Faculty of Resource Science and Technology

Isolation, Characterization and Bioactivities of Bioactive Compounds from *Etlingera* spp.

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DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature

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ABSTRACT

Etlingera spp. is one of the largest genera in the Zingiberaceae family, traditionally used by local communities in Borneo for medicinal purposes, culinary and as insect repellents. Despite its widespread traditional use, there is still lack of scientific evidence supporting its potential as a natural source of antioxidants and biopesticides. This study focused on the extraction, isolation and characterization of bioactive compounds from *Etlingera coccinea*, *Etlingera foetens* and *Etlingera nasuta*, as well as the evaluation of their antioxidant and repellent activities. Sequential solvent extraction was performed using hexane, dichloromethane and ethyl acetate. Isolation of bioactive compounds was conducted using thin layer chromatography and column chromatography, while characterization was carried out using gas chromatography - mass spectrometry (GC-MS), fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR). Essential oils were extracted using the hydrodistillation method and identified by GC-MS supported with Kovats index data. The extract yields ranged from 0.56 % to 14.19 %, while essential oils yields ranged from 0.08 % to 0.42 %. One slightly pure compound, 5-methyl-2-1-methylethenyl acetate was successfully isolated from ethyl acetate extract from *E. nasuta*. Major constituents of essential oils were β-chamigrene (57.41 %), α-copaene (51.63 %) and γ-elemene (31.91 %) in *E. coccinea*; octanal acetate (85.63 %), α-gurjunene (47.75 %) and β-cubebene (14.69 %) in *E. foetens*; and neo-intermedeol (49.89 %) and α-muurolene (27.82 %) in *E. nasuta*. Antioxidant screening based on DPPH free radical scavenging revealed significant activity in hexane extracts and leaves essential oils of *E. foetens* and *E. nasuta*, with IC_{50} values between 20.95 and 25.2 ppm. Similarly, *E. coccinea* rhizome essential oil and hexane and ethyl acetate extracts also possessed strong antioxidant potential. Repellent activity indicated that dichloromethane and ethyl acetate extracts of all three species demonstrated high

repellency (71.43 % - 75.00 %) against *Sitophilus oryzae*, with effectiveness observed within 1 - 3 hours. The findings support the ethnobotanical claims and suggest that these *Etlingera* species are promising candidates as natural antioxidants and biopesticides.

Keywords: *Etlingera*, bioactive compounds, essential oils, antioxidant activity, repellent activity

Pemisahan dan Pengkarakteran Bioaktiviti Sebatian Bioaktif dari Etlingera spp.

ABSTRAK

Etlingera spp. adalah salah satu genus terbesar dalam famili Zingiberaceae yang secara tradisional digunakan oleh komuniti tempatan di Borneo untuk tujuan perubatan, masakan dan sebagai penghalau serangga. Walaupun penggunaanya meluas secara tradisioal, masih terdapat kekurangan bukti saintifik yang menyokong potensinya sebagai sumber semula jadi antioksidan dan biopestisid. Kajian ini memberi tumpuan kepada pengekstrakan, pengasingan dan pencirian sebatian bioaktif daripada Etlingera coccinea, Etlingera foetens dan Etlingera nasuta, serta penilaian terhadap aktiviti antioksidan dan penghalau serangga. Pengekstakan berturutan menggunakan pelarut telah dijalankan dengan menggunakan heksana, diklorometana dan etil asetat. Pengasingan sebatian bioaktif dilakukan menggunakan kromatografi gas-spektrometri jisim (GC-MS), spektoskopi inframerah transformasi fourier (FTIR) dan resonans magnet nuklear (NMR). Minyak pati telah diekstrak melalui kaedah hidrodistilasi dan dikenal pasti menggunakan GC-MS yang disokong oleh indeks Kovats. Hasil ekstrak yang diperoleh adalah antara 0.56 % hingga 14.19 %, manakala minyak pati antara 0.08 % hingga 0.42 %. Satu sebatian hampir tulen, 5-metil-2-1-metiletenil asetat telah berjaya diasingkan daripada ekstrak etil asetat daripada E. nasuta. Komponen utama minyak pati ialah β-chamigrene (57.41 %), α-copaene (51.63 %) dan γ-elemene (31.91 %) dalam E. coccinea; oktanal asetat (85.63 %) dan α-gurjunene dan β-cubebene (14.69 %) dalam E. foetens; and neo-intermedeol (49.89 %) dan α-muurolene (27.82 %) dalam E. nasuta. Saringan aktiviti antioksidan menunjukkan aktiviti tinggi dalam ekstrak heksana dan minyak pati daun E. foetens dan E. nasuta dengan nilai IC_{50} antara 20.95 hingga 25.12 ppm. Minyak pati rizom E. coccinea serta ekstrak heksana dan etil asetat juga menunjukkan potensi antioksidan yang tinggi. Ujian penhalau

menunjukkan bahawa ekstrak diklorometana dan etil asetat daripada ketiga-tiga spesies mempunyai aktiviti penghalau yang tinggi (71.43 - 75.00 %) terhadap Sitophilus oryzae *dengan kesan diperhatikan dalam masa 1 hingga 3 jam. Penemuan ini menyokong dakwaan etnobotani dan mencadangkan spesies* Etlingera *ini sebagai calon berpotensi untuk pembangunan sebagai antioksidan semula jadi dan biopestisid.*

Kata kunci: Etlingera, *sebatian bioaktif, minyak pati, aktiviti antioksidan, aktiviti penghalau*

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LIST OF ABBREVIATIONS

°C	Celsius
J	Coupling constant
δ	Delta
DPPH	2, 2-diphenyl-1-picrylhydrazyl
E.	*Etlingera*
g	Gram
IC_{50}	Inhibitory concentration
km	Kilometer
L	Litre
m	Meter
mt	Metric ton
%	Percentage
v/v	Volume per volume
v/w	Volume per weight

CHAPTER 1

INTRODUCTION

1.1 Study Background

Natural products are a valuable source of bioactive compounds with significant potential for the development of new drugs and therapeutic agents. These secondary metabolites such as flavonoids, terpenoids and phenolic possess diverse biological activities including antioxidant, antimicrobial, anti-inflammatory and insect repellent properties (Cos *et al.,* 2006). With over 80 % of the global population relying on traditional medicine according to the World Health Organization (WHO), resistance to synthetic drugs is increasing and leading to the growing demand for safer and eco-friendly alternatives (Khadim *et al*., 2024).

The Zingiberaceae family (ginger family is one of the largest family within the Zingiberales order, comprising over 60 genera's and approximately 1 500 species globally, especially in Southeast Asia (Mohamad & Kalu, 2019). These aromatic and medicinal plants are used widely in food, cosmetics, traditional medicine and rituals. In Borneo, *Etlingera* is recognized for its rich biodiversity and has significant in local cultures such as traditional medicine, culinary and rituals (Salasiah *et al*., 2022). For an example, *Etlingera* found in tropical forests is traditionally used to treat ailments such as fever, infections, wounds and stomachache (Madavi *et al*., 2016). *Etlingera elatior* is the most studies species in this genus for their bioactivities (Chan *et al*., 2011). However, other species such as *E. coccinea*, *E. foetens* and *E. nasuta* have limited investigation regarding their phytochemical profiles and biological activities.

There is also increasing interest in plant-based repellents as eco-friendly alternatives to synthetic insecticides, which can harm non-traget species and environment. In tropical countries, post-harvest grain losses due to insect infestation range from 10 % to 30 % (Mesterházy *et al*., 2020), emphasizing the need for effective botanical repellents (Adeniyi *et al*., 2010).

This study aims to explore the phytochemical composition and biological potential of these studied species, contributing scientific evidence to support traditional claims and encouraging further research into their pharmacological and pest control applications.

1.2 Problem Statement

Etlingera spp. is a diverse genus within the Zingiberaceae family, traditionally utilized by local communities in Borneo for medicinal, culinary and insect-repelling purposes. While traditional knowledge supports the use of *Etlingera* spp., scientific validation of their bioactive compounds and bioactivities remain limited. The gap is significant leading to the growing demand for natural alternatives to synthetic antioxidants and pesticides, which are associated with environmental concerns, health hazards and the emergence of resistant pest species such as *Sitophilus oryzae*. The overreliance on chemical fumigants like phosphine has intensified the need for safe, effective and sustainable botanical alternatives.

Previous studies have shown that certain *Etlingera* species possess bioactive compounds with significant antioxidant and insect-repellent properties, suggesting their viability as natural substitutes for synthetic chemicals. With at least 40 species of *Etlingera* spp. have been recorded in Borneo including *E. coccinea*, *E. foetens* and *E. nasuta*, it is possible to reveal unique phytochemicals with potential industrial applications from

different species of *Etlingera* from different locations. These findings highlight the potential of *Etlingera* spp. as natural antioxidants and insect repellent to be further explored. However, the bioactive compounds and repellent efficacy of *Etlingera* spp. remain inadequately investigated. Exploring the bioactive constituents of *Etlingera* spp. may uncover novel compounds for sustainable applications.

