimicrobial properties, thereby reinforcing the pharmacological potential of the genus (Ghasemzadeh *et al.*, 2015; Lachumy *et al.*, 2010). While specific pharmacological evaluations of *E. coccinea* are limited, the similarity in phytochemical composition among *Etlingera* species indicates that *E. coccinea* may exhibit similar therapeutic properties. Therefore, thorough phytochemical characterisation is crucial to facilitate future bioactivity research and to confirm the medicinal potential of this under-researched species.

Recent studies have emphasised the diverse biological activities linked to secondary metabolites in *E. coccinea*, beyond traditional medicinal applications. An investigation into the allelopathic effects of *E. coccinea* extracts demonstrated inhibitory activity on seed germination and seedling growth, attributed to the presence of flavonoids, tannins, saponins, and terpenoids (Mohamad Zainurin *et al.*, 2021). While allelopathic activity is not directly associated with therapeutic applications, it offers indirect evidence regarding the biological efficacy of the species' secondary metabolites and underscores its significance as a source of bioactive compounds.

Additional evidence for the therapeutic potential of *E. coccinea* can be derived from research on closely related species within the genus. Recent studies on *E. elatior* have indicated notable antioxidant and anticancer properties linked to phenolic and flavonoid compounds, with variations affected by ecological and geographical factors (Asmilia *et al.*, 2025; Wen *et al.*, 2023). The close taxonomic and phytochemical similarities between *E. elatior* and *E. coccinea* suggest potential pharmacological relevance for *E. coccinea*, highlighting the necessity of thorough phytochemical profiling.

The documented therapeutic and biological properties of *Etlingera* species, encompassing antioxidant, antibacterial, anti-inflammatory, and several other

bioactivities, underscore the pharmacological potential of this genus and validate its traditional medicinal applications. Nevertheless, despite these encouraging results, a significant portion of the current evidence originates from initial bioactivity testing and research predominantly centred on volatile components, with insufficient attention to thorough chemical characterisation. Specifically, comprehensive data on non-volatile secondary metabolites and their direct correlation with certain biological functions is limited for *E. coccinea*. These limitations highlight the necessity for a more thorough and integrated assessment of the phytochemical composition of this species, hence validating the identification of critical research gaps addressed in the subsequent section.

2.4 Secondary Metabolites

The genus *Etlingera* exhibits a diverse chemical profile, as numerous studies have identified various volatile and non-volatile compounds among its species. Phytochemical studies indicate that this genus is primarily characterised by monoterpenes, sesquiterpenes, aldehydes, fatty acids, and diverse phenolic metabolites, which play a significant role in their ethnomedicinal relevance and biological functions.

A significant amount of research examines the volatile compounds of *Etlingera* species through GC-MS analysis. *E. elatior* has frequently been reported to contain β-caryophyllene, α-humulene, and 1,8-cineole as major compounds (Ghasemzadeh *et al.*, 2015; Maimulyanti & Prihadi, 2015; Mutmainah *et al.*, 2024). β-Caryophyllene is notably prevalent, with research suggesting concentrations ranging from 20–30 % within the essential oil composition. *E. littoralis* exhibits elevated concentrations of aldehydes, including decanal and dodecanal, as well as sesquiterpenes such as germacrene D, with decanal accounting for 15–20 % of the total volatile compounds (Arista *et al.*, 2023; Van, 2021). Genus-level evaluations consistently detect α-pinene, limonene, camphene, and germacrene D across various *Etlingera* species (A. & Mohammad, 2019; Nagappan *et al.*, 2017; Vairappan *et al.*, 2012), indicating a distinct chemotaxonomic pattern within the genus.

Conversely, there is a limited number of studies that have characterised non-volatile compounds through LC-MS. Available analyses of related species, such as *E. elatior*, indicate the presence of flavonoid glycosides, phenolic acids, and other semi-

polar constituents (Ghasemzadeh *et al.*, 2015; Resna *et al.*, 2021). The constituents exhibit a strong association with the antioxidant and medicinal properties documented for the genus.

Medical plants are high in secondary metabolites such as alkaloids, glycosides, flavonoids, insecticides, and steroids. These active metabolites have a high medical value and are widely used in the medicine and pharmaceutical industries (Marimuthu (a) Antonisamy & Sankara Raj, 2016). Medicinal plants have been used for therapeutic purposes across cultures for ages. They have been discovered and used in traditional medicine practices. Therefore, plants are functional foods as well as a major form of traditional medicine which gives influential act to the rural people in the isolated parts of developing countries (El-shemy, 2017). These plants have bioactive chemicals compounds that can have a beneficial effect on the well-being of humans (Fabricant & Farnsworth, 2001).

Secondary metabolites in plants are a large group of chemical compounds that typically are assumed to be generated by most plant species to deal with biotic and abiotic stress (Chomel *et al.*, 2016). It is not the main metabolites like primary metabolites which helps in the growth development and reproduction of an organism (Irchhaiya *et al.*, 2015). The presence of secondary metabolites in the plants shows the existing of its medicinal potential such as tannins, flavonoids, saponins, alkaloids that plays important roles to reveal the discovery of a therapeutic agent (Sonam *et al.*, 2017).

Among of the chemical constituents, terpene is one of the largest classes of natural products because of their interesting chemical diversity that have gained much attention due to their important physiological roles, ecological roles and broad pharmaceutical and industrial applications (Jiang *et al.*, 2016). Flavonoids are also an important class in natural products with polyphenolic structure and they are often found in fruits, vegetables and certain beverages (Panche *et al.*, 2016).

2.4.1 Alkaloids in *Etlingera species*

The most prevalent secondary chemical constituents are generally heterocyclic nitrogen atoms synthesised from amino acid precursors, wherein one or more hydrogen atoms are substituted by various radicals, predominantly including oxygen (Njoku *et al.*, 2011). The term alkaloids is derived from "alkaline," which referred to any

nitrogenous base (Kurmukov, 2013). The study by Lachumy *et al.*, (2010) conducted a phytochemical screening of 80% methanol extracts from dried *E. elatior* flowers. The test findings indicated an absence of alkaloids. A study on *E. elatior* flowers conducted by Maimulyanti and Prihadi, (2015) found that crude extracts of methanol and ethyl acetate exhibited no evidence of alkaloids. Khor *et al.*, (2017) validated the presence of alkaloids in the essential oils of *E. elatior* by phytochemical screening. Shahid-ud-daula *et al.*, (2019) examine the methanolic extracts of the leaves, stems, and rhizomes of *E. fimbriobracteata*. Nonetheless, all crude extracts produced negative results in alkaloid phytochemical screening. The review of *E. elatior* by Resna *et al.*, (2021) indicated the presence of alkaloids in certain areas of the plant. Alkaloids were discovered in the floral components of the plant (Sukandar *et al.*, 2018). Conversely, the stem sections revealed the presence of alkaloids in *n-hexane*, chloroform, and methanol extracts (S. S. Susilowati, S. Martono, S. Riyanto, 2011).

Methanolic extracts of leaves, stems, and rhizomes of *E. coccinea* were analysed for phytochemicals. The alkaloid test was conducted using three distinct reagents: Mayer, Wagner, and Dragendorff's, all of which yielded negative results (Shahid-Ud-Daula *et al.*, 2015). The investigation of *E. coccinea* leaf and stem Mohamad Zainurin *et al.*, (2021) similarly indicated that all extracts—*n-hexane*, ethyl acetate, and methanol—yielded unfavourable results in the phytochemical screening for the alkaloid test. Nonetheless, the work by Wahyuni *et al.*, (2021) reported the presence of alkaloids in *E. alba* rhizomes.

2.4.2 Flavonoids in *Etlingera species*

Polyphenols, often known as phenolic compounds, constitute a diverse and plentiful category of chemical substances present in plants. This extensive and broadly dispersed category encompasses a diverse array of chemicals that demonstrate significant structural and functional variation. Polyphenols are abundant in plant tissues, including fruits, vegetables, seeds, bark, and leaves, and they play significant roles in plant physiology and ecology (Bravo, 1998). Flavonoids are a class of polyphenolic phytonutrients categorised within the plant kingdom according to their chemical structure, encompassing anthocyanidins, flavanols, flavones, myricetin, flavanones, and kaempferol (Njoku *et al.*, 2011; Venkatanagaraju & Xavier, 2018). They are identifiable

by their distinctive 15-carbon skeleton, which acts as a structural basis for their various subclasses; the structural diversity of these subclasses enhances the versatility of flavonoids, enabling them to execute a range of biological functions (Panche *et al.*, 2016). Flavonoids possess several medicinal properties, including anticancer, antioxidant, anti-inflammatory, and antiviral activities, rendering them vital to human health (Ullah *et al.*, 2020).

According to Herrmann (1988), flavanols and flavones are found in all regions of the plant, and their synthesis is influenced by light conditions. They were significantly concentrated in leaves, and both chemicals increase carbon dioxide levels. Herrmann (1988) noted that ultraviolet B light intensities enhance the formation of phenolic compounds, specifically flavonoids. A significant concentration of these secondary metabolites accumulates in the vacuoles of epidermal cells, safeguarding the underlying tissue by absorbing ultraviolet wavelengths. Consequently, it may be asserted that leaves have a greater concentration of phenolic compounds compared to rhizomes or stalks/stems.

The investigation of the methanolic extract of *E. coccinea* leaves, rhizomes, and stems by Shahid-Ud-Daula *et al.*, (2015) revealed that the leaf extracts contain the highest flavonoid concentration, followed by the rhizomes and stems. The study by Mohamad Zainurin *et al.*, (2021) revealed minor differences in the dried *E. coccinea* leaf and stem, which were extracted by maceration with *n-hexane*, ethyl acetate, and methanol. All crude extracts exhibit the presence of flavonoids, except for the *n-hexane* stem extracts. However, tannins, which are a class of phenolic chemicals, exhibit negative results in both *n-hexane* stem extracts and methanol leaf extracts. This may be attributed to the higher quantity of flavonoids in the methanol extract of the leaves compared to the phenolic group, specifically tannins.

Lachumy *et al.*, (2010) employed maceration with 80 % methanol to extract the dried *E. elatior* flower. A phytochemical screening was conducted, revealing the presence of flavonoids. The research conducted by Maimulyanti and Prihadi, (2015) indicates that the maceration of fresh *E. elatior* flowers with methanol yields favourable outcomes in the phytochemical screening for flavonoids in both methanol and ethyl acetate extracts. Shahid-ud-daula *et al.*, (2019) conducted same studies on methanolic extracts of *E. fimbriobracteata*. The flavonoid content in leaves exceeded that in rhizomes and stems. The flavonoid concentration was four times greater than that

reported in their 2015 study on *E. coccinea*. The study by Wahyuni *et al.*, (2021) indicates the presence of flavonoids and phenolic compounds in the rhizomes of *E. alba* from Southeast Sulawesi. *E. alba* was extracted using maceration utilising 96 % ethanol in their investigation. The review by Resna *et al.*, (2021) indicates that the floral components of *E. elatior* contain flavonoids in methanolic and ethyl acetate extracts (Maimulyanti & Prihadi, 2015). The stem extract contained *n-hexane*, chloroform, and methanol (S. S. Susilowati, S. Martono, S. Riyanto, 2011).

2.4.3 Tannins in *Etlingera species*

Tannins are molecules of intermediate to high molecular weight and belong to a class of phenolic compounds present in varying quantities in numerous fruits and vegetables consumed by humans (Bravo, 1998; Ghosh, 2015). Tannins are compounds characterised by high hydroxylation that can create insoluble complexes with proteins and carbohydrates (Bravo, 1998). Tannins exhibit solubility in both water and alcohol and are present in the roots, bark, stems, and outer layers of plant tissues (Njoku *et al.*, 2011). Tannins exhibit antiviral, antibacterial, antiparasitic, anti-inflammatory, antiulcer, and antioxidant properties, indicating potential therapeutic applications (Marimuthu (a) Antonisamy & Sankara Raj, 2016). Tannins possess antioxidant properties and exhibit antiseptic characteristics attributed to their phenolic groups. Ayurvedic medicines derived from tannin-rich plants have been utilised in the treatment of disorders including leucorrhoea, rhinorrhea, and diarrhoea (Njoku *et al.*, 2011).

Tannins were present in the methanol extracts of *E. elatior* flowers but were undetected in the ethyl acetate extracts, as reported by Maimulyanti and Prihadi, (2015). According to the study by Mohamad Zainurin *et al.*, (2021), tannin exhibits positive results; however, contrasting outcomes are observed in *n-hexane* stem and methanol extracts of *E. coccinea* leaves. The study of *E. alba* rhizome from Southeast Sulawesi, extracted via maceration with 96 % ethanol, included a phytochemical screening for phenolic compounds, which yielded positive results (Wahyuni *et al.*, 2021). This can also be regarded as a tannin test, as it falls within the category of phenolic compounds. The study of dried flowers of *E. elatior*, extracted using 80 % methanol, revealed a positive presence of tannins as indicated by phytochemical screening (Lachumy *et al.*, 2010).

Khor *et al.*, (2017) investigated the essential oils of the *E. elatior* flower. The researchers conducted a phytochemical screening of phenols utilising the ferric chloride test, which also assessed the presence of tannins. The test results indicated an absence of tannin. The essential oils exhibited a higher content of flavonoids compared to phenolic compounds, as flavonoids are polyphenolic and can be categorised into various groups (Subedi *et al.*, 2014).

The review by Resna *et al.*, (2021) on the phytochemical and anti-inflammatory screening of all parts of *E. elatior* indicates that only the flower parts contain tannins. The methanolic and phenolic acid extracts demonstrate the presence of tannin and tannic acid, respectively (Maimulyanti & Prihadi, 2015).

2.4.4 Terpenoids in *Etlingera species*

Terpenoids represent a diverse category of naturally occurring compounds, primarily consisting of acyclic, bicyclic, or monocyclic hydrocarbons synthesised from five-carbon isoprene units (C_5H_8) (Chemat *et al.*, 2019; Kurmukov, 2013). Terpenoids represent a category of terpenes characterised by modified functional groups and the alteration or repositioning of oxidised methyl groups at different sites. The structural diversity of terpenoids provides them with a broad range of biological functions, rendering them globally important for therapeutic applications in the treatment of various disorders (Perveen, 2018). Natural lipids are present in all classes of living organisms, rendering them the most prevalent natural compounds (Kurmukov, 2013). Their physical qualities are diverse, primarily sourced from conifers and fruit pomace. Numerous terpenoids possess significant commercial value due to their diverse applications in food, medicine, cosmetics, hormones, and vitamins (Chemat *et al.*, 2019; Kurmukov, 2013).

The study examines the presence of terpenoids in *E. alba* rhizomes from Southeast Sulawesi (Wahyuni *et al.*, 2021). The study utilised 96% ethanolic crude extracts. The research conducted by Mohamad Zainurin *et al.* (2021) on dried *E. coccinea* stem and leaf identified the presence of terpenoids in all crude extracts, including *n-hexane*, ethyl acetate, and methanol, with the exception of the *n-hexane* leaf and ethyl acetate leaf extracts. In 2010, the presence of terpenoids in dried *E. elatior* flowers was reported by Lachumy *et al.* In her study, the *E. elatior* flowers were oven-

dried at 50 ºC, as noted by Mohamad Zainurin *et al.* (2021). The leaf and stem of *E. coccinea* were subjected to oven drying at 50 ºC for a duration of three days. High-temperature stress often increased the production of secondary metabolites; however, some studies indicated a reduction in these compounds in plants subjected to high-temperature stress (Shibata *et al.*, 1998). Secondary metabolites exhibit variations in response to elevated temperatures, influenced by species-specific factors and other conditions (Li *et al.*, 2020). Under heat-stress conditions, alterations in gene expression progressively contribute to the development of heat tolerance, manifesting as a plant's acclimation or adaptability to elevated temperatures (Hasanuzzaman *et al.*, 2017).

2.4.5 Saponin in *Etlingera species*

Saponins represent a diverse group of secondary metabolites widely distributed across the plant kingdom. The term saponin derives from *Saponaria vaccaria* (*Quillaja saponaria*), a plant abundant in saponins that has been historically utilised for soap production. Saponins generate a stable foam in aqueous solutions, such as soap, attributed to their 'soap-like' properties (Kurmukov, 2013; Njoku *et al.*, 2011).

Saponins are regarded as a significant component in traditional Chinese medicine, constituting the majority of the recorded biological effects. Saponins have been shown to reduce inflammation (Marimuthu (a) Antonisamy & Sankara Raj, 2016). Saponins are extensively marketed as dietary supplements and nutraceuticals. Saponins lower blood cholesterol levels, decrease cancer risk, and attenuate blood glucose response. A diet high in saponins may prevent dental cavities and platelet aggregation, treat hypercalciuria in humans, and act as an antidote for acute lead poisoning (Shi *et al.*, 2004).

Lachumy *et al.* (2010) conducted a phytochemical screening of the dried flowers of *E. elatior* and reported the presence of saponins in the crude extract obtained using 80% methanol. The study by Maimulyanti and Prihadi (2015) on *E. elatior* flowers found that saponins were present only in ethyl acetate extracts and absent in methanol extracts. The methanolic extracts of the leaves, stems, and rhizomes of *E. fimbriobracteata* were subjected to a frothing test to assess the presence of saponins, yielding a positive result (Shahid-ud-daula *et al.*, 2019). The review study by Resna *et al.* (2021) on the phytochemical and anti-inflammatory properties of *E. elatior* indicates

the presence of saponins in the stem methanol extracts, as well as in the methanol and ethyl acetate extracts of the flower (Maimulyanti & Prihadi, 2015; S. S. Susilowati, S. Martono, S. Riyanto, 2011).