To address these gaps, this study focuses on three selected species of *Etlingera* including *E. coccinea, E. foetens* and *E. nasuta* which are native to Borneo. The scope of study encompasses the phytochemical investigation of these species, specifically targeting the leaves, rhizomes and stems. This study involves isolation and characterization of bioactive compounds from leaves extract using chromatographic and spectroscopic techniques such as thin layer chromatography, column chromatography, gas chromatography - mass spectrometry, fourier transform infrared and nuclear magnetic resonance. Essential oils from leaves, rhizomes and stems parts analyzed using gas chromatography - mass spectrometry supported with Kovats retention index. Evaluation the antioxidant and repellent activities is carried out to determine the potential applications of *Etlingera* spp. using DPPH radical scavenging method and repellent activity against *S. oryzae*.

The findings from this study are expected to provide scientific evidence to support the traditional knowledge and highlight the potential of *Etlingera* spp. as sources of natural antioxidant an insect repellent for the food, cosmetic, pharmaceutical.

1.3 Objectives

The aims of this study are:

i. To isolate and characterize the compounds from the leaves extract of *Etlingera* spp. using chromatographic and spectroscopic techniques such as thin layer chromatography, column chromatography, gas chromatography - mass spectrometry (GCMS), nuclear magnetic resonance (NMR) spectroscopy and fourier transform infrared (FTIR)

ii. To identify and analyze the chemical constituents of essential oils extracted from leaves, rhizomes and stems of *Etlingera* spp. using gas chromatography mass spectrometry supported with Kovats retention index data

iii. To evaluate the antioxidant of crude extracts and essential oils from *Etlingera* spp. using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals and to assess repellent activity of extracts against rice weevils of *Sitophilus oryzae*, respectively to explore their potential as natural antioxidant and insect-repellent.

CHAPTER 2

LITERATURE REVIEW

2.1 Zingiberaceae

Zingiberaceae family belongs to Zingiberales order (Costaceae, Cannacea, Marantaceae, Musaceae, Strelitziaceae, Lowiceaceae and Helioniaceae), distributed in the tropics and subtropics with the highest diversity number of taxa especially in the Asian tropics. There are nine genera in the Zingiberaceae family including *Etlingera*, *Amomum* Roxb., *Alpinia* Roxb., *Plagiotachys* Ridl., *Hornstedia* Retz. and *Geostachys* (Baker) Ridl. (Salasiah *et al*., 2021). Zingiberaceae families are used for various purposes such as medicines, foods, food additives, beverages, ornamental plants, fragrances, cosmetics, in ritual and ceremonies locally and commercially all around the world. The most interested species from this family is gingers, perennial herbs belong to three tribes (Alpineae, Zingibereae and Hedychieae) because they produce aromatic rhizomes. Each rhizome is subterranean or above ground, produce leafy shoots, terminal inflorescences borne either on leafy shoots or on erect shoots near the base of the plants (Chan *et al.*, 2011). In Borneo, this species family belongs to Riedeliae and Alpineae tribes with at least 145 taxa from 13 genera (Mohamad & Meekiong, 2020; Salasiah *et al*., 2022). For *Etlingera*, about 15 (Peninsular Malaysia) and 42 (Borneo) spp. of *Etlingera* have been identified. The distribution of *Etlingera* is in lowland forests, 2 500 m above the sea level and tropical regions throughout the world. This genus is used traditionally as wound healing, antioxidant, condiments, vegetables, pickles, improve dry skin, treat fever, sore eyes, sore throat, jaundice, stomachache, food poisoning, gastric, rheumatism, sunstroke, respiratory problems and remove body odour after postpartum.

2.2 Phytochemistry of *Etlingera* spp.

The demand for bioactive compounds has grown local and globally, prompting exploration of both novel and known natural products. Among the Zingiberaceae family, *Etlingera* spp., particularly *E. elatior* have been a primary focus due to their rich phytochemical's profiles. Studies have identified several flavonoids from the leaves of *E. elatior*, including kaempferol and quercetin derivatives. Rhizome analysis revealed diarylheptanoids, labdane diterpenoids and steroids such as 1,7-bis(4-hydroxyphenyl)- -heptatrienones and stigmastane-type derivatives (Chan *et al*., 2011). Similarly, *E. fimbriobracteata* has demonstrated the presence of cardiac glycosides, saponins, and steroids through preliminary phytochemical screening (Shahid-Ud-Daula *et al*., 2019). However, other *Etlingera* species such as *E. coccinea, E. foetens*, and *E. nasuta* remain underexplored, indicating a significant gap in phytochemical investigations.

Within the Zingiberaceae family, terpenoids are the predominant components of essential oils. These compounds are broadly classified into terpenes, their oxygenated derivatives (terpenoids), and non-terpenoid constituents. Terpenoids are biosynthesized via the mevalonic acid pathway and are categorized based on the number of isoprene units, including monoterpenes (C_{10}), sesquiterpenes (C_{15}), and higher-order derivatives. Essential oils from *Etlingera* species are typically rich in both monoterpenes and sesquiterpenes such as α-pinene, limonene, cadinene, and caryophyllene. These terpenes can undergo enzymatic modifications resulting in terpenoids such as 1,8-cineole, linalool, α-terpineol, and nerolidol. The aerial parts, particularly the leaves of *Etlingera* species such as *E. fulgens* and *E. venusta*, are typically characterized by a high abundance of monoterpenes, followed by oxygenated monoterpenes and sesquiterpenes.

In addition to terpenes, non-terpenoid compounds such as phenylpropanoids are frequently identified in *Etlingera* essential oils. These compounds are synthesized from phenylalanine and serve crucial ecological roles including plant defence and interspecies signaling. According to Shahid-Ud-Daula and Basher (2019), major phenylpropanoids found in eight *Etlingera* species include elemicin, eugenol, and methyl eugenol. Other compound classes that have been identified in the genus include alcohols, ketones, carboxylic acids, and hydrocarbons such as dodecanoic acid and dodecyl acetate. The diverse chemical composition of *Etlingera* essential oils underpins their broad biological activities, including antioxidant, antimicrobial, insecticidal, and repellent properties, which highlights the importance of systematic extraction and chemical profiling.

For instance, Mahdavi *et al*. (2016) reported that essential oils extracted from *E. brevilabrum* leaves and stems contained up to 73.4 % monoterpene hydrocarbons, while stolons and rhizomes were dominated by oxygenated monoterpenes, reaching concentrations above 50 %. Notable constituents identified included perilla aldehyde, bornyl acetate, and *β*-pinene. In a related study, Khaleghi *et al*. (2012) identified *α*-terpineol and linalool as the primary components in the leaf and shoot oils of *E. venusta*, while its rhizome oils were rich in decanoic acid and cyclododecane. Conversely, the essential oil profile of *E. cevuga* was dominated by phenylpropanoids such as methyl eugenol and eugenol, along with significant amounts of monoterpenes and alcohols (Vahirua-Lechat & Mitermite, 2010).

These findings demonstrate the chemical richness of *Etlingera* species and underscore the need for further phytochemical studies, particularly in underexplored species, to fully harness their potential for pharmaceutical and agricultural applications.

2.3 Traditional and Commercial Uses of *Etlingera* spp.

Various species of the genus *Etlingera* (Zingiberaceae) are widely cultivated and utilized in traditional practices, particularly for culinary, medicinal and cosmetic purposes. Inner sheaths of leafy shoots from *E. coccinea*, *E. elatior*, *E. littoralis*, *E. sessilanthera*, *E. velutina*, and *E. rubromarginata* are traditionally consumed as condiments in local dishes (Poulsen, 2006). The fruits of *E. littoralis* are eaten either raw or cooked, depending on regional preferences. Several species have also been incorporated into cosmetic formulations; for example, the rhizomes of *E. baramensis* are used in the production of perfumes, while fruits of *E. elatior* and *E. pyramidosphaera* are formulated into shampoos (Poulsen, 2006). In addition, the rhizomes of *E. punica* serve as a spice in traditional cuisine.

The leaves and stems of *E. fimbriobracteata* are important culinary ingredients, and its ripe fruits valued for their sweet-sour flavour are consumed raw. The leaves of this species are also traditionally used to wrap rice, while its leaf sheaths are woven into mats for domestic use. Moreover, young shoots and inflorescences of various *Etlingera* species are consumed as vegetables (Shahid-Ud-Daula *et al*., 2016). In Aceh, *E. elatior* is used as flavour enhancer, culinary, ornamental plants (Wong *et al*., 2013). The flower buds are used in local delicacies sush as *anyang*, *urap*, *pecel* and *sayur asem* (Saudah *et al*., 2022) while in Malaysia, young inflorescences are used in traditional foods including asam laksa, nasi kerabu and nasi ulam (Chiang *et al*., 2010).

Medicinally, several *Etlingera* species are employed in traditional healthcare systems to treat rheumatism, jaundice, urinary ailments, headaches, stomachaches, snake bites, and diarrhea. Documented species with ethnomedicinal applications include *E. foetens*, *E. belalongensis*, *E. elatior*, *E. brevilabrum*, *E. pyramidosphaera*, and *E. sessilanthera* (Poulsen, 2006). Specifically, the inner sheaths of *E. brevilabrum* are applied to treat skin

irritations and itchiness, while juice extracted from the pseudostem of *E. labellosa* is traditionally used to relieve body aches (Poulsen, 2006).