The study of *E. coccinea* conducted by Shahid-Ud-Daula *et al.* (2015) utilised methanolic extracts from leaves, stems, and rhizomes for phytochemical screening. Saponins are present in all parts of the plants; however, the results contradict for the rhizome extract. In dried *E. coccinea* leaf and stem, all *n-hexane*, ethyl acetate, and methanol extracts tested positive for saponins, with the exception of the ethyl acetate extracts from both samples (Mohamad Zainurin *et al.*, 2021).

The identified literature gaps underscore the necessity for a thorough and systematic phytochemical examination of *E. coccinea*, especially concerning the identification of both volatile and non-volatile secondary metabolites. Existing studies offer valuable preliminary insights; however, the predominance of limited analytical approaches has constrained a comprehensive understanding of the species' chemical complexity. Addressing these gaps necessitates the use of strong and complementary analytical methods that can effectively differentiate various classes of phytochemicals. This section reviews the analytical methods utilised in phytochemical studies of *Etlingera* species, focussing on chromatographic and mass spectrometric techniques for qualitative compound identification.

2.5 Chemical Profiling Analysis

Research on *E. coccinea* is limited; however, existing studies consistently identify a core set of volatile constituents primarily composed of sesquiterpenes and aldehydes. Jems *et al.* (2021) conducted GC-MS investigations that identified β-caryophyllene and α-humulene as the predominant sesquiterpenes, along with significant amounts of the aldehydes decanal and dodecanal, which are responsible for the species' distinctive aroma. Daniel-Jambun *et al.* (2017) confirm the presence of β-caryophyllene and other sesquiterpenes in antimicrobial-active extracts. Additionally, Shahid-Ud-Daula *et al.* (2015) report the presence of fatty acid derivatives in hexane fractions.

The results align with patterns noted in other *Etlingera* species, thereby reinforcing the chemotaxonomic consistency within the genus. Nonetheless, *E. coccinea* is inadequately researched regarding non-volatile metabolites, as there are no

published LC-MS profiles specific to this species. Genus-level LC-MS studies suggest that *E. coccinea* may contain flavonoid glycosides and phenolic acids akin to those identified in *E. elatior* and related taxa (Ghasemzadeh *et al.*, 2015; Resna *et al.*, 2021; Lachumy *et al.*, 2010); however, this has not been experimentally validated.

Soil characteristics significantly influence plant growth, metabolism, and phytochemical composition. Soil type, nutrient availability, pH, and heavy metal content significantly affect the biosynthesis of secondary metabolites such as phenolics, flavonoids, alkaloids, and terpenoids. These compounds function as antioxidants, defence chemicals, or metal chelators, aiding plants in adapting to environmental stresses (Qaderi *et al.*, 2023).

Ranau is primarily defined by ultramafic or serpentine soils originating from ultrabasic rocks. The soils exhibit elevated levels of heavy metals, including nickel (Ni), chromium (Cr), cobalt (Co), and manganese (Mn), alongside a deficiency in essential nutrients such as calcium (Ca), phosphorus (P), and potassium (K). The pH is moderately acidic, and the soil texture is typically loamy and friable, which aids root penetration while presenting nutrient limitations (Ent, 2011).

Plants that have adapted to these soils frequently display distinct metabolic profiles in response to abiotic stress. Elevated metal concentrations lead to oxidative stress, which stimulates the synthesis of secondary metabolites, including phenolic compounds and flavonoids, known for their potent antioxidant properties. Alkaloids and other nitrogen-containing compounds are synthesised as components of the plant's defence strategy. Members of the *Etlingera* genus, such as *E. coccinea*, have been documented to possess flavonoids, phenolics, and various antioxidant compounds, which may be upregulated in reaction to the metal-rich soils of Ranau (Ent, 2011). This indicates that the soil chemistry of Ranau may elevate the concentration of stress-related phytochemicals in these species, thereby potentially augmenting their medicinal or bioactive value.

Tambunan primarily consists of alluvial and clay-loam soils derived from river deposits. The soils possess greater nutrient richness, increased organic matter content, and demonstrate slower drainage relative to the ultramafic soils of Ranau. The pH ranges from slightly acidic to neutral, and the texture is denser owing to increased clay content, influencing water retention and aeration (Simon *et al.*, 2017). Plants in Tambunan experience reduced metal-induced stress, resulting in a more balanced

production of secondary metabolites. The phytochemical composition of *Etlingera* species, including *E. coccinea*, is primarily determined by genetic factors and general environmental conditions, rather than by extreme abiotic stress. Flavonoids, phenolics, alkaloids, and terpenoids remain present, albeit in proportions characteristic of the species' standard metabolic processes (Jems *et al.*, 2021). Thus, although plants from Tambunan contain bioactive compounds, their concentrations may be less pronounced or specialised in comparison to those found in ultramafic soils.

The variations in soil characteristics between Ranau and Tambunan suggest that the phytochemical profiles of *Etlingera* species may differ considerably across these locations. The metal-rich and nutrient-poor soils of Ranau are expected to promote the synthesis of stress-induced compounds, thereby elevating antioxidant activity and the concentration of metal-chelating metabolites. In contrast, Tambunan's nutrient-dense and less stressful soils facilitate the production of standard secondary metabolites (Ent, 2011; Simon *et al.*, 2017). The variations are important for the selection of *Etlingera* species, especially *E. coccinea*, for use in medicinal, nutritional, or industrial applications, as soil conditions can affect the quantity and quality of bioactive compounds. Comprehending the relationships between soil and phytochemicals establishes a basis for future experimental investigations and supports the choice of suitable analytical techniques for quantifying and characterising phytochemical compounds.

The reviewed literature indicates that although the Zingiberaceae family and the genus *Etlingera* have been thoroughly examined regarding morphology and volatile phytochemistry, *E. coccinea* is relatively underexplored, especially concerning its non-volatile chemical constituents. Current research primarily focusses on GC-MS profiling of essential oils, with limited incorporation of LC-MS or alternative chromatographic methods. The lack of thorough chemical profiling and the weak correlation between morphological traits and phytochemical composition underscore notable research deficiencies. The identified gaps justify the current study, which seeks to utilise an integrated analytical approach to characterise both volatile and non-volatile constituents of *E. coccinea*, thus offering new insights into the phytochemistry of this under-researched species.

CHAPTER 3
METHODOLOGY

3.1 Materials

3.1.1 Scope of Works

E. coccinea was obtained from Poring Hotspring, Ranau, and Crocker Range Park, Tambunan, with authorisation from Sabah Park and the Sabah Biodiversity Centre (SaBC). The access licence has been issued, and the permission number is JKM/MBS.1000-2/2 Jld. 12(115). The sampling sessions were consistently conducted with the presence of designated Sabah Park Ranger, Mr. Duni Molidin. The DMS coordinates for the sampled areas were 6.0457° N, 116.7034° E for Ranau and 5.533° N, 116.100° E for Tambunan. Subsequently, it was segmented, and only the stalk portions were selected for study.

Ranau and Tambunan are districts situated in the interior region of Sabah. Both districts are located inside the Crocker Range. Rivers and streams are likely ubiquitous over Ranau's landscape. These bodies of water augment the aesthetic appeal of the environment and may be crucial for adjacent ecosystems. Tambunan, while possessing mountains akin to those in the Crocker Range, may exhibit a topology that diverges slightly from Ranau, highlighting hills and likely providing picturesque vistas adorned with verdant foliage.

3.1.2 Chemicals

The lists of chemicals used were *n-hexane* AR grade (QRec brand), methanol AR grade (QRec brand), chloroform AR grade (QRec brand), sodium sulphate anhydrous (Systerm brand), acetonitrile HPLC grade (Systerm brand), Dragendorff's reagent, Mayer's reagent, hydrochloric acid (Merck brand), sulphuric acid (Merck brand), magnesium powder, ferric chloride powder.

3.1.3 Instruments

The lists of instruments used were: Laborata 4000 – Eyela N-1100 rotary evaporator, HPLC Agilent 1200 Series equipped with G1322A degasser, G1311A quaternary pump and G1315D Diode-Array detector (DAD); Liquid Chromatography Mass Spectrometer (LC-MS) Vanquish/Thermo Scientific equipped with Impact II/Bruker Quadrupole Time-of-Flight (QTOF) MS system and Gas Chromatography Mass Spectrometer (GC-MS) Agilent Technologies 7890A GC system equipped with 5975C inert XL EI/CI MSD with Triple-Axis detector.

3.2 Sample Preparations

About 10 kg of freshly collected *E. coccinea* plants from both locations were washed under cold running tap water to remove any dirt on it. Then, the plants were cut into different parts leaving behind only its stalks parts. The stalks parts were then placed on a dry tissue paper and left for air-dry.

3.3 Extractions

3.3.1 Maceration Method

Upon drying the stalk samples from both locations, the stalks were segmented into small pieces and ground using a blender. Subsequently, they commenced the extractions. The extraction method employed was maceration using 10% aqueous methanol. The mass of the dry sample from Ranau (ECS-R) prior to extractions was 98.41g. The weight of the dry sample from Tambunan (ECS-T) prior to extractions was 394.08 g.

Maceration is typically conducted at ambient temperature. The *E. coccinea* stalks were immersed in a 10% aqueous methanol solution within a volumetric flask. Intermittent agitation might enhance the rate of extraction. After leaving one night, the residual plant samples (marc) were removed to separate them from the solvent. The separation was performed through filtration utilising a cloth. The plant samples remaining in the volumetric flask were subsequently supplemented with new methanol. The aforementioned steps were reiterated three times. The filtrates were subsequently

combined. The addition of anhydrous sodium sulphate to the pooled filtrates facilitates the removal of any residual water. The extracts were concentrated via a Laborata 4000 – Eyela N-1100 rotary evaporator at a temperature of 45 ˚C until the extract achieved a waxy consistency. The crude extracts were further concentrated by putting them in a desiccator with silica gel. The weight of the extracts was measured three times until a stable weight was achieved.

3.3.2 Partitioning of Crude Extract

Subsequently, the extracts were carried on performing solvent-solvent partitioning. The solvent-solvent partitioning process was referred with slightly modification from Emran T., *et al.* (2015). Dissolved methanol extracts with 10% aqueous methanol then poured into separating funnel. Then 150 mL of *n-hexane* will be poured by three stages into the separating funnel and then pooled them together leaving the methanol extract in the separating funnel. The step then repeated with 150 mL of chloroform. By adding anhydrous sodium sulphate to remove water in it. The extracts concentrated by using Laborata 4000 – Eyela N-1100 rotary evaporator at temperature of 45˚C until the extract turned into wax texture. The crude extract further concentrates by placing them inside a desiccator containing silica gel. Thus, there will be *n-hexane*, chloroform and methanol fractions obtained.

3.4 Thin Layer Chromatography (TLC)

The *n-hexane*, chloroform and methanol fractions were used to perform TLC. The type of TLC plates used were from Merck brand 20 × 20cm size of aluminium sheets bonded with silica gel $F_{254.}$ The fractions were spotted on the origin of the normal phase TLC. The solvent system used were *n-hexane* and ethyl acetate. The ratio of the solvent systems used were 100% *n-hexane*, 9:1, 7:3, 5:5, 3:7, 1:9 and 100% ethyl acetate. The solvent systems were placed on the development jars according to their ratios. The spotted TLC plates sketched with the origin and solvent front lines were placed inside the jar. Then the elusions on the TLC plates was examine under 254nm UV lamp.

3.5 Phytochemical Screening

3.5.1 Test for Alkaloids

The test for alkaloids was conducted by using two tests, namely Dragendorff's and Mayer's test. The reagents were as follows:

a. Dragendorff's test: Dragendorff's reagent (Potassium bismuth chloride) was added about 1mL; an orange red precipitate shows the presence of alkaloids.

b. Mayer's test: Mayer's reagent (Potassium mercuric iodide solution) was added about 1 mL. The presence of alkaloids will give a formation of cream-colored precipitates.

3.5.2 Test for Flavonoids

Methods of hydrochloric test were referred to (Maimulyanti & Prihadi, 2015). 1mL of diluted extracts were added with mixture of 0.1 g magnesium and 1 mL hydrochloric acid. A yellow or violet coloration disappears on standing.

3.5.3 Test for Terpenoids

A Salkowski test (Wahyuni *et al.*, 2021) were used with some modifications. About 50 mg of extracts was shaken with 1 mL of chloroform followed by the additions of 1 mL of sulphuric acid, H_2SO_4. A reddish-brown coloration presence of terpenoids.

3.5.4 Test for Tannin

About 0.5 mL of extracts was added with 1 mL of distilled water. A 4 to 5 drops of 1% ferric chloride, $FeCl_3$ were added into it. Black or blue-green coloration was taken into positive results for the presence of tannin (Peash *et al.*, 2017).

3.5.5 Test for Saponin

Methods was referred to (Peash *et al.*, 2017), 1 mL of extracts was added with 3 mL of distilled water and proceed of shaking for 5 minutes. A 1 mL of foam which persist within 10 minutes indicates the presence of saponin.

3.6 Chemical Profiling of *E. coccinea*

3.6.1 High Performance Liquid Chromatography (HPLC)

The method was referred to (Daniel-Jambun *et al.*, 2017) with some modifications. In this analysis, methanol and chloroform fractions were subjected to HPLC. The analysis was performed at Kompleks Makmal Sains dan Agroteknologi (KOMSAT), UiTM Kota Kinabalu, Sabah. The HPLC instruments used was Agilent 1200 Series, equipped with G1322A degasser, G1311A quaternary pump and G1315D Diode-Array detector (DAD). The column used was reverse phased Zorbax SB-C18 column with 4.6 × 250mm diameter and particle size of 5µm.

About 10 ppm from the fraction's concentrations were used to carried out this analysis. A gradient elusion was used with solvent systems of pure water (A) and acetonitrile (B). The gradient elusion was used at: 90 % A at 0–5 min, 760 % A at 5–10 min, 40 % A at 10–15 min, 20 % A at 15-20 min and 10 % A at 20-25 min. The solvents were filtered and degassed prior to use. The solvent flow rate was set at 1.0 mL/min, and the injection volume used was 20 µL. The maximal UV absorption peak: 200 to 400 nm. The UV-Vis were used in DAD; hence, the UV profiles were compared to the previous studies of Zingiberaceae family and *Etlingera* genus.

3.6.2 Gas Chromatography Mass Spectrometer (GC-MS)

The *n-hexane* fractions underwent GC-MS analysis at the Pusat Instrumentasi dan Perkhidmatan Sains (PIPS), UMS. The GC-MS analysis was conducted following the framework established by Maimulyanti & Prihadi (2015), with several alterations implemented. The fractions were analysed using an Agilent Technologies 7890A gas chromatograph coupled with an Agilent Technologies 5975C inert XL EI/CI mass spectrometer featuring a Triple-Axis detector. The study utilised a narrow bore capillary column HP-5ms (30 m in length, 0.25 µm phase thickness, 0.25 mm diameter), coupled with an Agilent 7693 autosampler.

The operational parameters for the GC-MS column were as follows: initial temperature 60 ºC, final temperature 220 ºC, heating rate 2 k/min, and total duration 20 min. Helium was utilised as the carrier gas. The chemicals were identified by correlating their mass spectra with those catalogued in the mass spectral library. This analysis

utilised the ChemStation Integrator NIST11 database.

3.6.3 Liquid Chromatography Mass Spectrometer (LC-MS)

The methanol and chloroform fractions underwent LC-MS analysis at the Institute of Biotechnology, UMS. The LC-MS approach was based on the work of Daniel-Jambun *et al.* (2017) with certain changes. The analysis was performed with an LC-MS Vanquish/Thermo Scientific system, integrated with an Impact II/Bruker Quadrupole Time-of-Flight (QTOF) mass spectrometer. C18 column (2.1 x 50 mm, 1.7-micron particles) utilising a solvent system of A – 0.10% formic acid and B – acetonitrile with 0.10% formic acid; gradient from 40% to 100% B over 2 minutes, followed by 100% B for 0.5 minutes. The parameters are a flow rate of 0.5 mL/min, a concentration of 1 mg/mL, and an injection volume of 5 µL. The methodology utilised electrospray ionisation (ESI) in positive ion mode, with a scanning range of 50 m/z to 1500 m/z. The detected plant metabolites were analysed by comparing their mass spectra to those documented in the mass spectral library database (Bruker Compass DataAnalysis 4.3 and National Centre for Biotechnology Information). The identified plant metabolites were contrasted with previous studies of the genus and the advantages of the metabolites in plants.

CHAPTER 4
RESULTS AND DISCUSSIONS

4.1 Percentage Yields of Extracts

This chapter describes the findings derived from the extraction, fractionation, and chromatographic analysis of *E. coccinea* stalk extracts sourced from Ranau and Tambunan, Sabah. All results are analysed comparably to fulfil the major purpose of this study, which is to assess the differences in metabolite profiles between two geographical regions employing various chromatographic techniques. The percentage yield of each extract was computed to assess the efficacy of the extraction and partitioning operations.

A maceration extraction process was conducted on samples from both Ranau and Tambunan. The dried stalk samples were immersed in 10 % aqueous methanol to obtain ECSM-R and ECSM-T extracts. A total of 98.41 g was extracted from the dried stalk sample obtained from Ranau. The dried stalk samples from Tambunan weighed 394.08 g. The yield percentages for Ranau and Tambunan extracts were 12.77 % and 11.08 %, respectively. After maceration with 10 % aqueous methanol, the crude extracts underwent liquid-liquid partitioning with *n-hexane* and chloroform to yield fractions of varying polarity. The weights of each fraction were documented post-solvent removal, and the percentage yield was computed appropriately.