Table 2.1: Traditional and commercial uses of *Etlingera* spp. (Poulsen 2006; Shahid-Ud-Daula *et al*., 2019)

Species	Parts/Usage	Diseases/Ailments	Foods	Industry
E. coccinea, E. elatior, E.littoralis, E. sessilanthera, E. velutina and *E. rubromarginata*	Inner sheaths of leafy shoots	No data available	Condiments	No data available
E. littoralis	Fruits	No data available	Eaten raw or cooked	No data available
E. baramensis	Rhizomes	No data available	No data available	Perfume
E. elatior and *E. pyramidosphaera*	Fruits	No data available	No data available	Shampoo
E. punica	Rhizomes	No data available	Spices	No data available
E. brevilabrum and *E. elatior*	Leaves	Medicine in children against long-lasting fever and cleaning wounds	No data available	No data available
E. brevilabrum	Inner sheaths	Treatment of skin problems and itchiness	No data available	No data available
E. brevilabrum	Juice of young shoots	Sore eyes	No data available	No data available
*E. foetens, E. belalongensis, E. elatior, E. brevilabrum, E. pyramidosphaer*a and *E. sessilanthera*	Externally	Rheumatism, jaundice, urinary ailments, headache, stomachache, snake bite and diarrhea	No data available	No data available
E. fimbriobracteata	Leaves and stems	No data available	Cooking ingredients	No data available
E. fimbriobracteata	Ripe fruits	No data available	Eaten raw and has a sweet-sour taste	No data available
E. fimbriobracteata	Leaves	No data available	Wrap rice	No data available
E. fimbriobracteata	Leaf sheaths	No data available	No data available	Mats
E. fimbriobracteata	Young shoots and young inflorescences	No data available	Vegetables	No data available
E. labellosa	Pseudo stem juice	Body ache	No data available	No data available

2.4 Bioactivities and Phytochemicals research gaps in the *Etlingera* genus

The *Etlingera* genus (Zingiberaceae) has been widely recognized for its ethnomedicinal relevance and diverse pharmacological properties. Several species, such as *Etlingera elatior*, have been extensively studied and reported to possess antioxidant, antibacterial, antifungal, tyrosinase inhibition, cytotoxic, and hepatoprotective activities (Chan *et al*., 2011; Meekiong & Teo, 2022). Likewise, *E. fimbriobracteata* and *E. brevilabrum* have demonstrated significant antioxidant and anticancer effects (Shahid-Ud-Daula *et al*., 2019; Meekiong & Teo, 2022). Despite these findings, a substantial number of species within the genus remain underexplored, with minimal scientific data available on their phytochemistry or bioactivities. Notably, *Etlingera coccinea*, *E. foetens*, and *E. nasuta* are among the species for which there is currently little to no published information regarding their chemical composition or biological effects. This gap in the literature presents an opportunity to investigate their antioxidant and potential repellent properties, which could uncover novel bioactive compounds and expand the therapeutic applications of the genus. Therefore, the present study aims to address this knowledge gap by exploring the phytochemical constituents and bioactivities of *E. coccinea, E. foetens* and *E. nasuta*.

Table 2.2: Varieties of *Etlingera* spp.

Species	Vernacular name	Distribution	Bioactive properties	Reference
E. elatior	*Kantan, Kenchala*	Southeast Asia	Antioxidant, Antibacterial**,** Antifungal activity, Tyrosinase inhibition activity, Cytotoxic activity and Hepatoprotective activity	Chan *et al*., (2011); Meekiong & Teo, 2022
E. baculutea	*Tubu kerinang* (Lunbawang)	Endemic to Borneo (Sabah and Sarawak), can be found at elevation more than 900 m altitudes	No data available	Meekiong & Teo, 2022
E. barioensis	*Tubu bua'saleh* (Kelabit)	Endemic to Sarawak	No data available	Meekiong & Teo, 2022
E. brevilabrum	-	Most common species in the Heart of Borneo, endemic to Borneo, Brunei, Kalimantan and Sabah	Anticancer	Meekiong & Teo, 2022
E. baramensis	No data available	Endemic to Borneo (Sarawak and Kalimantan)	No data available	Meekiong & Teo, 2022
E. burtii	No data available	Limestone of Mulu National Park at lower altitude	No data available	Meekiong & Teo, 2022
E. crispate	*Tubu saleh* (Kelabit), *Tubu kerinang* (Lunbawang)	Primary and moderately disturbed montane forests (above 900 m)	No data available	Meekiong & Teo, 2022

Cont. of Table 2.2.

E. inundata	*Ketimbang duduk* or *Senggang pelandok* (Iban)	Small streams in primary and secondary mixed dipterocarp forests, *kerangas* forests. Endemic to Borneo	No data available	Meekiong & Teo, 2022
E. kenyalang	*Tepus kenyalang*	Endemic to Sarawak	No data available	Meekiong & Teo, 2022
E. sessilanthera	*Tubu pelapad* (Kelabit)	Endemic to Borneo	Antimicrobial	Daniel- Jambun *et al*., 2017; Meekiong & Teo, 2022
E. fimbriobracteta	*Tukung* (Iban), *Layun* (Kelabit)	Riverbanks of Sg. Temburong and Sg. Belalong, Brunei Darussalam. Also, reported as an endemic plant in Sarawak and Brunei.	Antioxidant, anticancer	Shahid-Ud-Daula *et al*., 2019
E. pyramidosphaera	*Kenchala*	Common in reserve area and limestone hill of Borneo	Anticancer	Gobilik *et al*., 2000
E. littoralis	-	Malay Penisula, Gulf of Thailand and Andaman Sea Coasts.	Antibacterial	Chan *et al*., 2007; Chongkraijak *et al*., 2013
E. rubromarginata	*Ketimbang mansau* (Iban)	Mixed dipterocarp forests, primary or secondary forests mostly in swampy habitats near stream from 30 m to 850 n elevations. Endemic to Borneo	No data available	Meekiong & Teo, 2022

2.4.1 Antimicrobial and antibacterial activity

Since a long time ago, crude extracts from different parts of medicinal plants have been used to treat human diseases. The abundance of medicinal plants in the traditional knowledge increases the understanding in the medicinal plants' properties, safety and efficacy (Obeidat *et al*., 2012). Recent years, the concern on antimicrobial properties of essential oils and their major components has risen to be applied in the food industry. Essential oils are an alternative to synthetic preservatives due to their ability to inhibit foodborne pathogens and extend the shelf life of perishable products. Essential oils consist of various bioactive compounds mainly terpenes and polyphenol which exhibit antimicrobial properties to disrupt the bacteria cell membranes, inhibit enzyme activity, cause leakage of essential molecules that leads to bacterial cell death. However, essential oils are more effective against gram-positive bacteria than gram-negative bacteria due to differences in cell wall structures but some essential oils components are able to bypass the protective outer membrane of gram-negative bacteria. Although they show a significant effectiveness, the commercial use of essential oils is limited due to their strong flavours and odours (Agane *et al*., 2022). Naksang *et al*. (2020) assessed the antimicrobial activity, mode of action and volatile compounds of *Etlingera pavieana* rhizomes essential oil. The essential oil was extracted using hydrodistillation and analyzed using headspace solid-phase microextraction coupled with gas chromatography - mass spectrometry, 22 compounds were identified. Trans-anethole (78.54 %) and estragole (19.36 %) were their major components. Other compounds identified was β-myrcene, camphene, pinocarvone, camphor and α-pinene. Rhizomes essential oil of *E. pavieana* exhibited potent antibacterial activity against gram-positive foodborne pathogens including *Bacillus cereus*, *Staphlococcus aureus* and *Listeria monocytogenes*. The mode of action of *E. pavieana* rhizomes essential oil was analyzed

using synchroton fourier transform infrared (FTIR) microspectroscopy to study the essential oil effects on bacterial cells. The essential oil disrupted DNA / nucleic acids, protein and cell membrane leading to bacterial cell death. A significant change in bacterial molecular structures was confirmed using principal component analysis (PCA). However, this study only focused on one part of the plants. Lutfia *et al*. (2019) screened the antagonistic fungi of *Etlingera littoralis* rhizome from Sibolangit Forest, North Sumatra. Fresh rhizomes of *E. littoralis* and the endophyhtic fungi was isolated by the method of surface sterilization using ethanol and sodium hypochlorite followed by plating on potato dextrose agar supplemented by chloramphenicol whereas the antagonism assay by method of agar plug diffusion method evaluated fungal isolated against *Staphylococcus aureus,* Methicillin-resistant *S. aureus, Eschericia coli* and Enterophathogenic *E. coli* K11. The zone of inhibition indicated antagonistic activity. Six fungal isolates (Eel01SU, Eel02SU, Eel03SU, Eel04SU, Eel05SU and Eel06SU) were identified with various antagonistic activities against tested bacterial pathogens. Most fungal isolates exhibited strong activity against *Staphylococcus aureus* and making it the most sensitive strain in the study. Ee105SU demonstrated antibacterial activity against all tested pathogens. However, no specific bioactive compounds responsible for the antagonistic activity are identified in this study. Daniel-Jambun *et al*., (2017) investigated the antimicrobial properties of two ginger species, *Etlingera coccinea* and *Etlingera sessilanthera,* which are endemic to Borneo. This study used the sequential extraction method to obtain fractionation from hexane, dichloromethane, ethyl acetate, methanol and water and tested the antimicrobial activity (broth microdilution assay) using 7 strains of bacteria. Results revealed that two antimicrobial compound isolated trans-2-dodecenal and 8(17), 12-labdadiene-15,16-dial exhibited low MIC values (4 - 8 μg/mL) against *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*. However, this study lack of toxicity