The fractional yields for Ranau extracts were 8.40 %, 5.42 %, and 2.90 % for the methanol, chloroform, and *n-hexane* fractions, respectively. The Tambunan extracts give fractions of 3.72 %, 4.94 %, and 1.02% for methanol, chloroform, and *n-hexane*, respectively. The data indicates that methanol fractions from Ranau and chloroform fractions from Tambunan exhibited the highest yield for their respective locations.

The yield data from both sampling locations are consolidated in Table 4.1 and Table 4.2. The extracts derived from Ranau exhibited higher percentage yields than those obtained from Tambunan. These differences may relate to variations in environmental conditions, including altitude, soil characteristics, and local microclimate; however, further investigation is necessary to establish a definitive relationship.

Table 4.1
Percentage Of Yields From Poring Hot Spring, Ranau

Extraction in 10 % aq methanol	12.77 %
Methanol fraction extract (ECSM-R)	8.40 %
Chloroform fraction extract (ECSC-R)	5.42 %
n-hexane fraction extract (ECSH-R)	2.90 %

Table 4.2
Percentage Of Yields From Crocker Range Substation, Tambunan

Extraction in 10 % aq methanol	11.08 %
Methanol fraction extract (ECSM-T)	3.72 %
Chloroform fraction extract (ECSC-T)	4.94 %
n-hexane fraction extract (ECSH-T)	1.02 %

Percentage yield serves as a preliminary measure of extraction efficiency; however, it does not to convey details regarding the chemical composition or complexity of the extracted materials. Consequently, additional qualitative analysis was necessary to evaluate potential variations in metabolite profiles between samples from Ranau and Tambunan. TLC was utilised as an initial comparative method to visualise the distribution and polarity characteristics of the extracts before conducting more advanced chromatographic analyses.

4.2 Thin Layer Chromatography (TLC)

A preliminary qualitative TLC examination was performed to evaluate the complexity and polarity variations among the extracts before conducting advanced chromatographic investigations. TLC functions as a swift and economical technique to visualise the separation patterns of chemicals and to assess chemical similarities or differences among samples. This comparative investigation emphasised the significance of TLC in assessing whether extracts from various geographical regions displayed divergent chromatographic behaviours within the same solvent system. Variations in the quantity of spots, and spot intensities offer preliminary evidence of differences in metabolite composition.

The fractions were submitted to perform normal phase of TLC. The aluminum

TLC plates were bonded with silica. This makes the stationary phase polar and supposedly the mobile phase is non-polar. Initially, the solvent ratio used for this was 100 % *n-hexane* until 100 % ethyl acetate. However, the other ratio was not showing the better elution of the compound. Therefore, for Ranau sample only 6 solvent ratios preferred, which were 9:3, 7:3, 5:5, 3:7, 1:9 of *n-hexane* to ethyl acetate and 100 % ethyl acetate. As for Tambunan sample, there were 5 solvent ratios preferred which were 7:3, 5:5, 3:7, 1:9 of *n-hexane* to ethyl acetate and 100 % ethyl acetate.

Figures 4.1 and 4.2 illustrate the TLC profiles of *n-hexane*, chloroform, and methanol fractions from Ranau and Tambunan. Despite the overall similarity in spot patterns, modest discrepancies in Rf values and spot intensities were noted, indicating slight variations in compound distribution between the two locations. These observations warrant more comparative analysis employing HPLC, LC-MS, and GC-MS.

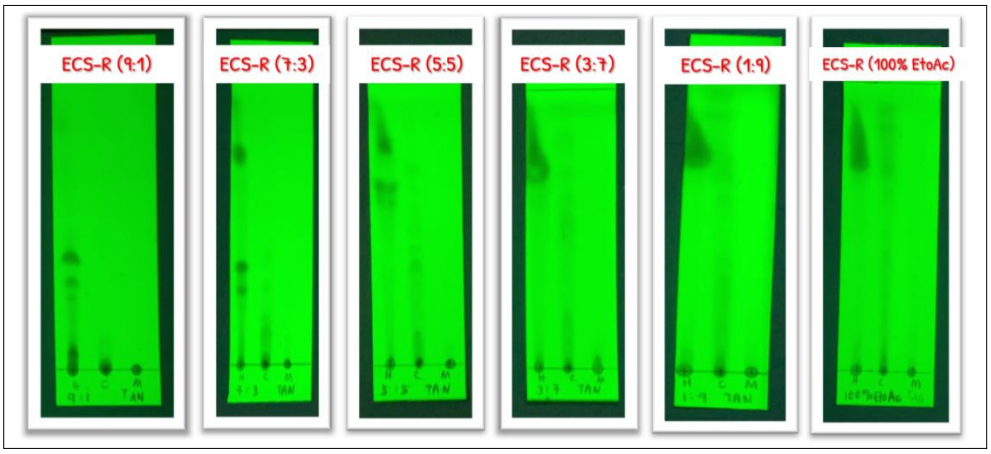


Figure 4.1 TLC Plates For *n*-hexane, Chloroform And Methanol Fractions From Ranau.

The TLC analysis in the Ranau area indicates that the ratios presented in Figure 4.1 for the fraction of *n-hexane* (ECSH-R) demonstrate effective elution towards the solvent fronts. ECSH-R demonstrates effective separations and intense spots at a 9:1 ratio; however, it did not achieve higher elution on the plate. This results from the strong retention of the compound by the stationary phase. Consequently, this compound is likely semipolar to slightly polar. At a 7:3 ratio, ESCH-R demonstrates effective separation with a pronounced spot; however, the elution is increasing at this stage. This ratio indicates that the eluted compound was more non-polar, as evidenced by its reduced retention on the stationary phase and greater mobility in the mobile phase. At

a 7:3 ratio, the chloroform fraction (ECSC-R) begins to exhibit separation, although it continues to elute at a lower level. The compound at this stage was still strongly retained, although its strength in the stationary phase was beginning to diminish. Their polarity began to align with that of the mobile phase. Furthermore, at the ratios of 5:5, 3:7, 1:9, and 100 % ethyl acetate, the elution fractions of ECSH-R and ECSC-R exceeded those of the prior ratios. Figure 4.1 illustrates that ECSH-R fractions at the specified ratio exhibit a highly intense spot. The compounds may exist in that ratio.

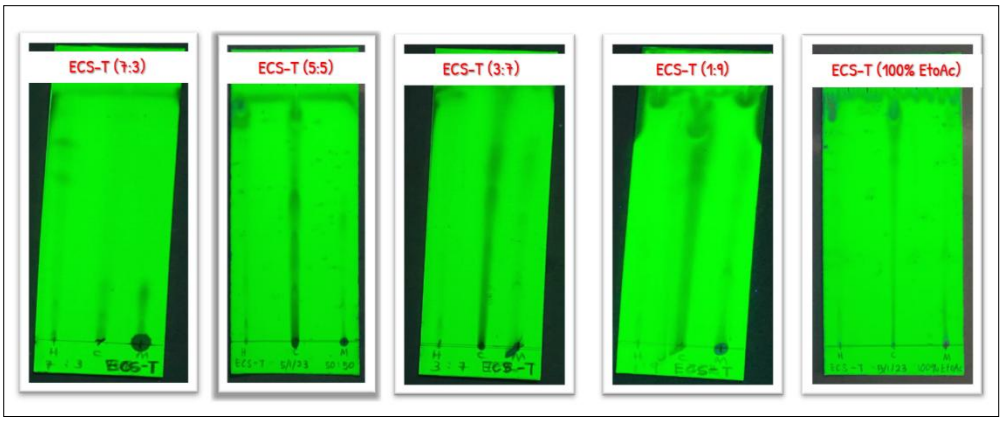


Figure 4.2 TLC Plates For *n*-hexane, Chloroform And Methanol Fractions From Tambunan.

In TLC for Tambunan, ECSH-T demonstrates effective separation and enhanced elution, although spots appear faded at a 7:3 solvent ratio. ECSC-T exhibits separations despite insufficient elution, indicating that the compound remains strongly retained by the stationary phase at this stage. At a 5:5 ratio, ECSH-T exhibited a pronounced spot at the top of the plate, suggesting that the eluted compound may be semipolar to polar in nature. The ECSC-T at this ratio exhibited poor separation until reaching the top of the plate, resulting in tailing with pronounced spots. The compound eluted at this ratio may be classified as nonpolar to semipolar. The elution of ECSH-T and ECSC-T for the remaining ratios of 3:7, 1:9, and 100 % ethyl acetate was consistent with that observed in the 5:5 ratio.

4.3 Phytochemical Screening

Qualitative phytochemical screening was conducted to identify the presence of principal classes of secondary metabolites, such as flavonoids, terpenoids, tannins,

alkaloids, and saponins. The analysis of each test relied on recognised colour reaction and precipitation scales documented in the literature. Phytochemicals are naturally occurring compounds present in plants. Plants contain a range of chemical compounds, such as phenols, flavonoids, alkaloids, terpenoids, and saponins. Phytochemical screening involves the identification of specific phytoconstituents present in different parts of a plant (Wachira *et al.*, 2024). Phytochemical screening is essential for the quantitative assessment and localisation of active components. The therapeutic properties of active groups are due to chemical substances that exert specific physiological effects on the human body (Suliman, 2018).

Six tests were conducted in the phytochemical screening of *E. coccinea* stalks. The phytochemical classes investigated for their presence included alkaloids, flavonoids, terpenoids, tannins, and saponins. The screening was conducted in test tubes containing the appropriate reagents. The alkaloid was synthesised using two distinct reagents. The presence of alkaloids in previous studies of the *Etlingera* genus accounts for this observation. Therefore, the objective was to reaffirm the presence of the class in the *E. coccinea* species specifically.

Flavonoids were detected by the emergence of a vivid yellow or orange hue upon the addition of reagents, as outlined by Herrmann, (1988). Terpenoids were validated by the development of a reddish-brown interface by the Salkowski test (Abayomi, 2009). Tannins were identified by the development of a blue-black or greenish precipitate upon interaction with ferric chloride, in accordance with the standard protocols established by (William Charles, 2009).

The results of the phytochemical screening for both Ranau and Tambunan extracts are presented in Table 4.3. Both sites exhibited flavonoids, terpenoids, and tannins, however alkaloids and saponins were not detected. The observed consistency in phytochemical classes indicates that regional variation did not markedly affect the presence or absence of principal metabolite groups, but disparities in compound abundance may still be present.

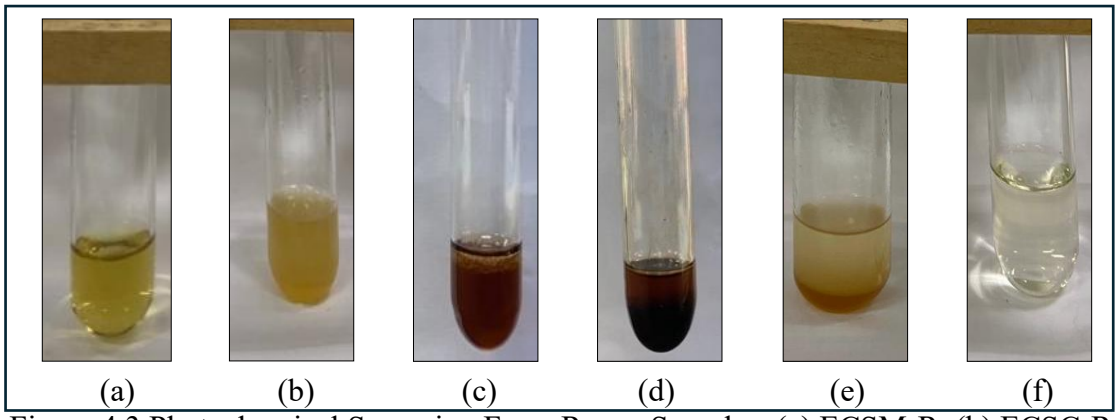
Table 4.3
Phytochemical Screening Test

PHYTOCHEMICAL SCREENING	FRACTIONS			
	ECSM-R	ECSC-R	ECSM-T	ECSC-T
Alkaloid (Dragendorff's Test)	-	-	-	-
Alkaloid (Mayer's Test)	-	-	-	-
Flavonoid (Hydrochloric acid test)	+	++	+	++
Terpenoid (Salkowski's Test)	++	++	++	++
Tannin ($FeCl_3$ Test)	+	+	+	+
Saponin (Froth Test)	-	-	-	-

Note: Qualitative approximation scale: '+' trace, '++' moderate, '-' absent

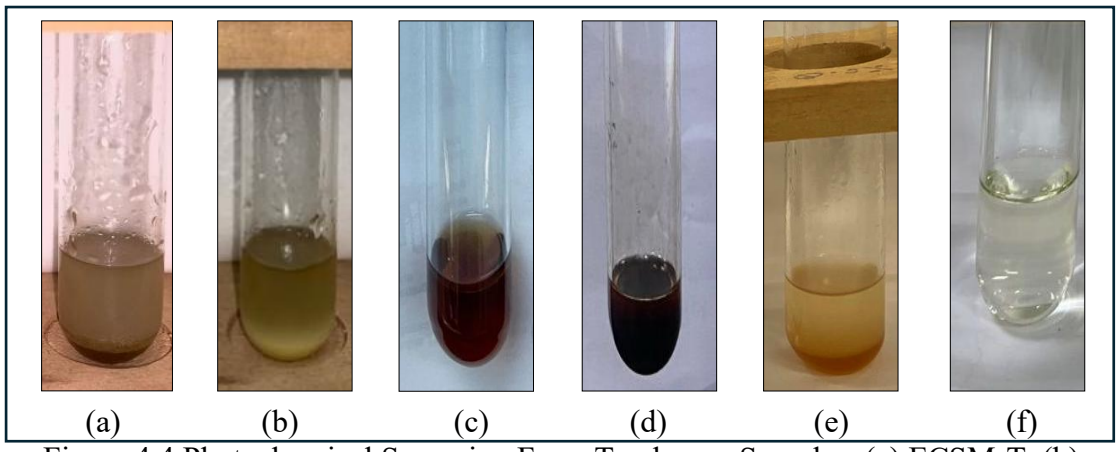
The screening revealed minimal amounts of methanol and chloroform from both locations examined. The insufficient quantity of hexane extracts precluded the possibility of conducting this screening. The data presented in the tables indicate that both fractions from Ranau and Tambunan exhibit positive results exclusively for flavonoids, tannins, and terpenoids. No presence of alkaloid compounds was detected in *E. coccinea* through both tests. The test tube containing Dragendorff's reagent did not change from its original state to an orange-red precipitate. The results were consistent with those observed in Mayer's test tube, showing no formation of cream-coloured precipitates. Therefore, alkaloids are absent. Similarly, saponin does not produce persistent foam within 10 minutes, indicating the absence of these compounds.

The screening test for *E. coccinea* stalks yielded positive results for flavonoids, terpenoids, and tannins. The chloroform fractions from Ranau and Tambunan were subjected to hydrochloric testing, resulting in the disappearance of colouration upon standing. The observations indicate the presence of flavonoids. However, the methanol fractions from both Ranau and Tambunan exhibit minimal yellow colouration that diminishes upon standing. Despite this, there remained a possibility that flavonoids were present in the fractions, as they could decolorise the yellow-colored fractions.



(a) (b) (c) (d) (e) (f)

Figure 4.3 Phytochemical Screening From Ranau Samples: (a) ECSM-R, (b) ECSC-R Tannin test; (c) ECSM-R, (d) ECSC-R Terpenoid Test; And (e) ECSM-R (f) ECSC-R Flavonoid Test.



(a) (b) (c) (d) (e) (f)

Figure 4.4 Phytochemical Screening From Tambunan Samples: (a) ECSM-T, (b) ECSC-T Tannin Test; (c) ECSM-T, (d) ECSC-T Terpenoid Test; And (e) ECSM-T (f) ECSC-T Flavonoid Test.

The screening test for terpenoids was confirmed in both the methanol and chloroform fractions of Ranau and Tambunan. The fractions tested with Salkowski reagents exhibited a reddish-brown colour, contrasting with the existing yellowish fractions. Finally, tannin screening revealed that both methanol and chloroform fractions from Ranau exhibited a faint blue-green colouration. The observation of colour changes was not significant; nonetheless, it alters the yellowish fractions to a pale blue-green hue. It was previously noted that the fractions may contain tannins. It was observed to have the capability to alter the initial colours to some extent. In the Tambunan fractions, significant changes to blue-green colouration were observed. In conclusion, the tannin content in Tambunan fractions exceeds that of Ranau.

4.4 High Performance Liquid Chromatography (HPLC)

HPLC research was conducted to evaluate the non-volatile metabolite profiles of *E. coccinea* stalk extracts sourced from Ranau and Tambunan. Ultraviolet detection was performed at 210 nm and 254 nm, which are standard wavelengths for the analysis of phenolic and flavonoid chemicals. References to alternate wavelengths have been excluded to eliminate uncertainty and maintain uniformity in data interpretation. Absorption in these areas is often linked to conjugated systems and aromatic structures characteristic of phenolic compounds found in *Etlingera* species.