assessment to evaluate the safety of the isolated compounds which is crucial for medicinal applications. Chan *et al*., (2007) studied the antibacterial properties from the methanolic leaf extracts of five *Etlinger*a species including *E. elatior*, *E. fulgens*, *E. maingayi*, *E. littoralis*, and *E. rubrostriata* from Peninsular Malaysia. Antibacterial screening showed that the extracts exhibited activity against gram-positive bacteria (*Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*) but not against gram-negative bacteria (*Eschericia coli*, *Pseudomonas aeruginosa* and *Salmonella cholerasuis*). The finding is not further to isolate the bioactive compounds that possess antibacterial properties. According to Ghasemzadeh *et al*. (2019), *E. elatior* demonstrated antibacterial activity. The study was done on *E. elatior* grown in different locations of Malaysia. Results revealed that ethanolic extract of *E. elatior* grown in Kelantan exhibited the strongest antibacterial activity compared to samples from Johor and Pahang. The extracts from Kelantan showed higher inhibition zones against bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella typhimurium*. The minimum inhibitiory concentrations (MIC) of Kelantan samples were lower, indicating greater potency in suppressing bacterial growth. In contrast, samples from Johor and Pahang had MIC values, suggesting weaker antibacterial effects. However, this study does not include in vivo studies to confirm the bioavailability of *E. elatior* for medicinal applications.

Several studies have demonstrated the promising antimicrobial and antibacterial potential of various *Etlingera* species, particularly against gram-positive bacteria. Crude extracts and essential oils from leaves ad rhizomes have shown significant inhibitory effects, with key compounds such as trans-anethole, trans-2-dodecenal and labdadiene derivatives being identified. The strength of activity appears influenced by both chemical composition and geographical origin. However, most research focused on a single plant part only; no

toxicity assessments were conducted for isolated compounds. A few studies isolated and characterized the exact bioactive molecules responsible for the bioactivities and no in vivo or clinical studies have been conducted to confirm therapeutic potential and bioavailability.

2.4.2 Antioxidant activity

An antioxidant is a molecule that can donate an electron to a free radical and scavenge the free radical by reducing its capacity to damage. Inhibition of cellular damage and antioxidants delay depends on their free radical scavenging property. Low molecular weight antioxidants can react with free radicals and discontinue chain reaction before the important molecule is damaged. Some of the antioxidants are produced from the metabolism pathway in our body such as glutathione, ubiquinol and uric acid but some of them are micronutrient antioxidants such as vitamin E (α-tocopherol), vitamin C (ascorbic acid) and β-carotene. Previous study revealed the antioxidants by focusing on the prevention of oxidation of unsaturated fats which leads to rancidity. However, the study of vitamins A, C and E as antioxidants brings towards the realization among the researchers, the vitamins are the major useful antioxidants in the biochemistry field (Sati *et al*., 2010). Different parts of the plant such as roots, leaves, stems, flowers, rhizomes and roots contribute to abundant of antioxidant compounds. Antioxidant reaction decreased the oxidative stress-induced carcinogenesis by direct scavenging of reactive oxygen either and or by inhibiting cell proliferation secondary to protein phosphorylation (Lobo *et al*., 2010). Antioxidant compounds are widely used for industrial applications such as preservatives in food and cosmetics and put off the degradation of rubber and gasoline. In food industries, free radicals are responsible for lipid oxidation that deteriorate foods during processing and storage. Thus, the addition of antioxidants in food and biological systems to scavenge free radicals become a worldwide interest. Synthetic materials antioxidants such as butylated hydroxyl anisole

(BHA), ethoxyquin and butylated hydroxytoluene (BHT) are widely used in food industries since the early century. A previous study shown, these synthetic antioxidants are produced toxic and harmful effects but eventually some of the secondary metabolites such as flavonoids, phenolics and polyphenolics show antioxidative effectivity. Therefore, antioxidants from plants are suitable to substitute synthetic antioxidants. Based on the epidemiological results, people who consumed fresh fruits and vegetables in their diet intake will have a less risk of cardiovascular diseases and certain cancer because the natural antioxidants will scavenge the free radical immediately after being process in our metabolic activities (Sati *et al*., 2010).

Chain breaking mechanism and removal of reactive oxygen species initiators by quenching chain initiating catalyst are the two main principles mechanisms of antioxidants that have been proposed. Antioxidants are affected by different mechanisms as well as electron donation, metal ion chelation, co - antioxidants or gene expression regulation. Antioxidants have working defence systems at various levels. Preventive antioxidants are the first line of defence that conquer the formation of free radicals by reducing hydroperoxides and hydrogen peroxides before transforming into alcohol and water without producing free radicals and some proteins sequester metal ions, decomposition of lipid peroxidase into alcohol can be done by glutathione peroxidase, glutathione - s - transferase, phospholipid hydroperoxide glutathione peroxidase (PHGPX) and peroxidase. PHGPX integrated bio - membranes by reducing hydroperoxides of phospholipids wherein the water is produced from glutathione peroxidase and catalase that reduces hydrogen peroxide. The second line of defence system consists of antioxidants that scavenge the active radicals to conquer chain initiation or break the chain propagation reactions. Endogenous radical scavenging antioxidants that have been discovered are vitamin C, uric acid, bilirubin,

albumin, thiols, vitamin E and ubiquinol. The third line of defence system is repairing and de novo antioxidants including proteolytic enzymes, proteinases, proteases and peptidases that present in cytosol and mitochondria of mammalian cells, recognizing, degrade and removing oxidatively modified proteins and preventing the accumulation of oxidized proteins. Oxidative damage can be secured by a DNA repair system that acts as a total defence system. Abundant of enzymes will repair the damaged DNA including glycolysis and nucleases (Krisnaiah *et al*., 2011).

Sabli *et al*., (2012) reported the antioxidant properties of selected *Etlingera* and *Zingiber* species (Zingiberaceae) from Borneo Island. Antioxidant activity was evaluated on methanolic extracts of rhizomes and stems of *Etlingera belalongensis*, *Etlingera velutina*, *Zingiber vinosum* and *Zingiber pseudopungens* using 1,1-diphenyl-2-picrylhydrazyl free radical scavenging (DPPH) assay, 2-2-azinobis-3-ethylbenzothioazaline-6-sulphonate radical scavenging (ABTS) assay and Ferric-reducing antioxidant power (FRAP). The results assessed using DPPH and FRAP assays were significant with $P < 0.05$. Mahdavi *et al.* (2017), investigated the chemical composition and antioxidant activity of *Etlingera sayapensis* A. D. Poulsen & Ibrahim. The essential oils of leaves, rhizomes and stems of *E. sayapensis* were obtained using a Clevenger-type apparatus and analyzed using gas chromatography - flame ion detection and gas chromatography - mass spectrometry. The antioxidant activitywas evaluated using DPPH radical scavenging activity, β-carotene bleaching and ferrous ion chelating ability. Leaves were abundant of carvone (21.38 %), cis-carveol (13.49 %), rhizomes were high in linalool formate (25.47 %) and eugenol (11.84 %) while stems were dominated with α-terpineol (39.86 %) and linalool formate (30.55 %). The leaves oil present as the highest potential of antioxidant among all of the antioxidant activity. However, this study focus only sample collected from a single location. More sampling

would strengthen the findings. Mendez *et al.* (2023) investigated the antioxidant activity of leaves and rhizomes of *Etlingera coccinea*. Leaves and rhizomes of *E. coccinea* were collected from Mount Malambo, Marilog District, Davao, Philippines. The samples were air-dried, ground into powder and extracted using absolute ethanol. The total antioxidant activity was evaluated using phophomolybdenum assay and ferric reducing power (FRAP). Results showed that leaves exhibited higher bioactivity than rhizomes in all assays with Total antioxidant activity (TAA) of leaves recording 12.76 ± 0.31 mg AAE/g compared to 0.85 ± 0.12 mg AAE/g in the rhizomes. The Reducing Power (RP) also demonstrated a pronounced difference, with the leaves showing 9.37 ± 1.88 mg GRPE/g and the rhizomes 0.28 ± 0.07 mg GRPE/g. A perfect positive correlation ($r = 1$, $p < 0.001$) among TPC, TAA, and RP indicates a strong association between phenolic content and antioxidant capacity. However, other plant parts should be included and no biological testing in the study.

Studies on various *Etlingera* and *Zingiber* species have consistently demonstrated notable antioxidant properties, particularly in leaves extracts. Antioxidant activities were confirmed using multiple assays (DPPH, ABTS, FRAP, *β*-carotene bleaching, and phosphomolybdenum), with leaves generally showing higher activity than rhizomes or stems. Key bioactive compounds such as carvone, cis-carveol, linalool formate, and α-terpineol were identified in essential oils. While findings highlight the antioxidant potential of *Etlingera* species, most studies are limited by single-location sampling and lack of biological activity testing, indicating the need for broader sampling and in vivo validation.