The HPLC chromatograms of methanolic extracts from Ranau and Tambunan displayed analogous retention time patterns, suggesting the presence of similar classes of polar chemicals in both samples. Despite the overall chromatographic profiles being essentially analogous, some discrepancies in peak intensities were noted. These discrepancies indicate variability in the relative abundance of compounds rather than the presence of unique metabolites specific to either geographical area. The chromatographic profiles of chloroform extracts from Ranau and Tambunan exhibited nearly comparable peak distributions and retention characteristics. The detection of absorption bands at 210 nm and 254 nm aligns with other studies detailing flavonoid and phenolic compounds in *Etlingera* species and other members of the Zingiberaceae family. Due to the absence of validated standards, chemical identities were not determined merely based on retention times or UV absorption properties.

The analysis was conducted using a gradient from 90 % pure water to 100 % acetonitrile, encompassing all solvent ratios. However, the peaks exhibit insufficient separation. The mobile phase was subsequently altered to include only odds ratios and ultimately adjusted to the current ratio. Operating this analysis proved challenging due to the overheated detector lamp. The analysis successfully produced the optimal chromatogram. The gradient elution was implemented as follows: 90 % A from 0 to 5 minutes, 70 % A from 5 to 10 minutes, 40 % A from 10 to 15 minutes, 20 % A from 15 to 20 minutes, and 10 % A from 20 to 25 minutes. The concentrations of mobile phase solvents are illustrated in the graph below.

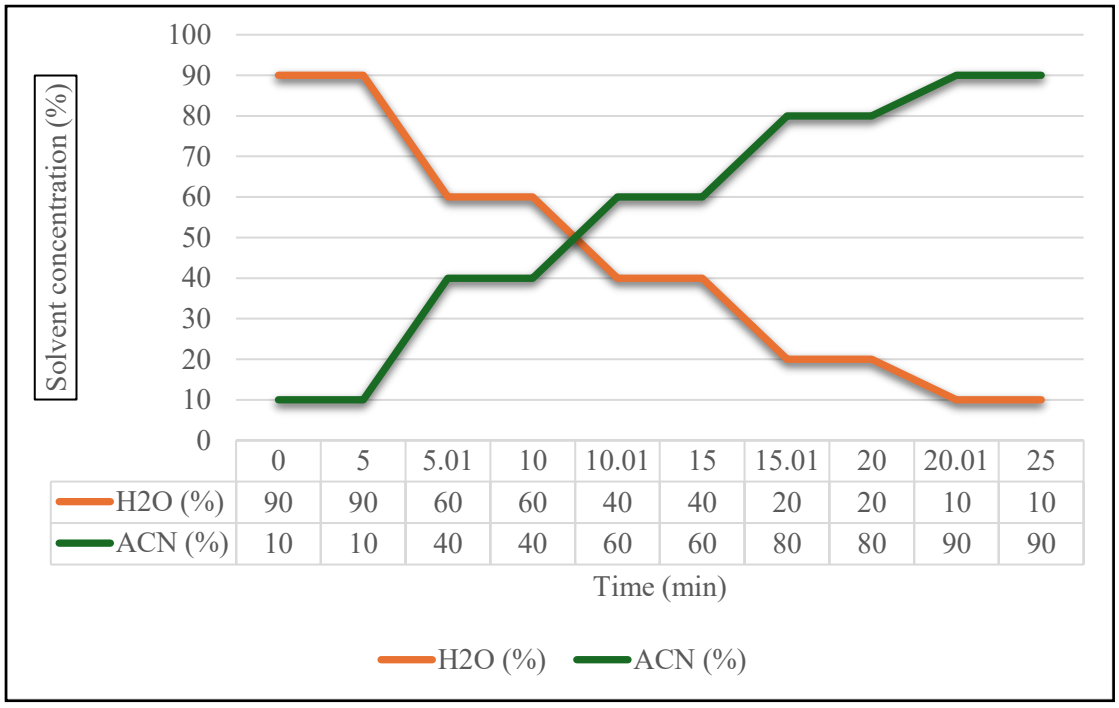


Figure 4.5 Graph Of HPLC Solvent System

4.4.1 Comparisons of HPLC Analysis of ESCM-R and ECSM-T

The methanol fraction of Ranau and Tambunan was analysed using HPLC. Three UV detection wavelength signals were utilised: 210 nm, 225 nm, and 254 nm. The signals utilised were informed by prior studies on the *Etlingera* genus, which predominantly employed similar wavelength signals in HPLC analysis. This analysis of ECSM-R focusses solely on the wavelength signal at 210 nm with a band slit of 8, which exhibits a UV-Visible spectrum profile. During ECSM-T, measurements were taken at 210 nm with a band slit of 8, 225 nm with a band slit of 8, and 254 nm with a band slit of 4. The UV-Visible profile resulted from the measured absorption or transmission of discrete wavelengths of UV-Visible light by the compounds. The chromatogram for ECSM-R and ECSM-T is presented below.

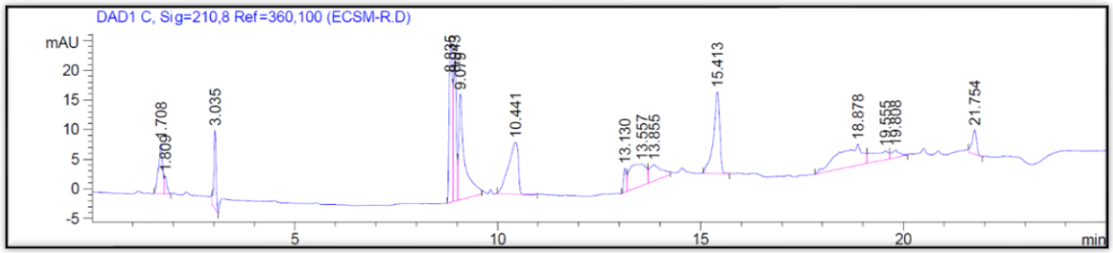


Figure 4.6 ECSM-R Chromatogram

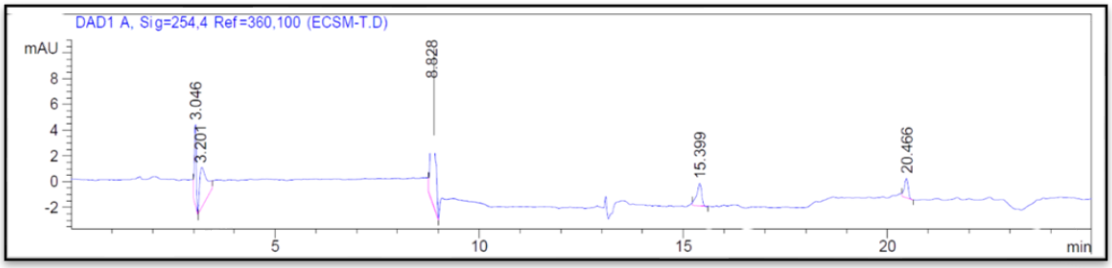


Figure 4.7 ECSM-T Chromatogram 254,4 nm

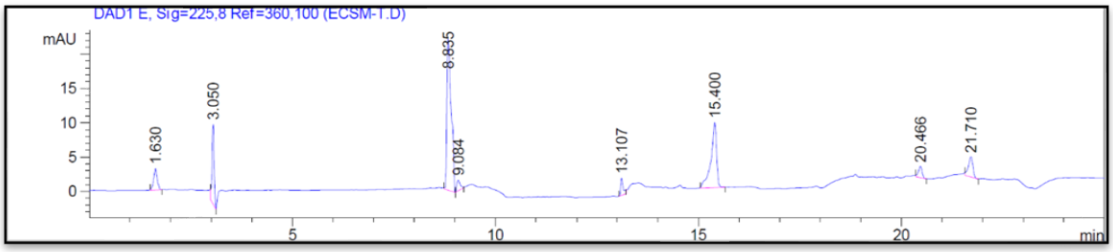


Figure 4.8 ECSM-T Chromatogram 225,8 nm

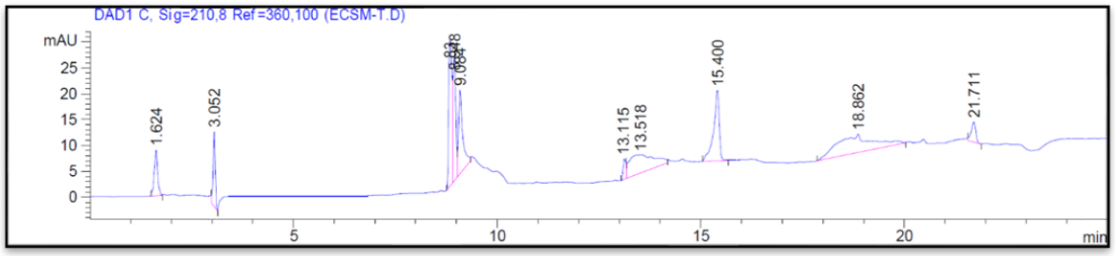


Figure 4.9 ECSM-T Chromatogram 210,8 nm

By referring to the figures above of chromatograms of ECSM-R and ECSM-T, it shows that the compounds from both locations have almost the same peaks in each chromatogram. Despite the differences of the wavelengths signal of UV absorption, the peaks were not too diverted from the retention time of each signal. There was consistency of compounds that eluted from both locations.

Table 4.4
Percentage Area Of Each Retention Time In ECSM-R And ECSM-T

Fractions	Signal	Retention Time (min)	Percentage Area (%)
ECSM-R	210,8	3.035	3.8683
		8.835	11.6532
		8.943	9.3738
		9.079	14.0314
		13.13	1.4164
ECSM-T	210,8	3.052	5.1499
	225,8	8.835	44.3092
		8.948	11.0553
	210,8	9.084	11.1019
		13.115	1.8104

The initial analysis of ECSM-R revealed a peak at 3.035 minutes, corresponding to a percentage area of 3.8683 %. Comparing the other location, ECSM-T's peak occurs at 3.052 minutes, which elutes 17 seconds later than ECSM-R. At 3.052 minutes, the calculated percentage area was 5.1499 %. The percentage area of ECSM-T exhibited a marginal increase. The class of chemical compounds was likely similar to that in ECSM-R, but in greater quantity. Subsequently, a comparison of the two peaks at identical retention times of 8.835 minutes is conducted. The peaks originated from various fractions and signals of UV absorption. The percentage area exhibited a significant difference. Fractions of ECSM-T exhibited 44.3092 % of area in 225.8 signals, whereas ECSM-R demonstrated 11.6532 % in 210.8 signals. The compounds that absorbed *UV-Vis*ible wavelengths at 225 nm were more abundant than those in ECSM-R, which exhibited lighter absorption at 210 nm.

At 8.943 and 8.948 minutes for ECSM-R and ECSM-T, the percentage areas were 9.3738 % and 11.0553 %, respectively. Both originated from identical UV absorption signals at 210 nm. The fractions of ECSM-T constitute the predominant percentage area within the range of 8.940 to 8.950 minutes. The recorded times were 9.079 minutes for ECSM-R and 9.084 minutes for ECSM-T. The area percentages for the two locations were 14.0314 % and 11.1019 %, respectively. This figure indicates that the compounds in ECSM-R elute 0.005 minutes faster than those in ECSM-T, suggesting a higher presence of semi-polar compounds in ECSM-R compared to ECSM-T. In conclusion, the retention times of ECSM-R and ECSM-T were 13.115 and 13.130 minutes, respectively, with corresponding area percentages of 1.8104 % and

1.3337 %. The area percentage in ECSM-R was marginally greater than that in ECSM-T. The elution of compounds was increasingly favouring ECSM-T.

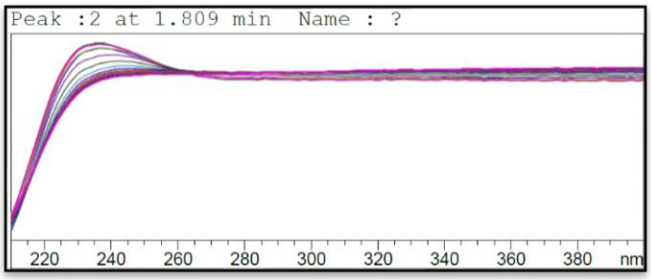


Figure 4.10 ECSM-R UV-Vis Spectrum Profile At 1.809 min

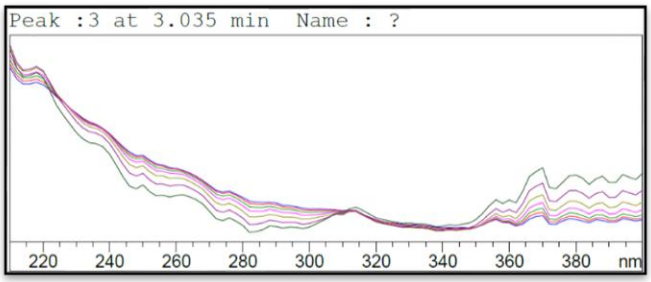


Figure 4.11 ECSM-R UV-Vis Spectrum Profile At 3.035 min

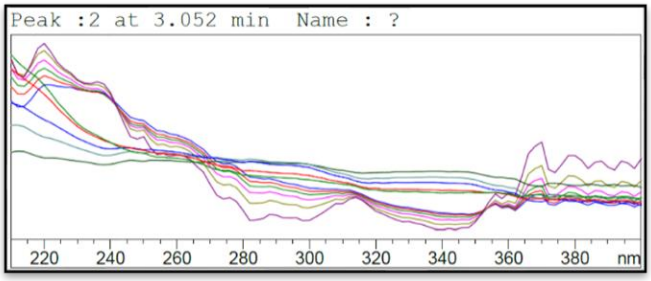


Figure 4.12 ECSM-T UV-Vis Spectrum Profile At 3.052 min

The maximum wavelength for the three UV-Visible spectrum profiles ranged from 200 to 240 nm. For a duration of 1.809 minutes, the maximum wavelength recorded was 240 nm. Kalaichelvi (2017) reported that flavonoid derivatives are potentially found at absorption maxima within the wavelength range of 230-285 nm. According to Malik *et al.*, (2018), terpenoid compounds exhibit a UV-Vis peak value at a wavelength of 248.0 nm in ethanolic extracts of *P. juliflora* pods. Both extracts yielded positive results in the phytochemical test for terpenoids, indicating a potential presence of flavonoids and terpenoids in ECSM-R at 1.809 minutes.

The UV-Visible spectrum profiles from ECSM-R and ECSM-T at 3.035 and 3.052 minutes exhibited similar shapes with multiple lines. It can be inferred that they

likely belong to the same class of compounds. The maximum wavelength for the two retention times ranged from 200 to 220 nm. Marković & Tošović, (2015) identified serratin, a flavonoid derivative, at a wavelength of 200 nm in their study. This analysis does not confirm the presence of a compound. It is probable that it contains compounds from the flavonoid class. The maximum UV-Visible absorptions of flavonoids and phenolic acids ranged from 202 to 254 nm (Christian *et al.*, 2016).

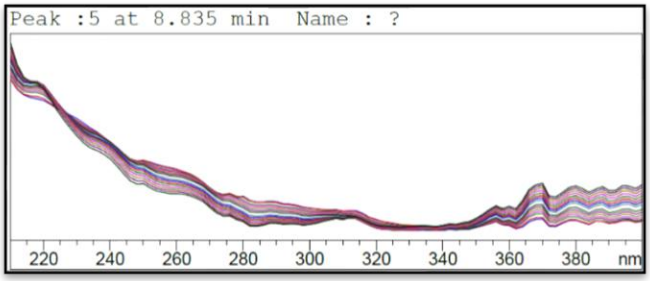


Figure 4.13 ECSM-R UV-Vis Spectrum Profile At 8.835 min

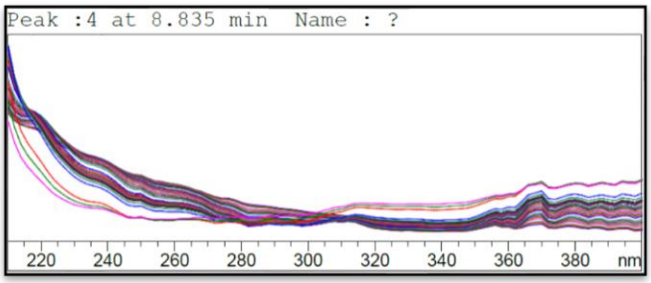


Figure 4.14 ECSM-T UV-Vis Spectrum Profile At 8.835 min

The UV-Visible spectrum profiles from ECSM-R and ECSM-T at 8.835 minutes exhibited similar shapes characterised by multiple lines. They were likely to possess the same class of compounds. The maximum wavelength corresponding to these two retention times was approximately 210 nm. Marković and Tošović (2015) identified a flavonoid derivative at a wavelength of 200 nm in their study. This analysis does not confirm the presence of a compound. It is probable that it contains compounds belonging to the flavonoid class. The maximum UV-Visible absorptions of flavonoids and phenolic acids ranged from 202 to 254 nm (Christian *et al.*, 2016).