2.4.3 Repellent activity

Two most abundant genus *Sitophilus* in the tropic, sub-tropic and warm zones are *Sitophilus oryzae* (L.), rice weevil and *Sitophilus zeamais* (Mostch.) (Devi *et al*., 2017). The invasions of both species may be facilitated the establishment of secondary colonizers, mites and stored product pathogens (Devi *et al*., 2017; Trematerra *et al*., 2007). In recent days, most of the rice industry are depending on the synthetic pesticide but the synthetic pesticide has developed resistance in *Sitophilus oryzae* populations and harmful towards the ecosystem. The usage of synthetic pesticide would cause an adverse effect on human health such as acute toxicity, cancer and endocrine systems problem (Murugesan *et al*., 2021). As a matter of that, insect pests contributed to the loss of crops and industrial products especially in African country at the stage of production and storage. Yearly, about 10 to 30 % of all food products in African countries are destroyed by insects. *S. oryzae* or known as rice weevil, is one of the most popular widespread and destructive insects. Due to the widely usage of synthetic pesticide, it has led the researchers to develop an eco-friendly pest control. Current studies on the insecticidal activities of plant extracts and essential oils have been evolved and suggested that they could be explored as an alternative to replace many synthetic insecticides that have been marketed as based on the phytochemical and chemical profiling of the plants have been reported for their insecticidal potential. Indeed, the compounds that present in the plant extracts and essential oil have seemed to be able to react with the physiology and the behaviour of insects and lead to their death (Maazoun *et al*., 2019). Essential oils application has been increased as they have demands from the organic farmers and consumers that are environmentally conscious. Also, bioactive components in the essential oils have insecticidal, nematocidal and antifungal properties which led to the concern of their residue on stored grains or in water dye to their highly volatile constituents

and environmentally non-persistent (Khani *et al*., 2017). To best of our knowledge, the insecticidal activity on *Etlingera* spp. is less likely being reported. Hence, plant extracts and essential oils are potentially to carry out insecticidal activity. Rice weevils (*S. oryzae*) could be the most significant species that caused loss to grain and rice (Tian *et al*., 2023). This study interested to study the *S. oryzae* because of Malaysia's temperature and humidity. Despite the available data, there is limited research on the pest control management of *S. oryzae* in Malaysia.

Kingdom, phylum, class, order, family and subfamily of *S. oryzae* was shown in Table 2.3. *Sitophilus oryzae,* or known as rice weevil is in the kingdom and animalia of phylum: antropoda; class: insecta; order: Coleoptera, family: Curculionidae, subfamily: Curculionidae; subfamily: Dryopthorinae; genus: *Sitophilus* and species: *S. oryzae.* The life cycle of *S. oryzae* is eggs, early larvae, pupae, young adults and adults respectively (Annis **& Morton, 1997).**

Table 2.3: Kingdom and animalia of *S. oryzae*

Kingdom	Animalia
Phylum	Arthropoda
Class	Insecta
Order	Coleoptera
Family	Curculionidae
Subfamily	Dryophthorinae
Genus	*Sitophilus*
Species	*S. oryzae*

A study reported by Guo *et al*., (2015) reported the chemical composition and insecticidal potential of the essential oil extracted from *Etlingera yunnanensis* rhizomes. The study focused on its effectiveness in controlling two major stored product insect pests, *Tribolium castaneum* (red flour beetle) and *Liposcelis bostrychophila* (booklice). Essential oil was obtained from hydrodistillation, analyzed using gas chromatography - mass spectrometry (GC-MS) and the repellency test was evaluated to determine the ability of *E. yunnanensis* essential oil to repel the two stored products, *T. castaneum* and *L. bostrychophila*. The essential oil was abundant in estragole (65.2 %), β-caryophyllene (6.4 %), 1,8-cineole (6.4 %) and limonene (5.2 %). *E. yunnanensis* rhizome essential oil showed strong repellency against *T. castaneum,* even at low concentration (0.13 nL/cm^2) it achieved 36 % repellency after 4 hours of exposure. However, long-term environmental impact and safety for human exposure require further investigation. Until now, there is limited study done on *Sitophilus* genus. Hence, there is a need for further investigations into the repellency and insecticidal potential of *Etlingera*-derived essential oils against Sitophilus species, particularly for stored products pests.

2.4.4 Insecticidal activity

Botanical insecticides contain bioactive compounds that have insecticidal properties and provide minimal risks to human health and environment compared to synthetic chemical insecticides. Efficacy of plant extracts and essential oils must be investigated to reduce the insect resistance and regulatory restrictions on synthetic pesticides (Khan *et al*., 2016).

Anju *et al*. (2018) reported the larvacidal activity of essential oil extracted from *Etlingera fenzlii*, an endemic species from the Andaman Nicobar Islands. The plant is traditionally used by the local Shompens tribe as a bee repellent for honey collection. This study investigated the effectiveness of the essential oil against *Aedes aegypti*, the primary mosquito vector for dengue fever. The oil was extracted from fresh leaves and tested on mosquito larvae at concentrations ranging from 5 - 50 ppm following the World Health Organization method procedure. Results showed higher mortality rates among the treated larvae compared to the control group with an LC_{50} value of 11.22 ppm. This indicates that the oil is highly effective as a natural mosquito control agent. However, this study does not analyse the chemical constituents that responsible for the insecticidal effects which help optimize formulation or application strategies. Siregar *et al*., (2019) studied the insecticidal properties of *Kecombrang* (*Etlingera elatior*) as a larvacide to control *Aedes* aegypti using completely randomized design. The leaves and flowers of *E. elatior* were collected from Mount Leuser National Park and *Aedes aegypti* larvae (Instar III) were obtained from a research institute. The plant samples were dried under UV light and ground into powder. Maceration was performed using 75 % ethanol, followed by filtration and evaporation to obtain concentrated extracts. The study tested different concentrations of Kecombrang extract (0.5 % to 1.75 %) against mosquito larvae. Results showed *E. elatior* flower ethanolic extract significant larvacidal activity with LC_{50} value of 0.053 %. The flower extract was

more effective than the leaf extrat with lower LC_{50} and LC_{95} compared to leaves extract. However, the study does not expore whether repeated exposure to *E. elatior* extract could lead to resistance in *Aedes Aegypti* populations over time. de Lira Pimentel *et al*., (2023) explored the insecticidal potential of essential oil from *Etlingera elatior* inflorescences against *Sitophilus zeamais*, a pest responsible for significant corn kernel losses. The essential oil, extracted through hydrodistillation. The essential oil of *E. elatior* inflorescences exhibit insecticidal properties due to the presence of dodecanal and dodecanol as their major components via fumigation, contact toxicity and ingestion tests. Dodecanal is more potent in fumigant activity and dodecanol is more effective in contact toxicity and antinutritional activity. These compounds inhibit the pest's amylase activity, disrupting digestion and feeding. *E. elatior* essential oil provide a safer alternative with lower environmental toxicity compared to synthetic pesticides. However, this study not testing the degradation rate and persistence of the essential oil and its major compounds in different storage environments.

In conclusion, studies on various *Etlingera* species highlight their promising insecticidal potential, particularly against key pests such as *Aedes aegypti* and *Sitphilus zeamais*. Essential oils and extracts from *E. fenzlii* and *E. elatior* have demonstrated significant larvacidal and insecticidal activities, with low LC_{50} values indicating high potency. These findings support the traditional use of *Etlingera* plants as natural repellents and suggest their potential as safer and eco-friendly biopesticides. However, existing studies have limitations, including the lack of chemical profiling in some cases, limited assessment of resistance development and insufficient investigation into environmental persistence. Further research is needed to isolate active compounds, evaluate long term effectiveness and understand ecological impacts to optimize their application in pest management strategies.

2.5 Selected *Etlingera* genus

In this study, three endemic *Etlingera* native to Borneo have been chosen from different locations in Kuching, Sarawak, Malaysia. These species were *Etlingera coccinea*, *Etlingera foetens* and *Etlingera nasuta*. The genus *Etlingera* (Zingiberaceae) is known for its morphological diversity and ecological adaptations, particularly among species native to Borneo and Southeast Asia (Poulsen, 2006). *Etlingera foetens* is distinguished by its radical inflorescence, which emerges directly from the rhizome at ground level and comprises fleshy, bright red floral bracts that envelop smaller, less conspicuous flowers. A unique and notable trait of *E. foetens* is the emission of a strong, foul odor from the inflorescence, an uncommon characteristic among *Etlingera* species, hence the specific epithet "foetens" (Poulsen, 2006). The leaves are distichously arranged, lanceolate to oblong-lanceolate, glabrous, and leathery with prominent midribs and parallel venation. The pseudostems, formed by tightly sheathed leaf bases, are erect and cylindrical, reaching heights of up to 2 meters. The rhizomes are aromatic and fibrous, externally yellowish-brown with a bright orange to yellow interior.

In contrast, *Etlingera coccinea* typically exhibits terminal inflorescences that emerge above ground at the tip of leafy shoots, forming a more prominent and upright floral display. The bracts and flowers are also bright red but lack the fetid odor found in *E. foetens* (Mendez *et al*., 2023). The leaves of *E. coccinea* are large, lanceolate, and somewhat broader and thinner in texture, while the pseudostems are robust and tall. The rhizomes are aromatic, though less intensely scented than those of *E. foetens*.

Etlingera nasuta is morphologically distinguishable by its elongated, "nasal-like" floral tube structure, from which the species derives its name (Poulsen, 2006). Its inflorescence is terminal and odorless, with a more elongated and delicate floral morphology

compared to the compact, ground-level inflorescence of *E. foetens*. The leaves are narrower and longer, with a softer texture, and the pseudostems tend to be thinner. Rhizomes of *E. nasuta* are slender and paler in colour but still aromatic.

These morphological distinctions are crucial for accurate species identification and reflect potential differences in ecological roles and secondary metabolite profiles. The presence of radical inflorescences and unique volatile traits in *E. foetens* suggest specialized ecological interactions and indicate a promising avenue for phytochemical and biological studies.

2.5.1 Overview of Etlingera coccinea

E. coccinea (Figure 2.1) vernacular names are *Tuhau* (Kadazan Dusun), *Belusut bauh*, *Titei Ian* (Kenyah), *Ketokong* (Kayan), *Tipu* (Bidayuh), *Tepus* (Iban) *Tubu nanung*, *Tubu'tana*, *Tubu'layun*, *Tubu tamanom* (Kelabit) (Jems *et al*., 2021; Meekiong & Teo, 2022). *E. coccinea* is a terrestrial herb and primarily grows at the elevation between 300 to 1 400 m in the secondary forest at bright-lit to deeply shaded locations along streams with moist to wet soil. This species is widely distributed in Java, Sumatra, Thailand, Peninsular Malaysia, Borneo and Philippines (Naive *et al*., 2018; Jems *at al*., 2021). As stated by Jualang *et al.* (2015), leafy shoots with stout rhizome of *E. coccinea* produced sting smell when being crushed. Also, the indigenous ethnic groups in Sabah, especially, Kadazan Dusun used it to flavour their local dishes. As reported by Naive *et al*. (2018), the pith of leafy shoots is eaten as condiment, vegetable or pickles in Borneo and Java. Their fruits are edible and an aromatic scent can be obtained from their seed oil. It also has been utilized for traditional remedies such as stomachache, food poisoning and gastric (Mahdavi *et al*., 2017). According to Joseph & Godoong (2023), *E. coccinea* has been extracted using various methods including cold maceration method, maceration, hydrodistillation using Clevenger apparatus and sequential solvents extractions. Studies that have been conducted on this species were on allelophatic potential on seed germination and growth of mung bean and Siam weed, anti-candida study, volatile chemical composition, protective effect against autoxidation-induced ox brain homogenate antibacterial and antimicrobial activities. However, there is limited report on their antioxidant and no report for repellent activity yet. Previous studies have investigated the volatile constituents of essential oils extracted from the rhizomes of *Etlingera coccinea*. Vairappan *et al*. (2012) and Nagappan *et al*. (2017) extracted essential oils using hydrodistillation and analyzed the chemical profiles using gas

chromatography - mass spectrometry (GC-MS). Vairappan *et al*. (2012) reported nine compounds in the rhizome, including 3-Thujanone, Borneol, Camphor, Cedr-9-ene, L-Calamenene, Caryophyllene oxide, α-Bisabolol, α-Epi-muurolol, and Cycloartanyl acetate. In a separate study, Nagappan *et al*. (2017) identified seven volatile compounds, with Borneol and Aromadendrene oxide among the major components. These results demonstrate the chemical complexity of *E. coccinea* rhizomes and provide a basis for further investigation into their potential applications. However, there is still limited study on its antioxidant and repellent activities.

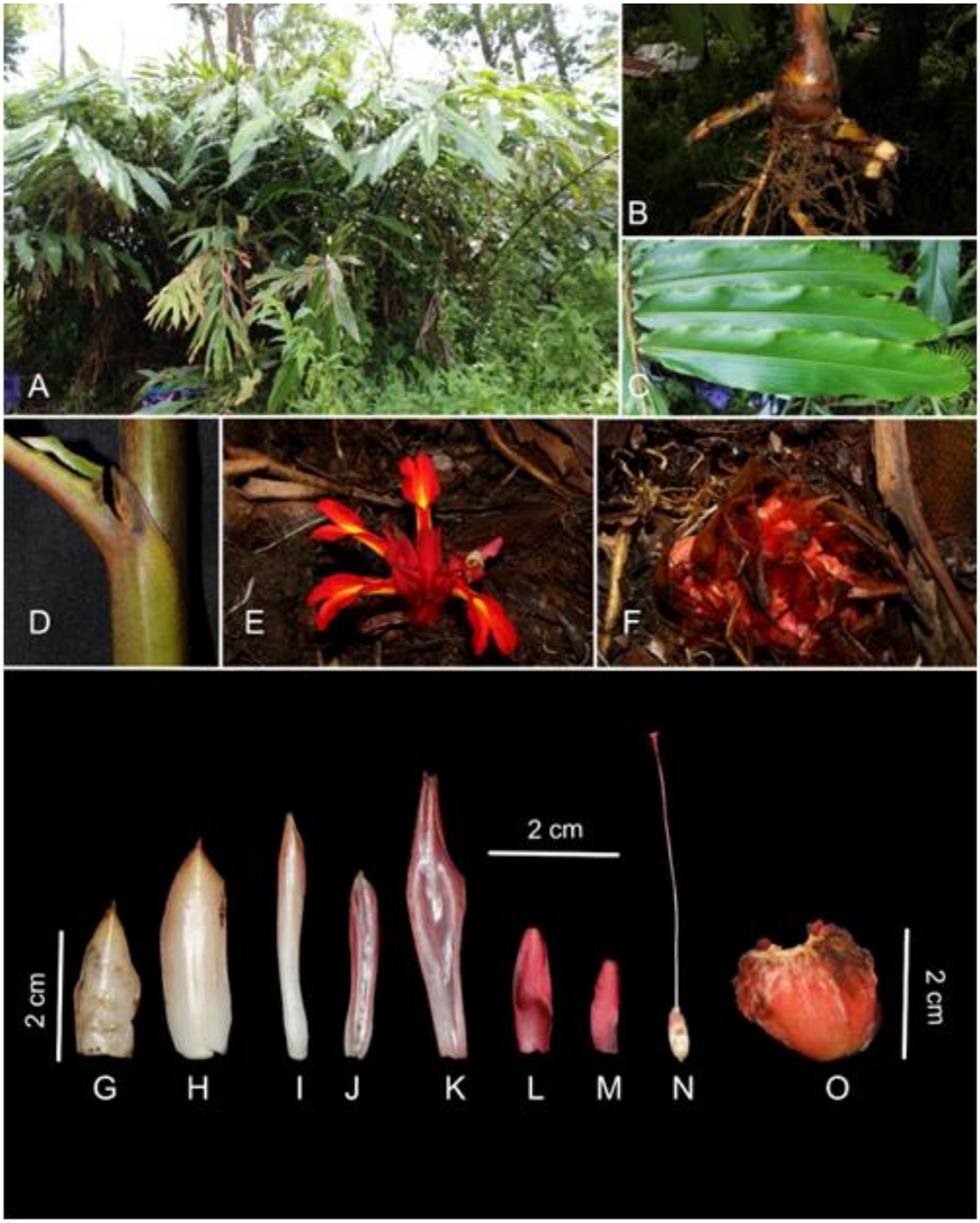


Figure 2.1: *Etlingera coccinea* : A. Habit, B. Rhizome, C. Leaves, D. Detail of ligule, E. Inflorescence, F. Infructescence (Naive *et al*., 2018)

2.5.2 Overview of Etlingera foetens

Ibanese people utilized this species as a dog repellent, its vernacular name *Tepus uduok* resemblance as dog ginger (Figure 2.2). It has long creeping rhizomes up to 80 cm apart and widely distribute in the Southeast Asia, including Java, Sumatra, Peninsular Malaysia, Thailand and Borneo. It can be recognized by its deeply reticulate and broad leaf bases, plain red flowers where the dorsal lobe of corolla does not cover the anther, elongated and broad labellum also produced strong smell when crushed. Its epithet named means smelly and the smell of varied parts of the vegetative parts is certainly very strong (Meekiong & Teo, 2022). However, there is limited literature reported for this species.

Figure 2.2: *Etlingera foetens* : A. Inflorenscence, B. Habitat, C. Leaves, D. Rhizomes

2.5.3 Overview of Etlingera nasuta

*Etlingera nasuta (*Figure 2.3) or known locally as *Ketimbang kampung* is a species with a long-creeping rhizome up to 1 m apart, epithet name means long nosed that refers to the elongated red labellum. Leafy shoots are shorted in the *kerangas* and swampy areas (1 - 1.2 m tall). Most of them are distributed in the hilly slopes at 3 - 4 m tall. This species is endemic to Borneo. Salasiah *et al*. (2022) studied the chemical composition of *E. nasuta* rhizome essential oil. The essential oil was obtained by hydrodistillation using Clevenger apparatus analyzed the chemical composition using gas chromatography - mass spectrometry. Major component of the rhizomes essential oil was eucalyptol (20.78 %) and α-terpineol (11.97 %). Other components identified in the *E. nasuta* rhizomes essential oil were *cis*-carveol, methyl eugenol and (2*E*)-dodecenal. However, no bioactivities were further tested from the study, highlighting the potential of *E. nasuta* need to be explored.