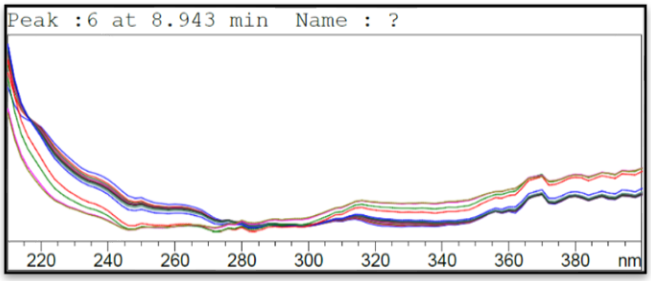


Figure 4.15 ECSM-R UV-Vis Spectrum Profile At 8.943 min

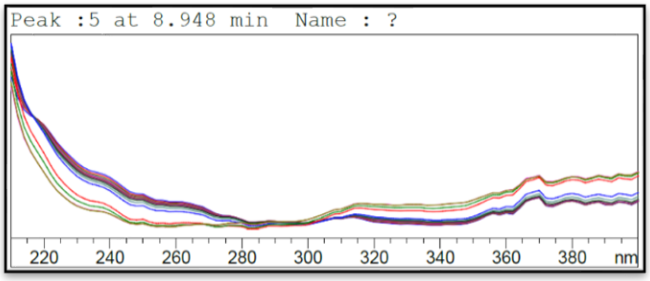


Figure 4.16 ECSM-T UV-Vis Spectrum Profile At 8.948 min

The fractions of ECSM-R and ECSM-T at 8.943 and 8.948 minutes exhibited identical shapes characterised by multiple lines. The maximum wavelength corresponding to these two retention times was approximately 210 nm. Maximum wavelengths of approximately 210 nm have been identified as potential indicators of flavonoid derivatives, as noted in previous studies (Kalaichelvi, 2017; Marković & Tošović, 2015). The maximum UV-Visible absorptions of flavonoids and phenolic acids ranged from 202 to 254 nm (Christian *et al.*, 2016). Additionally, condensed tannins were observed in the studies conducted by (Anburaj & Jothiprakasam, 2017).

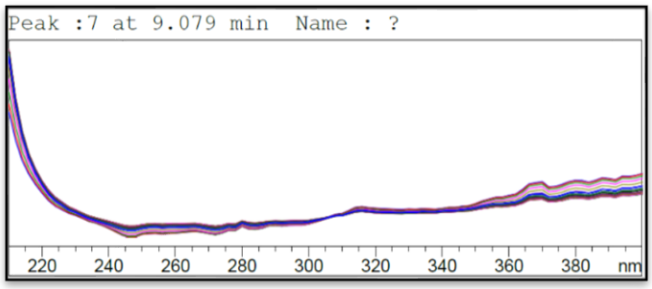


Figure 4.17 ECSM-R UV-Vis Spectrum Profile At 9.079 min

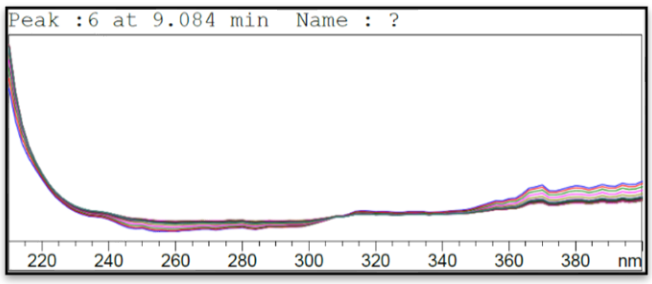


Figure 4.18 ECSM-T UV-Vis Spectrum Profile At 9.084 min

The fractions of ECSM-R and ECSM-T at 9.079 and 9.084 minutes were similar to those at 8.943 and 8.948 minutes. The shapes exhibited similarity, characterised by multiple lines. The maximum wavelength for the two retention times was approximately 210 nm. Maximum wavelengths of approximately 210 nm have been identified as potential indicators of flavonoid derivatives, as noted in previous studies (Kalaichelvi, 2017; Marković & Tošović, 2015). The maximum UV-Visible absorptions of flavonoids and phenolic acids ranged from 202 to 254 nm (Christian *et al.*, 2016). Condensed tannins were observed at 213 nm in the studies conducted by Anburaj and Jothiprakasam (2017).

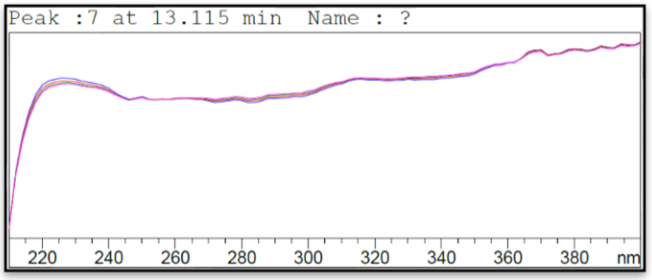


Figure 4.19 ECSM-T UV-Vis Spectrum Profile At 13.115 min

The maximum wavelength for the UV-Visible spectrum profile was approximately 220 nm. For a duration of 1.809 minutes, the maximum wavelength recorded was 240 nm. Kalaichelvi (2017) reported that flavonoid derivatives are potentially found at absorption maxima within the wavelength range of 230-285 nm. Additionally, gallic tannin and terpenes were detected in the range of 217 to 220 nm (Christian *et al.*, 2016).

4.4.2 Comparisons of HPLC Analysis between ECSC-R and ECSC-T

The analysis involved subjecting the chloroform fraction of Ranau and Tambunan to HPLC. Three UV detection wavelength signals were utilised: 210 nm, 225 nm, and 254 nm. The signals utilised were derived from prior studies on the *Etlingera* genus, which predominantly employed identical wavelength signals in HPLC analysis. This analysis of ECSC-R and ECSC-T focusses solely on the wavelength signal at 210 nm with a band slit of 8, which exhibits a UV-Visible spectrum profile. The chromatogram for ECSC-R and ECSC-T is presented below.

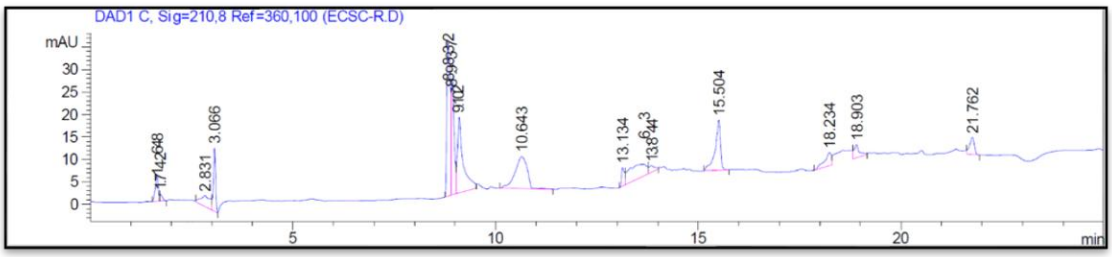


Figure 4.20 ECSC-R Spectrum

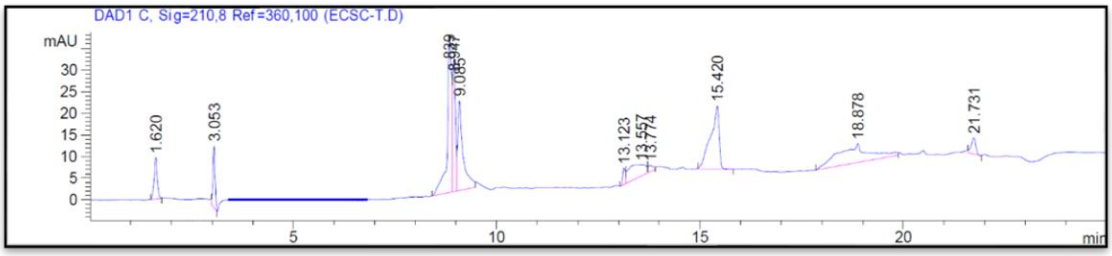


Figure 4.21 ECSC-T Spectrum

The chromatograms of ECSC-R and ECSC-T indicate that the compounds from both locations exhibit nearly identical peaks in each chromatogram. In the comparisons of fractions, the UV absorption wavelengths were consistent, and the peaks exhibited minimal deviation from the retention time of each signal. Compounds that eluted from both locations exhibited coherence.

Table 4.5
Percentage Area Of Each Retention Time In ECSC-R And ECSC-T

Fractions	Signal	Retention Time (min)	Percentage Area (%)
ECSC-R	210,8	3.066	4.9163
		8.832	18.8336
		8.937	11.1122
		9.102	15.2019
ECSC-T	210,8	3.053	3.961
		8.839	20.6683
		8.947	11.5383
		9.085	14.4978
		13.123	1.3337

Initially, for ECSC-R, the peak observed at 3.066 minutes represented a percentage area of 4.9163 %. In comparison to the other location, ECSC-T's peak occurs at 3.053 minutes, which elutes 16 seconds later than ECSC-R. At 3.053 minutes, the calculated percentage area was 3.961 %. The percentage area of ECSC-R was marginally greater. The class of chemical compounds was likely the same as in ECSC-R, but in greater total quantity. Subsequently, a comparison of the two peaks with similar retention times at 8.832 and 8.839 minutes revealed that ECSC-R and ECSC-T yielded 18.8336 % and 20.6683 %, respectively. The fractions from Tambunan were approximately 1.80 % higher than those from Ranau.

At 8.937 minutes for ECSC-R and 8.947 minutes for ECSC-T, the percentage areas were 11.1122 % and 14.4978 %, respectively. Both originated from identical signals of UV absorption at 210 nm. The fractions of ECSC-T comprise the majority of the percentage area within the range of 8.930 to 8.950 minutes. The recorded times were 9.102 minutes for ECSC-R and 9.085 minutes for ECSC-T. The area percentages for the two locations were 15.2019 % and 14.4978 %, respectively. This figure indicates that the compounds in ECSC-R were formed later than those in ECSC-T, yet likely contained a higher proportion of semi-polar compounds compared to ECSC-T. Finally, the retention time of ECSC-T was recorded at 13.130 minutes, with a percentage area of 1.14164 %. The retention times of ECSM-R and ECSM-T were 13.115 minutes and 13.130 minutes, respectively, with corresponding area percentages of 1.8104 % and 1.3337 %. The area percentage in ECSM-R exceeded that of ECSM-T marginally. The percentage area for both ECSM-T and ECSC-T was identical. The polarity of methanol

and chloroform fractions may be nearly identical.

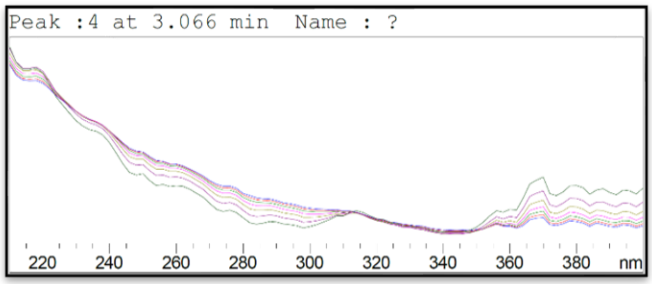


Figure 4.22 ECSC-R UV-Vis Spectrum Profile At 3.066 min

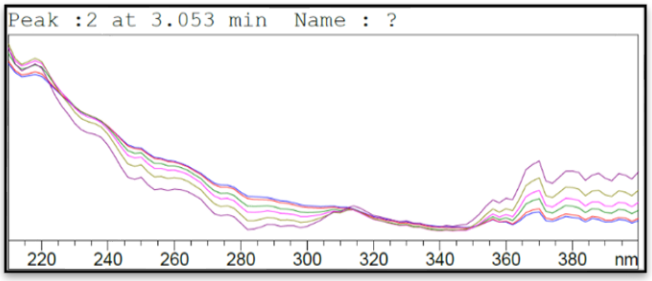


Figure 4.23 ECSC-T UV-Vis Spectrum Profile At 3.053 min

The UV-Visible spectrum profiles from ECSM-R and ECSM-T at 3.066 and 3.053 minutes exhibited similar shapes, characterised by numerous lines. This suggests that they were probably derived from the same class of chemicals. The maximum wavelength for the two retention durations ranged from 200 to 220 nm. Marković and Tošović (2015) identified serratin, a flavonoid derivative, at a wavelength of 200 nm. This analysis does not substantiate the classification as a compound. It is probable that it contains flavonoids. The maxima of UV-Visible absorptions for flavonoids and phenolic acids ranged from 202 to 254 nm (Christian *et al.*, 2016).

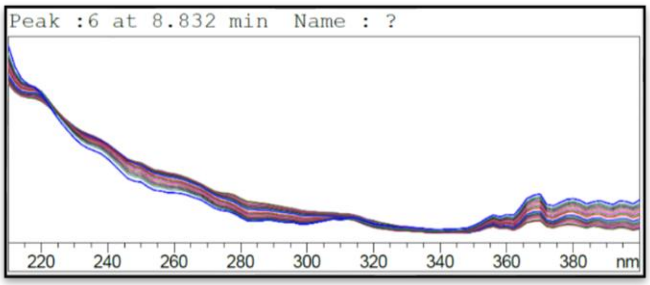


Figure 4.24 ECSC-R UV-Vis Spectrum Profile At 8.832 min

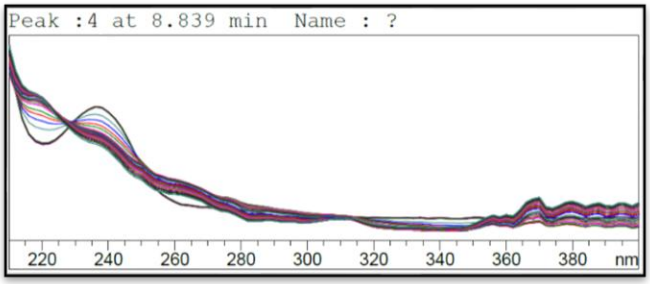


Figure 4.25 ECSC-T UV-Vis Spectrum Profile At 8.839 min

The maximum wavelength for the two UV-Visible spectrum profiles discussed above ranged from 200 to 240 nm. The maximum wavelength was approximately 210 nm over a duration of 8.832 minutes. According to Christian *et al.* (2016), the maxima *of UV-Vis* absorptions for flavonoids and phenolic acids range from 202 to 254 nm. At 8.839 minutes, two maximum wavelengths were observed. The range was from 210 to 240 nm. Kalaichelvi (2017) indicates that flavonoid derivatives can be detected at absorption maxima ranging from 230 to 285 nm wavelength. Terpenoid compounds with a wavelength of 248.0 nm for UV-Vis peak values of ethanolic extracts from *P. juliflora* pods were identified by Malik *et al.* (2018).

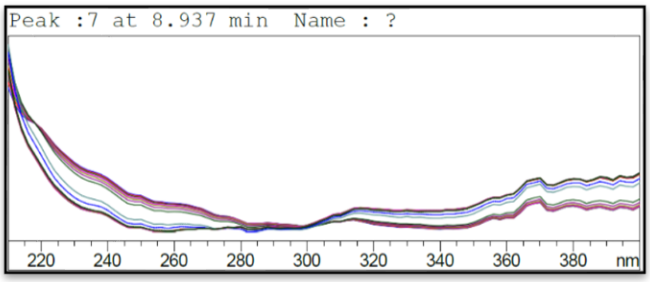


Figure 4.26 ECSC-R UV-Vis Spectrum Profile At 8.937 min

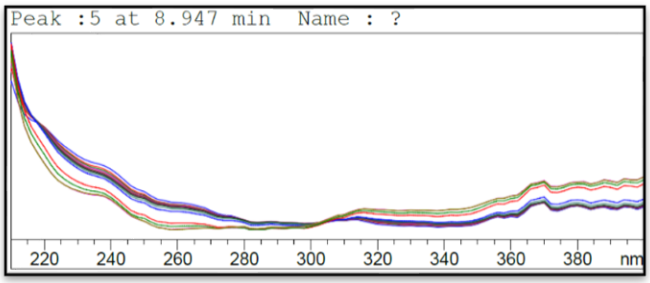


Figure 4.27 ECSC-T UV-Vis Spectrum Profile At 8.947 min

The fractions of ECSM-R and ECSM-T at 8.937 and 8.947 minutes exhibited identical shapes, characterised by numerous lines. The maximum wavelength for both retention times was approximately 210 nm. Flavonoid derivatives may exhibit

maximum wavelengths in the range of 210 nm (Kalaichelvi, 2017; Marković & Tošović, 2015). The maximum UV-Visible absorptions of flavonoids and phenolic acids range from 202 to 254 nm (Christian *et al.*, 2016). Investigations by Anburaj and Jothiprakasam (2017) also identified the presence of condensed tannin.

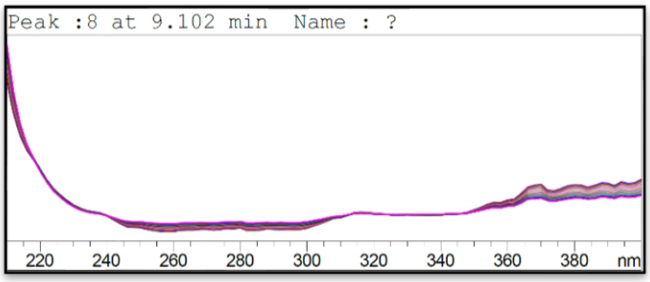


Figure 4.28 ECSC-R UV-Vis Spectrum Profile at 9.102 min

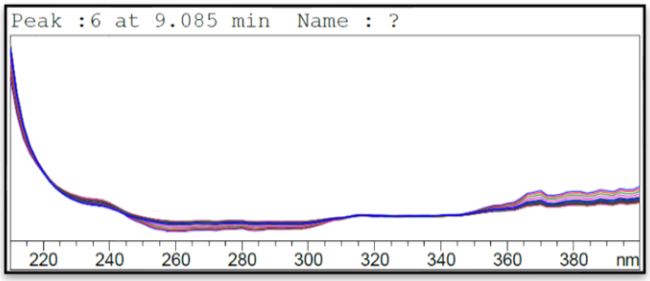


Figure 4.29 ECSC-T UV-Vis Spectrum Profile at 9.085 min

The fractions of ECSC-R and ECSC-T at 9.102 and 9.085 minutes were similar to those at 8.937 and 8.947 minutes. Their shapes were identical, characterised by numerous lines. The maximum wavelength for the two retention durations was approximately 210 nm. Kalaichelvi (2017) and Marković & Tošović (2015) indicate that flavonoid derivatives may exhibit maximum wavelengths in the range of 210 nm. The maximum UV-Vis absorptions of flavonoids and phenolic acids range from 202 to 254 nm (Christian *et al.*, 2016). Furthermore, condensed tannin was identified at 213 nm in the study conducted by Anburaj and Jothiprakasam (2017).

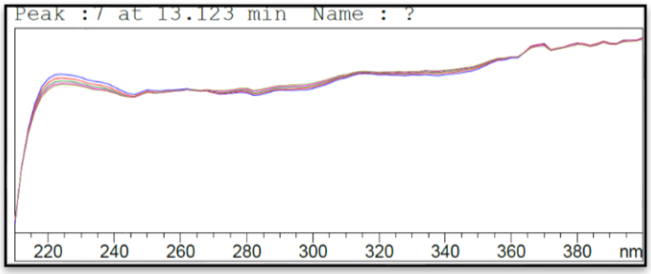


Figure 4.30 ECSC-R UV-Vis Spectrum Profile at 13.123 min

The maximum wavelength for the UV-Visible spectrum profile of ECSC-R at 13.123 minutes was approximately 220 nm. Kalaichelvi (2017) indicates that flavonoid derivatives can be detected at absorption maxima within the wavelength range of 230 to 285 nm. Gallic tannin and terpenes were identified within the wavelength range of 217 to 220 nm (Christian *et al.*, 2016).

The HPLC results indicate that the non-volatile metabolite profiles of *E. coccinea* stalk extracts from Ranau and Tambunan are qualitatively analogous. The identified chromatographic differences are confined to fluctuations in peak strength, reinforcing the assertion that geographical variation between the two regions does not yield significant qualitative disparities in the non-volatile chemical composition of *E. coccinea*. These results further validate the application of LC-MS analysis for supplementary qualitative profiling, as elaborated in section 4.5.

4.5 Liquid Chromatography Mass-Spectrometry (LC-MS)

LC-MS research was conducted to provide a comparative assessment of polar and semi-polar compounds in *E. coccinea* stalk extracts from Ranau and Tambunan. The findings are analysed in relation to the mass spectrum data acquired (Figures 4.31–4.34) and the provisional metabolite list outlined in Table 4.6. This investigation involved compound identification using spectral interpretation of the obtained mass spectra and database-assisted matching utilising the analytical center's processing software. The preliminary identification relied mostly on observed m/z values, suggested molecular ions, and comparisons with documented metabolites from *Etlingera* species and associated Zingiberaceae plants. Due to the absence of legitimate reference standards, all chemicals enumerated in Table 4.6 are considered preliminary identifications.

The LC-MS dataset was incomplete due to the lack of total ion chromatograms (TICs), which were not supplied by the analysis centre. Thus, the analysis of LC-MS results in this work relies exclusively on the accessible mass spectra, rather than on the integration of chromatographic peaks. This constraint hinders quantitative comparison and obstructs accurate evaluation of relative compound abundance. Nonetheless, the mass spectrum data continues to be valuable for qualitative and comparative metabolite analysis.

Four distinct extracts were subjected to LC-MS analysis: ECSM-R, ECSM-T, ECSC-R, and ECSC-T. Multiple compounds were identified as plant metabolites through the library analysis. Eleven compounds were identified, with the majority occurring in these four categories of extracts.

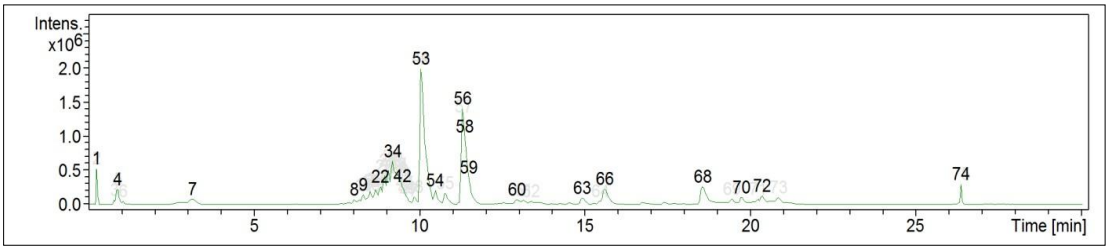


Figure 4.31 ECSM-R LC-MS Spectrum

The LC-MS spectra of methanolic extracts from Ranau and Tambunan (Figures 4.31 and 4.32) exhibited analogous fragmentation patterns, indicating the presence of similar classes of polar secondary metabolites in both samples. Numerous significant ions identified in both spectra align with mass ranges typically linked to phenolic and flavonoid chemicals documented in *Etlingera* species. Minor discrepancies in ion intensities and the detection of low-abundance ions were noted, suggesting potential fluctuations in metabolite concentration rather than the existence of distinct location-specific molecules.

Table 4.6
ECSM-R Plant Metabolites

No.	Compounds	Library	Molecular weight (g/mol)	RT (min)
1	Betaine Aldehyde	Plant Metabolites	201.1728	0.8
2	Phloridzin	Plant Metabolites	274.1027	1.1
3	Salicyl amide	Plant Metabolites	148.0158	15.6
4	4-hydroxyhippuric acid	Plant Metabolites	148.0158	15.6
5	Phthalic acid	Plant Metabolites	204.0786	15.6
	Phthalic acid	Plant Metabolites	300.1343	15.6

6	3-hydroxy-2-methylbutyric acid	Plant Metabolites	662.4467	19.7
	3-hydroxy-2-methylbutyric acid	Plant Metabolites	536.5288	20.3
7	Phytosphingosine	Plant Metabolites	564.5598	20.8
8	2-phenylbutyric acid	Plant Metabolites	564.5598	20.8
9	Phenylacetic acid	Plant Metabolites	564.5598	20.8
10	2-phenylacetamide	Plant Metabolites	564.5598	20.8

Ten types of compounds were identified in ECSM-R extracts. The compounds include betaine aldehyde **(1)**, phloridzin **(2)**, salicyl amide **(3)**, 4-hydroxyhippuric acid **(4)**, phthalic acid **(5)**, 3-hydroxy-2-methylbutyric acid **(6)**, phytosphingosine **(7)**, 2-phenylbutyric acid **(8)**, phenylacetic acid **(9)**, and 2-phenylacetamide **(10)**.

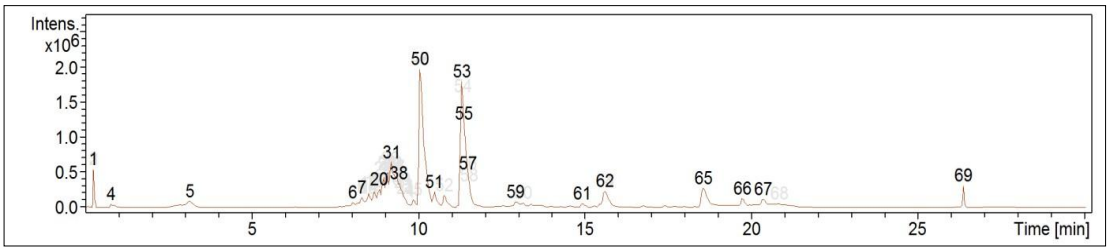


Figure 4.32 ECSM-T LC-MS Spectrum

Table 4.7
ECSM-T Plant Metabolites

No.	Compounds	Library	Molecular weight (g/mol)	RT (min)
1	Betaine aldehyde	Plant Metabolites	201.1727	0.8
5	Phthalic acid	Plant Metabolites	148.0159	15.6
	Phthalic acid	Plant Metabolites	204.0786	15.6
	Phthalic acid	Plant Metabolites	300.1341	15.6
6	3-hydroxy-2-methylbutyric acid	Plant Metabolites	662.4468	19.7
7	Phytosphingosine	Plant Metabolites	536.5284	20.4
8	2-phenylbutyric acid	Plant Metabolites	123.0798	26.4
9	Phenylacetic acid	Plant Metabolites	123.0798	26.4

10	2-phenylacetamide	Plant Metabolites	123.0798	26.4
11	3-methoxytyramine	Plant Metabolites	123.0798	26.4

In ECSM-T extracts, there were 8 types of compounds found. The compounds are betaine aldehyde **(1)**, phthalic acid **(5)**, 3-hydroxy-2-methylbutyric acid **(6)**, phytosphingosine **(7)**, 2-phenylbutyric acid **(8)**, phenylacetic acid **(9)**, 2-phenylacetamide **(10)** and 3-methoxytyramine **(11)**.

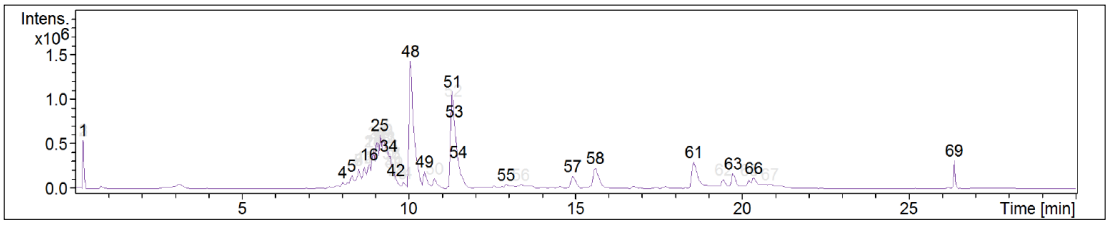


Figure 4.33 ECSC-R LC-MS Spectrum

Table 4.8
ECSC-R Plant Metabolites

No.	Compounds	Library	Molecular weight (g/mol)	RT (min)
3	Salicyl amide	Plant Metabolites	148.0163	15.6
4	4-hydroxyhippuric acid	Plant Metabolites	148.0163	15.6
5	Phthalic acid	Plant Metabolites	204.079	15.6
	Phthalic acid	Plant Metabolites	300.1344	15.6
7	Phytosphingosine	Plant Metabolites	530.4011	20.2
	Phytosphingosine	Plant Metabolites	564.5598	20.8
8	2-phenylbutyric acid	Plant Metabolites	123.0795	26.4
10	2-phenylacetamide	Plant Metabolites	123.0795	26.4
9	Phenylacetic acid	Plant Metabolites	123.0795	26.4
11	3-methoxyramine	Plant Metabolites	123.0795	26.4

In ECSC-R extracts, there were 8 types of compounds found. The compounds are salicyl amide **(3)**, 4-hydroxyhippuric acid **(4)**, phthalic acid **(5)**, phytosphingosine **(7)**, 2-phenylbutyric acid **(8)**, phenylacetic acid **(9)**, 2-phenylacetamide **(10)** and 3-methoxytyramine **(11)**.

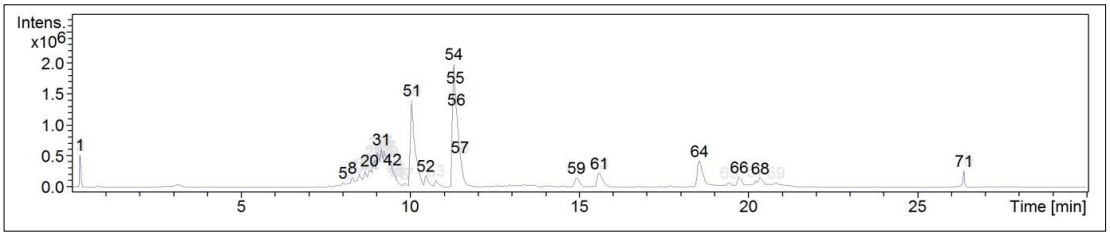


Figure 4.34 ECSC-T LC-MS Spectrum

Table 4.9
ECSC-T Plant Metabolites

No.	Compounds	Library	Molecular weight (g/mol)	RT (min)
5	Phthalic acid	Plant Metabolites	148.0163	15.6
3	Salicyl amide	Plant Metabolites	148.0163	15.6
4	4-hydroxyhippuric acid	Plant Metabolites	148.0163	15.6
5	Phthalic acid	Plant Metabolites	204.0791	15.6
	Phthalic acid	Plant Metabolites	300.1346	15.6
6	3-hydroxy-2-methylbutyric acid	Plant Metabolites	662.4474	19.7

Four types of compounds were identified in ECSC-T extracts. The compounds include salicyl amide **(3)**, 4-hydroxyhippuric acid **(4)**, phthalic acid **(5)**, and 3-hydroxy-2-methylbutyric acid **(6)**.

The chloroform extracts from Ranau and Tambunan (Figures 4.33 and 4.34) displayed analogous mass spectral patterns. The identified ions significantly overlapped between the two regions, corroborating the finding that the semi-polar metabolite composition of *E. coccinea* stalks is qualitatively analogous irrespective of geographical origin. The observed spectral differences were confined to fluctuations in signal intensity and the emergence of small ions, potentially caused by environmental factors impacting metabolite production.

This LC-MS analysis revealed that not all compounds have been previously reported from the Zingiberaceae family, the *Etlingera* genus, or the *E. coccinea* species. Only a limited number of compounds were reported. Recent studies by Ali *et al.*, (2018) and Pham *et al.*, (2020) elucidate the chemical composition of the rhizome of *Kaempferia galanga L.*, a member of the Zingiberaceae family. This study identified phthalic acid in *Kaempferia* galanga rhizomes, enhancing our comprehension of the chemical diversity in this medicinal plant. Phthalic acid is a prevalent chemical

compound with numerous industrial applications and is also present in nature, often as a metabolic byproduct of specific organisms. This is a naturally occurring metabolic byproduct that is extensively utilised in industry. It has also been reported to possess anti-bacterial, anti-fouling, and anti-fungal activities (Pham *et al.*, 2020).

Nag *et al.*, (2022) examined the rhizomes of *Amomum subulatum*, a member of the Zingiberaceae family, and identified phytosphingosine. Phytosphingosine is a sphingoid base found in plant tissues, significant for its involvement in various physiological processes, such as cell signalling and the regulation of lipid metabolism. The identification of phytosphingosine in *Amomum subulatum* rhizomes adds to the existing literature on the chemical diversity within the Zingiberaceae family and highlights the possible medicinal significance of these plants. 2-Phenylacetamide was recently identified in the Zingiberaceae family within *E. elatior* flowers through the use of 80% aqueous ethanol extracts. This compound may be significant in antibacterial activity (Fernandes *et al.*, 2020; Nag *et al.*, 2022).

Currently, other compounds identified as plant metabolites in the library have not been reported to correspond with those of the Zingiberaceae family or the *Etlingera* genus. Recent botanical studies have provided significant insights into the chemical composition of various plant species, revealing the presence of notable substances that may impact human health and ecological interactions. Betaine aldehyde, phloridzin, 4-hydroxyhippuric acid, 2-phenylbutyric acid, phenylacetic acid, and 3-methoxytyramine have been identified in various plant species, illustrating the diversity of secondary metabolites across botanical groups. However, some were reported to contain the same compound derived from other plants.

Betaine aldehyde is present in all plants and in the human body. Betaine aldehyde is an organic compound categorised as a tetraalkylammonium salt. These compounds are characterised by the presence of a nitrogen atom bonded to four alkyl groups, resulting in a quaternary ammonium centre. Betaine aldehyde is significant in the metabolism of amino acids such as glycine, serine, and threonine. Betaine aldehyde functions as a crucial intermediary in these pathways (Wishart *et al.*, 2022). The betaine aldehyde dehydrogenase (BADH) enzyme performs various functions in plants. It is crucial for fragrance production, abiotic stress response, and the antibiotic-free selection of transgenic plants. BADH catalyses the oxidation of betaine aldehyde to produce glycine betaine. Glycine betaine functions as a significant osmolyte, playing a critical

role in the ability of plants to respond to environmental stressors (Golestan Hashemi *et al.*, 2018; Singh *et al.*, 2022).

Panche *et al.*, (2016) and Zabidi *et al.*, (2019) identified phloridzin in the roots of *Curculigo latifolia*. Phloridzin, a dihydrochalcone glycoside, is primarily linked to apple trees (*Malus domestica*) but has also been identified in the root tissues of *Curculigo latifolia*. This discovery expands our comprehension of phloridzin's distribution across the plant kingdom and prompts enquiries regarding its potential relevance in non-apple plant species. Sánchez-Medina *et al.*, (2023) identified 4-hydroxyhippuric acid in coffee (*Coffea spp*.). This molecule, derived from hydroxybenzoic acid derivatives, plays a role in coffee's intricate chemical composition and may affect its flavour and health-related benefits. The presence of 4-hydroxyhippuric acid in coffee highlights the intricate chemistry of this widely consumed beverage and its importance beyond mere flavour.

Peng *et al.*, (2022) identified 2-phenylbutyric acid in the roots of sunflower (*Helianthus annuus*), representing a significant discovery. This molecule is recognised for its role in plant defence mechanisms and stress responses, illustrating the dynamic chemical interactions between sunflower plants and their environment. Investigating the presence and function of 2-phenylbutyric acid in sunflower roots opens new avenues for research into the compound's environmental and physiological implications. Phenylacetic acid is closely associated with polyphenols, as demonstrated by studies on plant-based supplements that reported significant absorption of various polyphenolic components and metabolites in plasma. Plant products contain a high concentration of phytochemicals, particularly polyphenols, which have been extensively studied (Rechner *et al.*, 2002). A comprehensive characterisation of 3-methoxytyramine could enhance understanding of neurological disorders linked to abnormal dopamine transmission, including Parkinson's disease, dyskinesia, and schizophrenia. Furthermore, 3-methoxytyramine inhibits catecholaminergic activity in the brain (Sotnikova *et al.*, 2010).