Figure 2.3: *Etlingera nasuta* inflorescence

As a result, based on the literature review, *Etlingera* species demonstrated notable antioxidant activity in extracts or essential oils, often focusing on single plant parts or extract types. In contrast, the present study employs a comprehensive approach by analyzing the essential oils from leaves, rhizomes, and stems, as well as crude leaf extracts obtained through sequential solvent extraction (hexane, dichloromethane, and ethyl acetate). This methodology allows for a more refined investigation of bioactive constituents across a polarity gradient. By incorporating multiple plant parts and extraction methods, this study not only broadens the phytochemical understanding of *E. coccinea*, *E. foetens*, and *E. nasuta*, but also identifies specific fractions or oils with the highest antioxidant and repellent activities, which were not fully addressed in previous studies. For instance, Guo *et al*. (2015) reported the chemical composition and insect-repellent activity of *Etlingera yunnanensis* rhizome essential oil, which was rich in estragole (65.2%), *β*-caryophyllene (6.4%), 1,8-cineole (6.4%), and limonene (5.2%). The oil exhibited strong repellency against *Tribolium castaneum*, achieving 36% repellency at a low concentration of 0.13 nL/cm² after 4 hours of exposure. However, their study was limited to just two insect species and did not explore effects on the *Sitophilus* genus, a major pest of stored grains. Additionally, most previous studies focused on single plant parts, used limited extraction methods, and often lacked biological activity testing beyond antioxidant assays, while also being constrained by single-location sampling. To address these gaps, the present study investigates the chemical composition, antioxidant, and repellent bioactivities of *Etlingera coccinea*, *E. foetens*, and *E. nasuta*. Essential oils were obtained from the leaves, rhizomes, and stems, while crude extracts from leaves were prepared through sequential solvent extraction using hexane, dichloromethane, and ethyl acetate. This approach allows for polarity-based fractionation, facilitating the identification of compounds with potential functional applications. By

expanding species coverage, plant parts examined, and bioactivities tested including the underexplored *Sitophilus* genus, this study contributes a more holistic understanding of the chemical and functional diversity within the *Etlingera* genus.

CHAPTER 3

METHODOLOGY

The Figure 3.1 provided a brief overview of the extraction, isolation, structural eclucidation and evaluation of their bioactivities processes applied to *Etlingera coccinea*, *E. foetens* and *E. nasuta*. It outlines the key steps involved in obtaining and identifying bioactive compounds from leaves crude extracts and various plant parts essential oils, followed by the evaluation of their antioxidant and repellent activities.

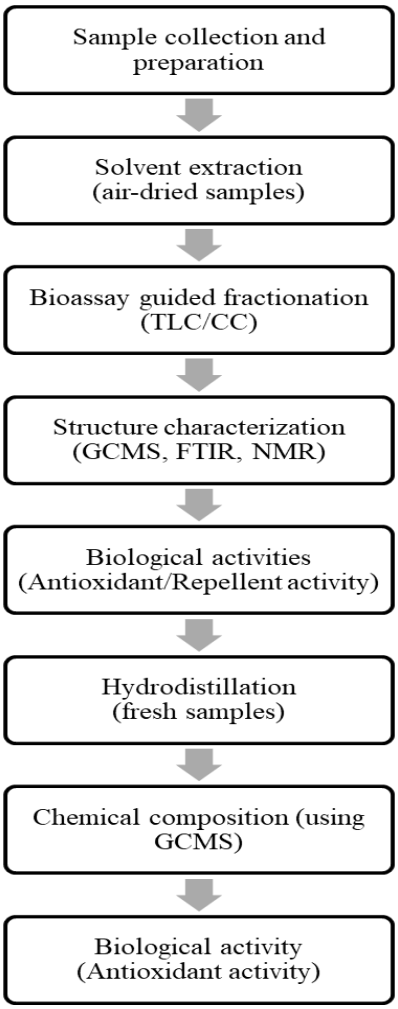


Figure 3.1: A brief summary in extraction, isolation, structural elucidation, chemical composition analysis and bioactivities of *Etlingera coccinea*, *E. foetens* and *E. nasuta*

3.1 Sample Collection and Preparation of Crude Extracts

Selected local *Etlingera s*pecies (*E. coccinea, E. foetens and E. nasuta*) were collected from Bau and Samarahan area. The plant specimens were taxonomically identified and authenticated by a taxonomist from the Faculty of Resource Science and Technology, UNIMAS. The voucher specimens were not prepared and deposited in the university herbarium due to the limited resources. Fresh leaves of samples (500 - 600 g) were thoroughly washed with tap water and rinsed with distilled water to remove dirt. The cleaned samples were then air-dried at room temperature for 3 - 5 days. The dried leaves were ground into fine powder using mechanical blender and transfer into 2 L conical flasks. The powdered plant materials were extracted sequentially with a series of solvents of increasing polarity: hexane (non-polar), dichloromethane (semi-polar) and ethyl acetate (polar) at room temperature according to the method done by Mahdavi *et al*. (2017) with slight modifications. Each extraction was conducted at room temperature for 72 hours using a sample to solvent ratio of 1:3 (w/v). The extraction process was repeated by topping up with fresh solvent of the same type until the filtrate turned colourless, indicating complete extraction in order to obtain nonpolar, semi polar and polar fractions as shown in Figure 3.2. Different solvents extract different bioactive compounds (Sasidharan *et al*., 2011). After each extraction cycle, the solvent fractions were filtered and concentrated under reduced pressure using a Buchi, rotary evaporator (Figure 3.2). The mass and percentage yield of crude extracts were recorded and stored in airtight bottles at 4 ^{o}C in a refrigerator for further use.

The percentage yield of crude extract was calculated using Equation 3.1:

$$\text{Percentage yield (w/w \%)} = \frac{X_1}{X_0} \times 100 \qquad \text{(Equation 3.1)}$$

where, X_1 = Weight of crude extracts (g) and X_0 = Weight of powdered sample before extraction (g).

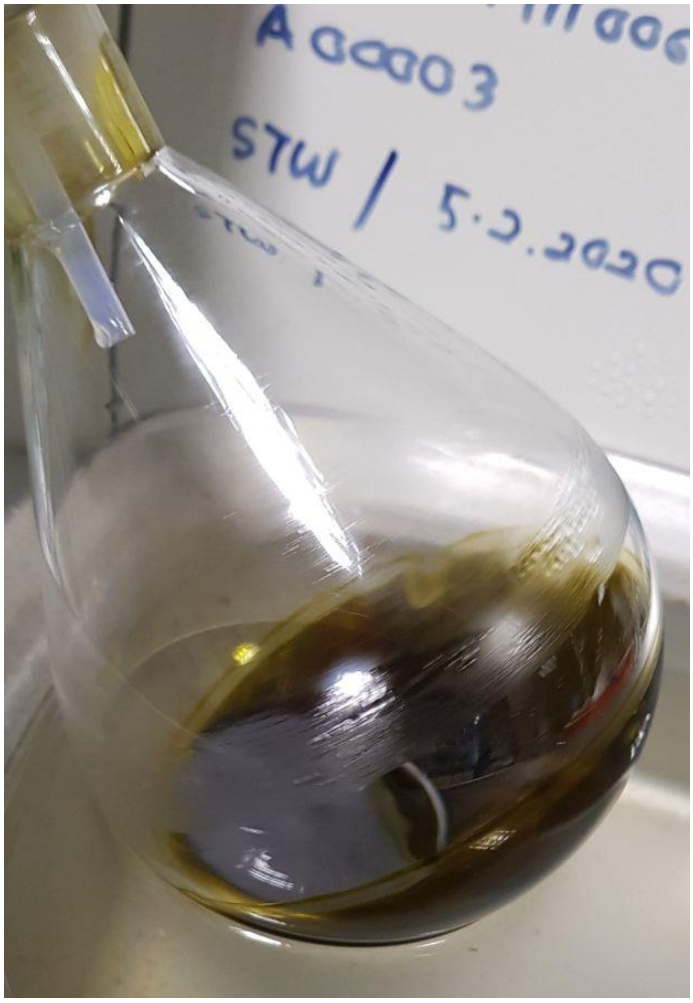


Figure 3.2: Concentrated crude extract, brown viscous liquid

3.2 Extraction of Essential Oils

Fresh leaves, rhizomes and stems of *Etlingera* spp. (100 - 400 g) were chopped into small pieces and subjected to hydrodistillation unit using Clevenger-type apparatus for 4 - 6 hours at the distillation rate of 1 - 2 drops per second (Nur-Anwariah *et al*., 2011; Khaldi *et al*., 2017). The essential oils were then kept in a small vial and dried over anhydrous sodium sulphate. The essential oils were transferred into another vial covered with aluminium foil and parafilm, stored at 4 ℃ prior to GCMS analysis (Faridahanim *et al*., 2007). Figure 3.3 showed the schematic diagram of the hydrodistillation using Clevenger type apparatus. In this setup, the plant materials such as leaves, rhizomes and stems are placed inside the round bottom flask along with 1 L distilled water. The flask is heated using a heating mantle until the water boils and produce steams. This steam carries the volatile essential oils components from the plant material into a condenser, where it cooled and condensed back into liquid form. The condensed mixture of water and essential oil then flows into the Clevenger-type apparatus, where separation occurs based on differences in density. Typically, the essential oil floats on the water surface, although some oils may sink depending on their density. The water phase is recycled back into the boiling flask, allowing continuous distillation. Meanwhile, the essential oil accumulates in the graduated section of the Clevenger arm, where it can be measured and collected for further analysis.