This method guarantees that the tabulated results precisely represent the confidence level of the LC-MS analysis and prevents overinterpretation of mass accuracy. While a limited quantity of ions seemed to be identified solely in a certain geographical area, these discrepancies could not be substantiated with high confidence and should so be regarded with caution. The updated Table 4.6 and the corresponding

spectral data consistently demonstrate that the LC-MS profiles of *E. coccinea* stalk extracts from Ranau and Tambunan are qualitatively analogous, with discrepancies mainly due to variations in signal intensity rather than distinct chemical composition, aligning with the comparative aim of this study.

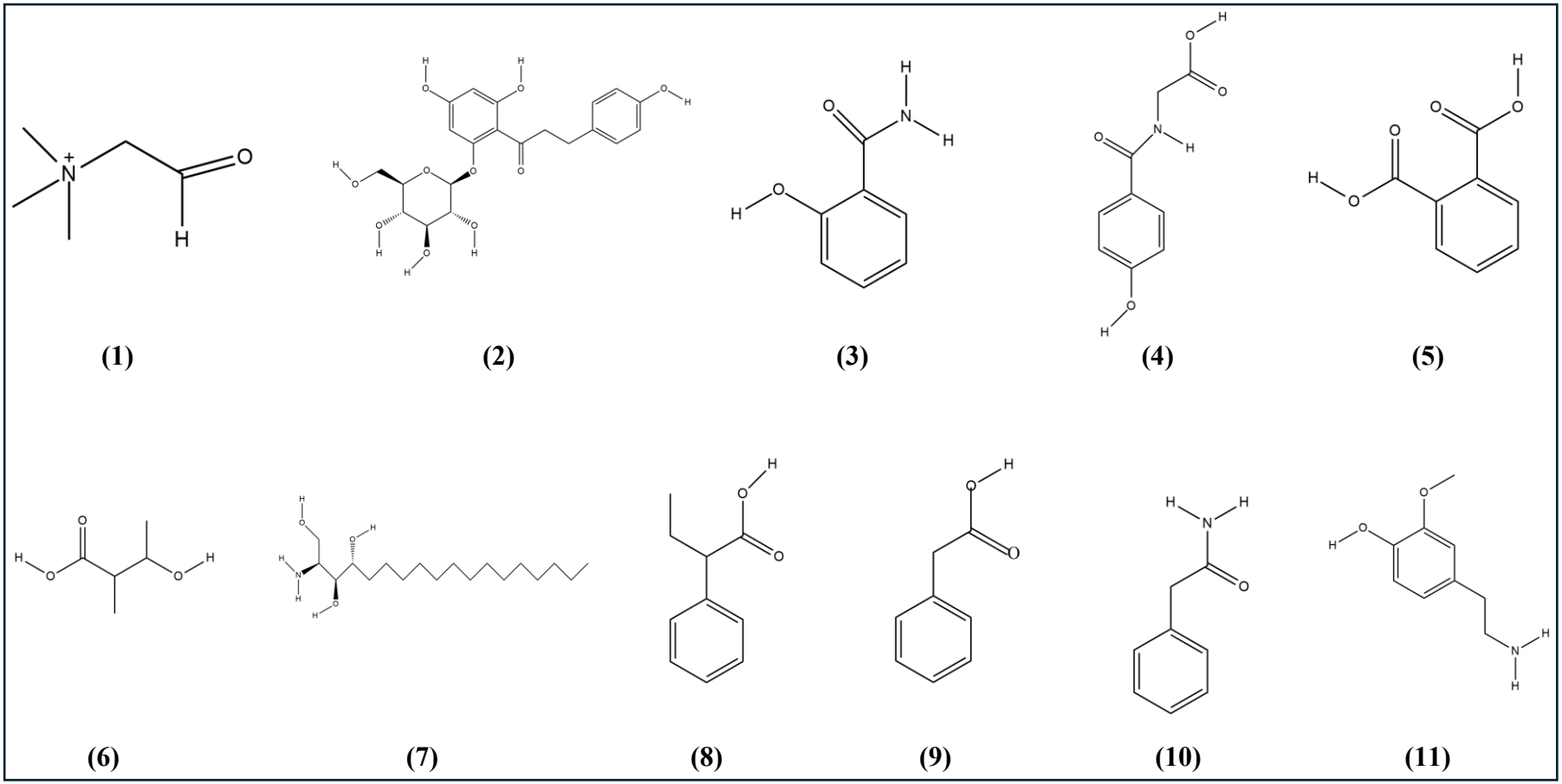
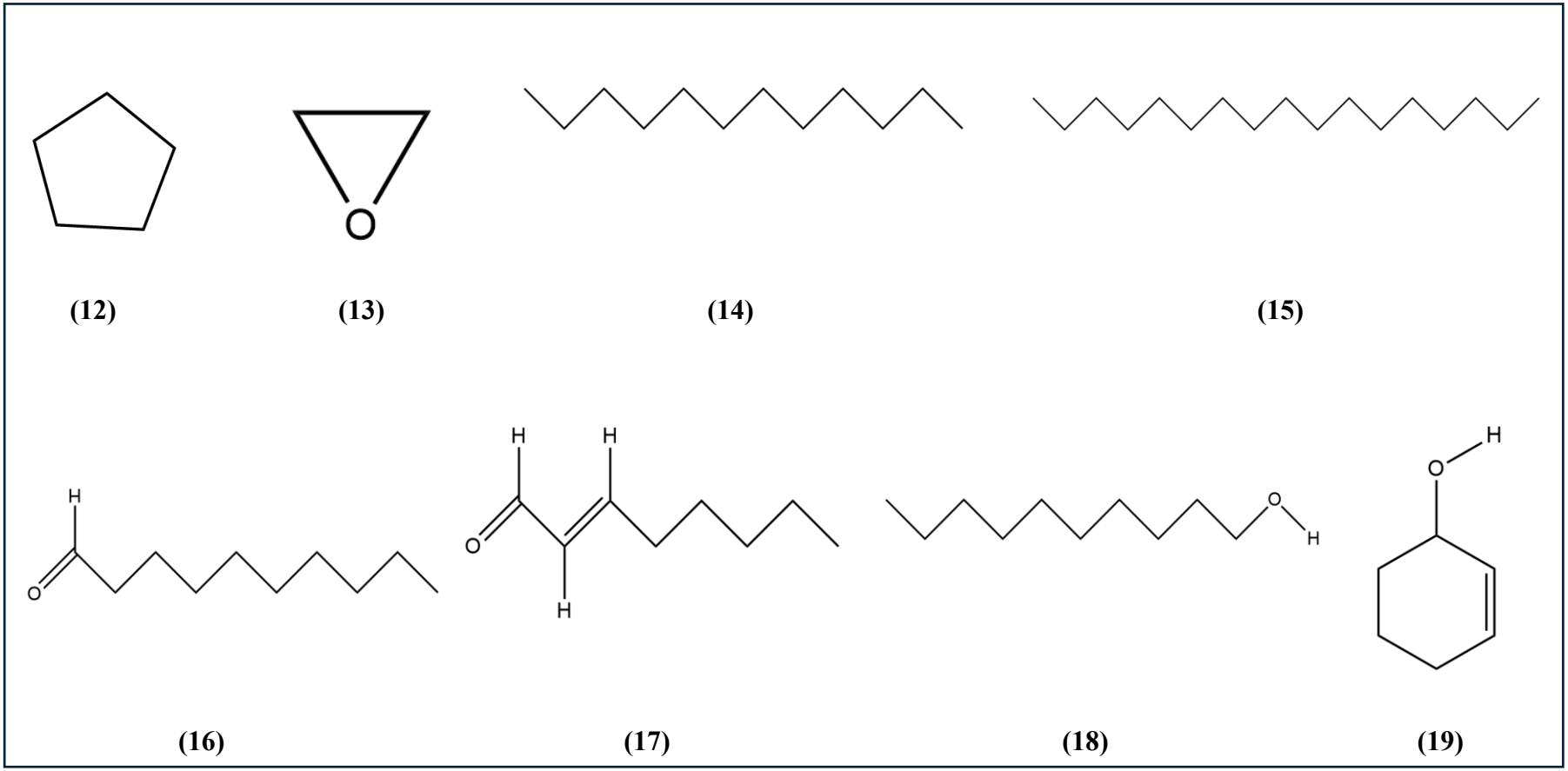


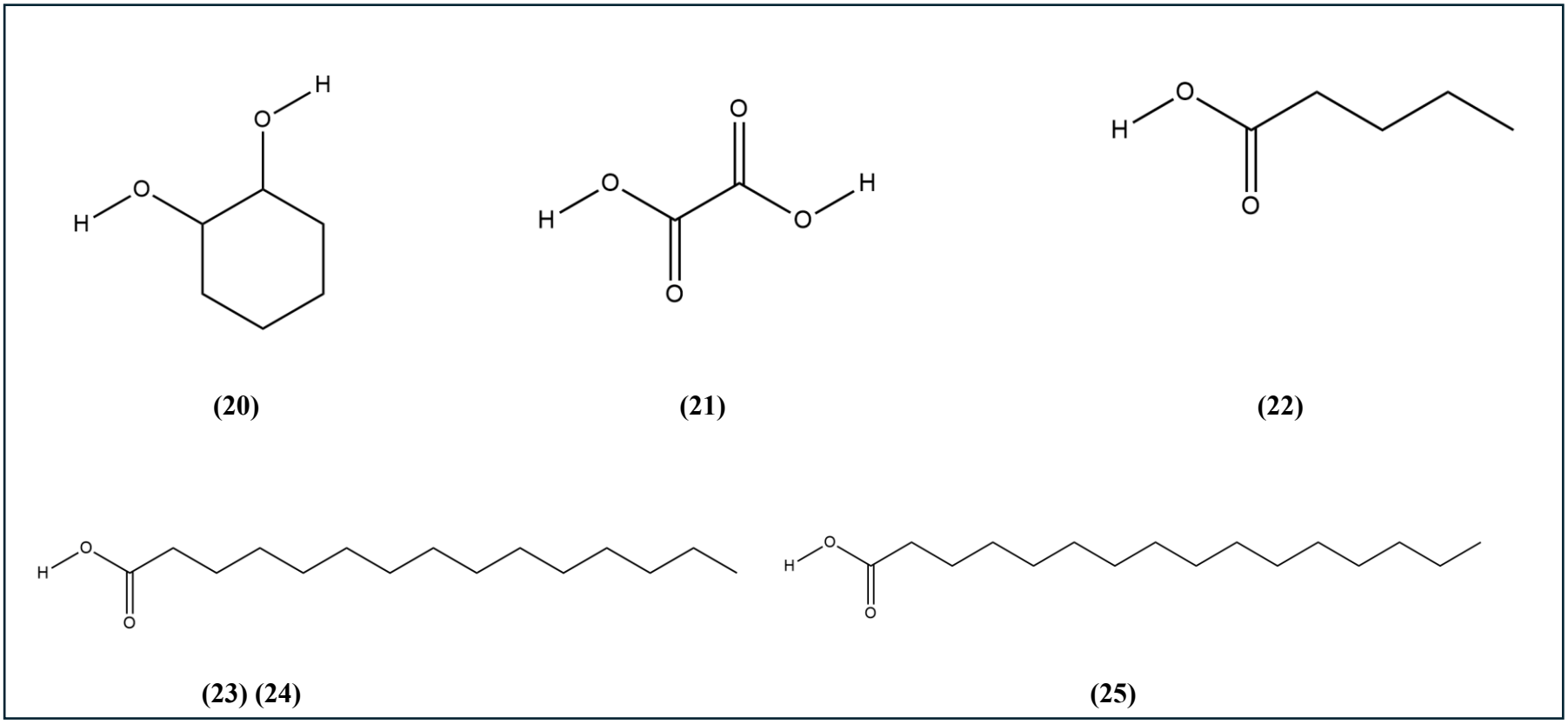
Figure 4.35 Chemical Structure Detected In LC-MS Library (1-10)



(12) **(13)** **(14)** **(15)**

(16) **(17)** **(18)** **(19)**

Figure 4.36 Chemical Structure Detected In GC-MS Library (12-19)



(20) **(21)** **(22)**

(23) (24) **(25)**

Figure 4.37 Chemical Structure Detected In GC-MS Library (20-25)

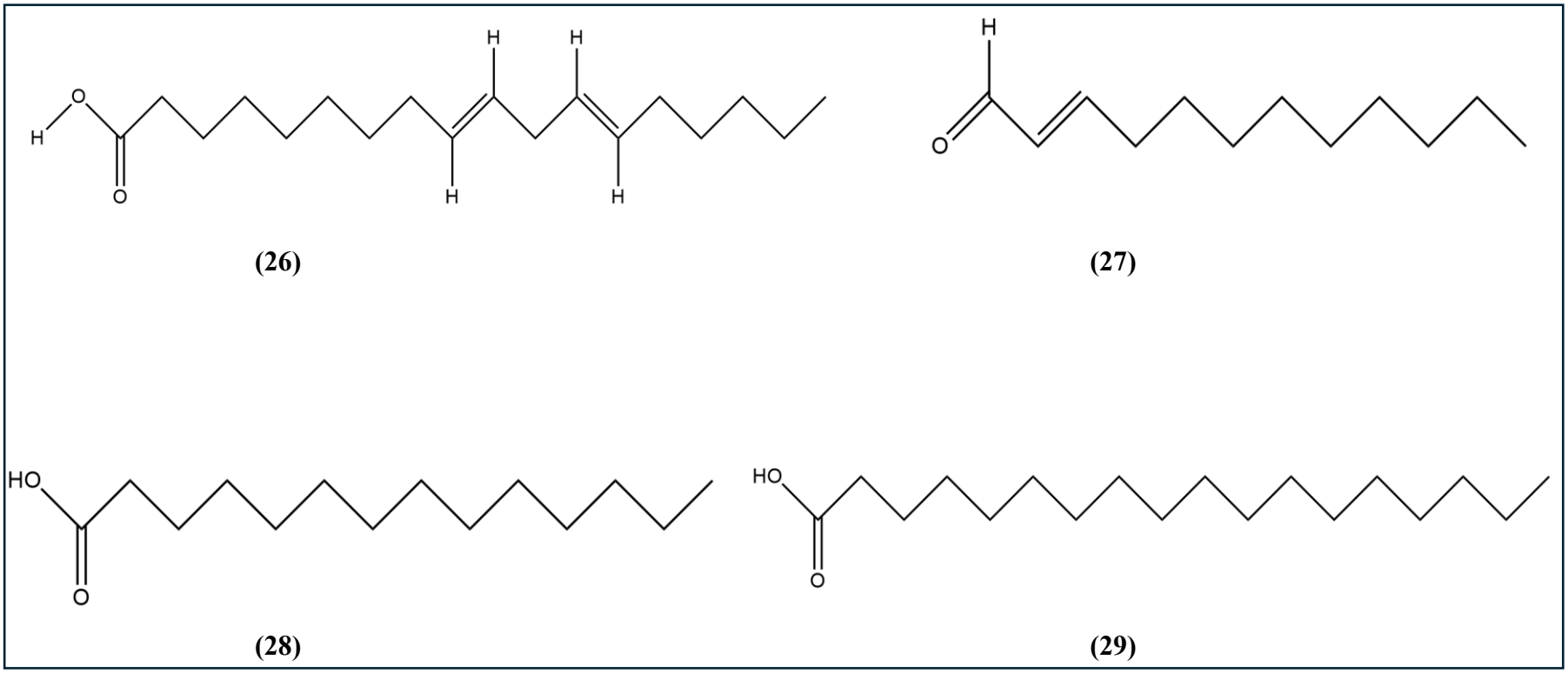


Figure 4.38 Chemical Structure Detected In GC-MS Library (26-29)

4.6 Gas Chromatography Mass-Spectrometry (GC-MS)

GC-MS utilised to characterise the volatile and semi-volatile components in the *n-hexane* extracts of *E. coccinea* stalks from Ranau and Tambunan. This part discusses the comparative volatile profiling of the two geographical regions, with specific experimental conditions outlined in Chapter 3. Volatile compounds were tentatively identified by matching mass spectra with the NIST mass spectral library. The proposed compound identities were assessed for chemical plausibility utilising molecular data from the PubChem database. Due to the absence of authentic reference standards or retention index data, all compounds are classified as tentative.

The volatile compounds identified in *n-hexane* extracts from both regions are presented in Table 4.7, along with their relative percentage composition. Both Ranau and Tambunan samples exhibited several major volatile constituents, demonstrating a significant similarity in their volatile profiles. The comparative analysis indicated that the Ranau extract displayed a marginally greater diversity of volatile compounds than the Tambunan extract; nonetheless, the predominant constituents were predominantly common to both samples. The differences between the two regions were mainly noted in the relative percentage composition, rather than in the presence or absence of specific compounds. Variation may be influenced by environmental factors, such as altitude and local growing conditions, which affect secondary metabolite biosynthesis.

The GC-MS analysis was conducted on two separate extracts, ECSH-R and ECSH-T, derived from different sources but prepared in the same manner. GC-MS is a widely employed analytical method in chemistry for the identification and characterization of chemical constituents in complex mixtures. The extracts were analyzed via GC-MS to ascertain their chemical composition and identify potential bioactive components. The identification of chemicals in the Zingiberaceae and *Etlingera* genera within the ECSH-R and ECSH-T extracts illustrates the complexity of their chemical composition and indicates potential therapeutic significance. The findings underscore the significance of these extracts as potential sources of bioactive compounds with diverse therapeutic applications. The comprehensive GC-MS analysis of ECSH-T revealed significant insights into its chemical composition, identifying three distinct functional groups: alkanes, aldehydes, and organic acids. The comprehensive GC-MS analysis of ECSH-R identified a diverse array of chemical elements, revealing

four distinct functional groups of compounds: alkanes, aldehydes, alcohols, and organic acids. Each category includes a diverse array of chemical species, each possessing distinct structural characteristics and potential biological activity.

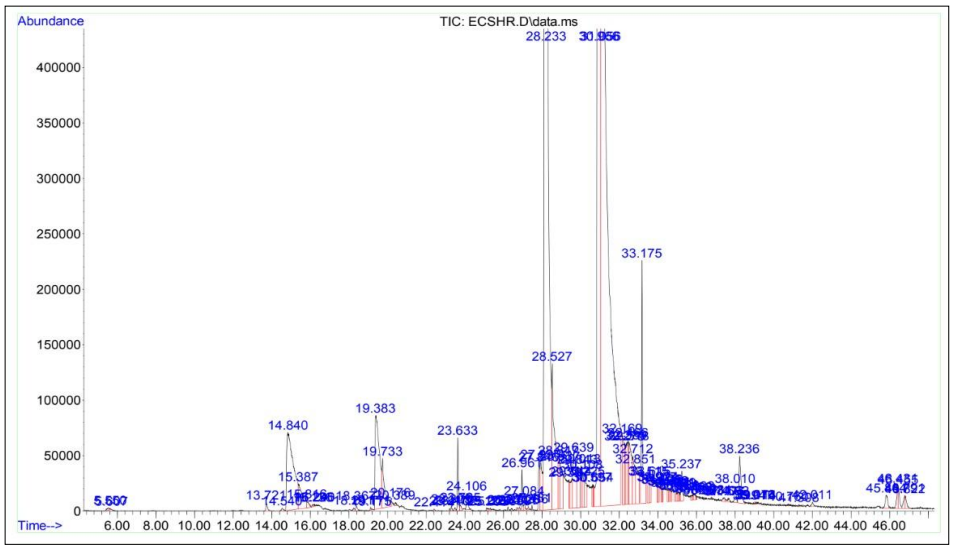


Figure 4.39 ECSH-R GC-MS Spectrum

Table 4.10
ECSH-R Plant Metabolites

No.	Compounds	RT (min)	% Area
Alkane			
12	cyclopentane	14..841	2.05
13	oxirane	18.366	0.04
14	dodecane	19.734	0.54
15	heptadecane	23.636	0.27
Aldehyde			
16	decanal	13.72	0.05
17	2-octenal	20.174	0.03
Alcohol			
18	1-decanol	14.538	0.02
19	2-cyclohexen-1-ol	19.384	1.55
20	1,2-cyclohexanediol	23.304	0.02
Organic Acid			
21	oxalic acid	19.173	0
27	pentanoic acids	25.204	0.01
36	hexadecanoic acids	27.481	0.01

39	n-hexadecanoic acid	28.231	30.47
44	pentadecanoic acid	29.547	0.23
52	9,12-octadecadienoic acid	30.966	27.77

The GC-MS analysis of ECSH-R displayed a complex chromatogram featuring 98 peaks, each representing a unique chemical entity or molecule. Analysis of these peaks through comparison with spectrum libraries identified multiple plant metabolites, highlighting the chemical diversity of ECSH-R. Given the scarcity of research on the metabolites of *E. coccinea*, the compounds identified in ECSH-R through GC-MS analysis were compared with those documented in earlier studies concerning the Zingiberaceae family, the *Etlingera* genus, and specifically, the *E. coccinea* species. This comparative approach aims to utilize existing knowledge from analogous botanical taxa and species to enhance the understanding of the chemical composition of ECSH-R and its potential bioactive constituents. A total of 15 compounds were identified from the comprehensive data obtained through GC-MS analysis of ECSH-R, as previously reported in another study. The substances identified in previous studies within the Zingiberaceae family, specifically the *Etlingera* genus and *E. coccinea* species, constitute certain chemical compounds present in ECSH-R.

A comprehensive GC-MS analysis of the Ranau hexane extract revealed the existence of four distinct functional groups. Cyclopentane **(12)** was the initial compound. The study by Arista *et al.*, (2023) utilized Cardamom, a member of the *Amomum* genus within the Zingiberaceae family. Cyclopentane was found abundantly in the stems of Cardamom. The subsequent compounds discussed are oxirane **(13)** and dodecane **(14)**, as noted in the study by Maimulyanti and Prihadi (2015). This study investigates the flower of *E. elatior*. *E. coccinea* and *E. elatior* belong to the same family and genus, *Etlingera*. The study by Ma *et al.*, (2015) identified heptadecane **(15)** as a compound present in the essential oils of *Kaempferia galanga L. Kaempferia galanga L.* belongs to the Zingiberaceae family. Subsequently, two compounds were identified as aldehydes. Decanal **(16)** was the primary component of the basal stem oils identified in the study by Ud-Daula *et al.* (2016) on *E. fimbriobrateata*, which belongs to the same genus as *E. coccinea*. 2-octenal **(17)** was identified in the study by Jems *et al.* (2021) concerning the chemical composition of *E. coccinea*.

As for alcohol class of compounds, there were 3 compounds were identified,

which were 1-decanol **(18)**, 2-cyclohexen-1-ol **(19)** and 1,2-cyclohexanediol **(20)**. For 1-decanol and 2-cyclohexen-1-ol, they were studied by (Jems *et al.*, 2021) and (Arista *et al.*, 2023) respectively. In the study from (Kulyal *et al.*, 2021) regarding on *Curcuma longa* L. a member from Zingiberaceae, 1,2-cyclohexanediol were reported.

As in class of compounds of organic acids, there were 6 compounds identified. Compounds hexadecanoic acid **(23)**, n-hexadecanoic acid **(24)**, pentadecanoic acid **(25)**, and 9,12-octadecadienoic acid **(26)** were reported from the study of (Nurjannah *et al.*, 2023). Their study was regarding the secondary metabolites of Zingiberaceae family. As for 9,12-octadecadienoic acid were reported by (Maimulyanti & Prihadi, 2015) study as well. In the study of *E. elatior* rhizomes by Barbosa *et al.*, (2017) in their study of Zingiberaceae, oxalic acid **(21)** were reported. In 2018, pentanoic acid **(22)** were reported by Trimanto & Hapsari in their study on *Etlingera megalocheilos*.

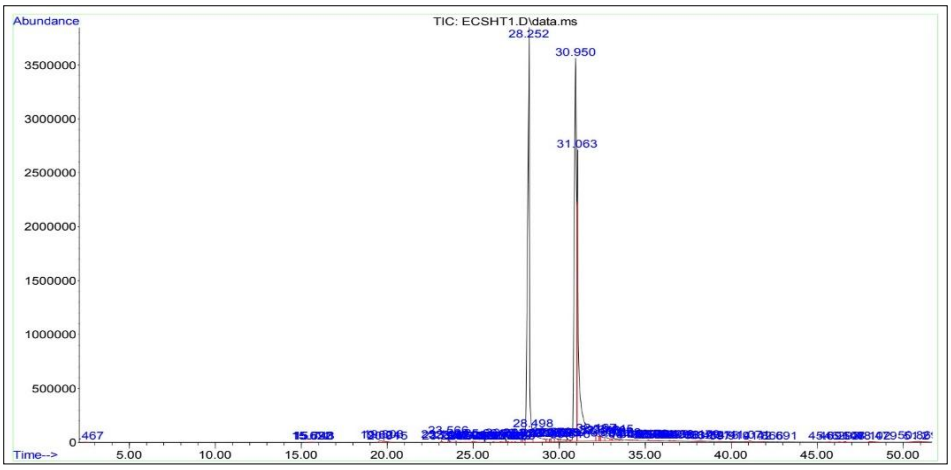


Figure 4.40 ECSH-T GC-MS Spectrum

Table 4.11
ECSH-T Plant Metabolites

No.	Compounds	RT (min)	% Area
Alkane			
15	heptadecane	23.567	0.15
13	oxirane	23.996	0.07
Aldehyde			
27	2-dodecenal	19.625	0.22
Organic Acid			
21	oxalic acid	25.221	0.02
23	hexadecanoic acids	27.435	0.02

23	n-hexadecanoic acid	28.253	31.97
28	tetradecanoic acid	28.253	31.97
29	octadecanoic acid	28.499	2.21
26	9,12-octadecadienoic acid	30.948	35.27

The GC-MS analysis of ECSH-T identified a total of 75 peaks. Nine compounds have been similarly reported in the previous study. Initially, two compounds were identified as belonging to the alkane class of compounds. Heptadecane **(15)** was reported by Ma *et al.* (2015) in their study of essential oils derived from *Kaempferia galangal L*. Oxirane **(13)** was also identified in ECSH-T. Only one compound was identified as an aldehyde. 2-Dodecenal **(27)** was reported by Daniel-Jambun *et al.* (2017). Oxalic acid **(21)**, as identified in the study by Barbosa *et al.* (2017), was also found in ECSH-T. The study of Zingiberaceae plants reported four compounds. Hexadecanoic acid **(23)**, n-hexadecanoic acid **(24)**, tetradecanoic acid **(28)**, and 9,12-octadecadienoic acid **(26)** were identified. 9,2-octadecadienoic acid was also reported by Maimulyanti and Prihadi (2015). Octadecanoic acid **(29)** was identified by Pham *et al.* (2020) in their study of the *Kaempferia* L. genus.

Several compounds identified above exhibited beneficial properties. Heptadecane has been previously reported to possess anti-microbial, antioxidant, and anti-cancer properties. GC-MS analysis of roselle calyx revealed the presence of compounds that were identical to those found in *E. coccinea*. Hexadecenoic acid has been reported to possess anti-inflammatory and anti-cancer properties (Pham *et al.*, 2020). Hexadecanoic acid has been previously reported to possess anti-androgenic and hemolytic properties as a 5-alpha reductase inhibitor. N-hexadecanoic acid exhibits similar properties, including antioxidant and hypo-cholesterolemic effects, and contributes to the regulation of swelling in the human body. 9,12-octadecadienoic acid possesses the previously mentioned properties, in addition to anti-histaminic, insecticidal, anti-eczema, and anti-acne effects. Pentadecanoic acid has been reported to possess antioxidant properties. Octadecanoic acid exhibits anti-tumor properties (Gavamukulya *et al.*, 2015).

The volatile compounds identified in this study align with those previously documented in *Etlingera* species and the Zingiberaceae family, where terpenoids and

associated hydrocarbons are frequently recognised as primary components. The presence of these compound classes in both regions reinforces the conclusion that geographical variation between Ranau and Tambunan does not lead to significant qualitative differences in the volatile metabolite profiles of *E. coccinea* stalks. GC-MS analysis indicates that the volatile profiles of *E. coccinea* stalk extracts from Ranau and Tambunan are qualitatively similar, with differences primarily observed in relative abundance. The findings support the earlier HPLC and LC-MS results and strengthen the conclusion that geographical origin has a minimal impact on the overall metabolite composition of *E. coccinea* under the examined conditions.

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

The stalks of *E. coccinea* were effectively extracted, concentrated, and fractionated into three solvent polarities, followed by thorough analysis using phytochemical screening and chromatographic methods. TLC (TLC) profiling was performed to identify the most efficient solvent systems based on their elution behaviour on TLC plates. All fractions were subsequently examined using High Performance Liquid Chromatography (HPLC), Liquid Chromatography–Mass Spectrometry (LC-MS), and Gas Chromatography–Mass Spectrometry (GC-MS) to characterise the secondary metabolites found in this medicinal plant.

The extraction of *E. coccinea* stalks obtained from Ranau and Tambunan involved soaking the samples in 10% aqueous methanol, resulting in crude extracts with percentage yields of 12.77% and 11.08%, respectively. Each crude extract was further partitioned into methanol, chloroform, and *n-hexane* fractions. The percentage yields of the Ranau fractions were 8.40% for methanol, 5.42% for chloroform, and 2.90% for *n-hexane*, whereas the Tambunan fractions yielded 3.72%, 4.94%, and 1.02%, respectively. The methanolic fraction from Ranau and the chloroform fraction from Tambunan exhibited the highest yields.

Qualitative studies of all fractions were performed utilising TLC profiling and phytochemical screening. In the samples from Ranau and Tambunan, solvent systems comprising *n-hexane* and ethyl acetate in ratios of 7:3, 5:5, 3:5, and 1:9 demonstrated the highest efficacy in separation. These solvent systems were then utilised as benchmarks for HPLC solvent optimisation, with gradient elution favoured to enhance chemical resolution.

Phytochemical analysis identified flavonoids, tannins, and terpenoids in all fractions from both sites. Conversely, alkaloids and saponins were absent in all examined fractions. The results reveal a uniform phytochemical profile characterised by phenolic and terpenoid chemicals in both geographical areas.

LC-MS analysis found metabolites previously documented in the Zingiberaceae family, namely phthalic acid and phytosphingosine. Both substances were identified in the methanolic and chloroform extracts from Ranau, signifying a varied metabolite makeup. Conversely, samples from Tambunan contained phthalic acid but had no significant quantities of phytosphingosine. The regional variations in metabolite profiles indicate possible effects of environmental circumstances, genetic diversity, or ecological factors on the generation of secondary metabolites.

Alongside Zingiberaceae-related metabolites, LC-MS analysis identified various compounds frequently documented in other plant families, such as betaine aldehyde, phloridzin, salicyl amide, 4-hydroxyhippuric acid, 3-hydroxy-2-methylbutyric acid, phenylacetic acid, 2-phenylacetamide, and 3-methoxytyramine. The pharmacologically significant metabolites were identified in diverse combinations within the examined extracts. The chloroform extract from Ranau exhibited a comprehensive metabolite profile, encompassing nearly all previously recognised chemicals, with the exception of betaine aldehyde, phloridzin, and 3-hydroxy-2-methylbutyric acid. This extract uniquely contained 3-methoxytyramine, which was not present in the equivalent methanolic extract. These findings underscore the impact of solvent polarity on extraction efficacy and metabolite selectivity.

The analysis of Tambunan extracts demonstrated unique compositional features. The methanolic extract had a complicated metabolite profile, absent only phloridzin, salicyl amide, and 4-hydroxyhippuric acid. In contrast, the chloroform extract from Tambunan exhibited a more restricted composition, containing only salicyl amide, 4-hydroxyhippuric acid, and 3-hydroxy-2-methylbutyric acid. This fraction lacked several metabolites, including betaine aldehyde, phenylacetic acid, 2-phenylacetamide, and 3-methoxytyramine. Moreover, both Tambunan extracts were devoid of phloridzin, which was found in the Ranau methanolic extract. The GC-MS study of the Ranau *n-hexane* extract identified a variety of functional groups, such as alkanes, aldehydes, alcohols, and organic acids. Alkane chemicals, including oxirane, dodecane, heptadecane, and cyclopentane, have been documented in numerous Zingiberaceae species, validating the efficacy of GC-MS in profiling non-polar plant elements.

Aldehyde chemicals identified in the Ranau extract comprised 2-octenal, a substance previously documented in *E. coccinea* and other *Etlingera* species, suggesting common chemical traits among the genus. Alcohol compounds, including 1-

decanol and 2-cyclohexen-1-ol, were found, corroborating previous research on *E. coccinea* and other Zingiberaceae species, such as *Amomum compactum* and *Curcuma longa*. Organic acids identified in the Ranau *n-hexane* extract comprised oxalic acid and pentanoic acid, substances previously documented in *Etlingera* species and *Zingiber officinale*. These findings further illustrate the chemical complexity and variety of organic acids within the Zingiberaceae family.

GC-MS study of Tambunan *n-hexane* extracts indicated comparable functional groups, albeit with diminished compound variety. The alkane group comprised heptadecane and oxirane, both of which were also identified in Ranau extracts. The aldehyde group was only represented by 2-dodecenal, a chemical previously documented in *E. coccinea*. Furthermore, the organic acid group encompassed tetradecanoic acid, a molecule documented in *Zingiber officinale*, hence reinforcing chemical resemblances within the family. This work underscores the need of thorough phytochemical and chromatographic investigations in clarifying the chemical composition of therapeutic plants. The changes in metabolite profiles among solvents and geographical locations highlight the intricacies of plant secondary metabolism and affirm the promise of *E. coccinea* as a source of bioactive chemicals with significant medicinal implications.

5.2 Recommendations

The findings contribute to the study of plant chemistry and have implications for pharmacology, biological activities, and phylogeny. Ongoing research into the chemical elements of Zingiberaceae families aims to elucidate their chemical profiles, which may provide insights into their ecological roles and potential applications within the plant kingdom. Additional investigation into the phytochemical profiles of Zingiberaceae family plants may yield important insights regarding their pharmacological properties and possible medical applications.

Similar to the *E. coccinea* species, additional medical benefits are anticipated to be identified in the coming years. In addition to the increased research on essential oils, the crude extracts of *E. coccinea* demonstrate promising results. Therefore, the chemical compounds present in crude extracts of *E. coccinea* present significant opportunities for identifying the primary compounds and structures within these extracts.

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