The percentage yield (v/w) of essential oil was calculated by using this formula (Khor *et al*., 2017):

$$\text{Percentage yield (v/w \%)} = \frac{\text{Volume of oil collected (mL)}}{\text{Weight of fresh sample (g)}} \times 100 \qquad \text{(Equation 3.2)}$$

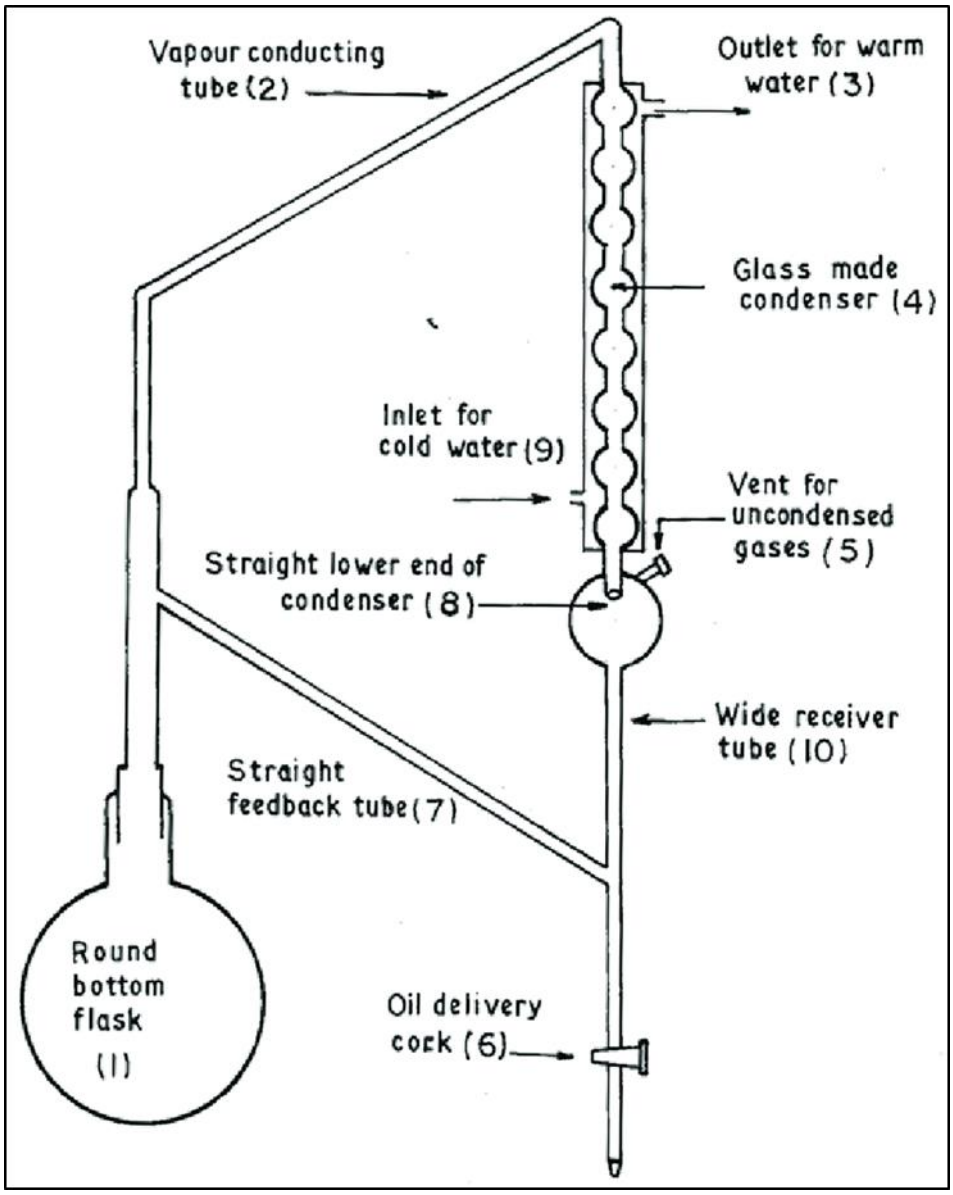


Figure 3.3: Schematic diagram of hydrodistillation using Clevenger-apparatus (Guha & Nandi, 2019)

3.3 Isolation and Purification of Bioactive Compound(s)

Isolation and purification of compound(s) was performed using thin layer chromatography (TLC) and column chromatography (CC) techniques. The TLC techniques was first carried out to determine the best solvent system for further separation of compounds into CC. According to Rasul (2018), thin layer chromatography was used as guidance for isolation and setting the parameters for column chromatography such as possible solvent system ratio. Silica or alumina (more polar) acts as stationary phase and organic solvents (less polar) used as mobile phase. Column chromatography was then carried out for the effective separation of crude plant extracts into individual components in pure form based on their polarity. Silica gel packed in a column (stationary phase) and the eluent acts as mobile phase after introducing the extracts on top of the stationary phase. Compounds were present at different rates depends on their affinity for the stationary phase and the polarity of the solvent system. These fractions were then collected and combined based on TLC profiles and further purified to obtain pure compound for subsequent structural elucidation.

3.3.1 Thin Layer Chromatography (TLC) technique

The TLC analysis of crude extract was carried out according to the method described by Rajvaidhya *et al*. in 2014. The sample solution was spotted on a TLC plate (1.5 cm x 1.5 cm x 1.5 cm) using an open-end glass capillary tube. The TLC plate was developed in different ratio of solvent system in closed chamber consisting of hexane and ethyl acetate to obtain best separation for various constitutes of the extracts. The developed plate was air dried and observed under visible and UV light. Various separated spots were noted as their R_f values. The potassium permanganate solution was used as staining reagent to stain invisible spots under UV light. The staining reagent was prepared by mixing potassium permanganate (1.5 g), potassium carbonate (10 g) and 10 % sodium hydroxide solution (v/v) (1.25 mL) into distilled water (200 mL) (Cai, 2014). The best solvent system ratio was selected to monitor the elution of column chromatography. The retention factor of each visible spot was calculated using the following equation:

$$\text{Retention factor } (R_f) = \frac{\text{Distance travelled by spot (cm)}}{\text{Distance travelled by solvent system (cm)}} \times 100 \qquad \text{(Equation 3.3)}$$

3.3.2 Column Chromatography (CC)

The column chromatography (CC) was adapted from the method desrcibed Mohamad *et al*. (2005) with modifications. It involved packing the column with silica gel as a stationary phase and a mixture of hexane and ethyl acetate as a mobile phase. Preparation of silica slurry was carried out by adding approximately 50 g of silica gel into suitable solvent system consisting of hexane and ethyl acetate. The silica gel slurry was carefully transferred into the column and left overnight to allow proper packing and more stable prior to separation. Exactly 1 g of crude extract was dissolved in small amount of dichloromethane and added carefully on the top of column. The column was developed by adding more solvent system on the top of column and fractions of 10 - 15 mL were collected in each test tube. The composition of fractions was analysed using TLC plate. Fractions with similar number of spot(s) were combined and concentrated under reduced pressure by using rotary evaporator. Fraction appeared as one spot during TLC analysis was proceeded to GCMS analysis and other spectroscopic techniques. Meanwhile, fraction containing several spots during TLC analysis was subjected to further purification before proceeding to spectroscopic analysis.

3.4 Structure Elucidation of Isolated Compounds and Characterization of EO

Several spectroscopic techniques such as gas chromatography - mass spectrometry (GCMS), fourier transform infrared spectrometry (FTIR) and nuclear magnetic resonance (NMR) spectroscopy were utilized to characterize the isolated compounds(s). Identification of chemical components in essential oil was determined based on GCMS analysis.

3.4.1 Gas Chromatography Mass Spectrometry (GCMS)

The samples were analyzed using GC-MS model Agilent 7890 B equipped with capillary column HP - 5 MS (30 m x 250 μm x 0.25 μm) coupled with a quadrupole mass spectrometer. Other GC-MS model was Shimadzu (QP2010) coupled with mass spectrometry fitted with non - polar DB - 5 (30 m x 0.25 mm i.d. x 0.25 μm film thickness) fused silica capillary column (Khaldi *et al*., 2017). Exactly 1 μL of sample was diluted into 200 μL of *n*-hexane (gas chromatography grade). Sample injection was performed by the auto-sampler by injecting 1 μL of the diluted sample using the split injection mode (Marsal *et al*., 2011; Umaru *et al*., 2019). The temperature of the column, initially 50 °C (1 min hold), was increased to 280 °C at the rate of 5 °C min^{-1} and maintained at this temperature for 10 min. Injection and detector temperature were programmed at 280 °C and 300 °C, respectively. Helium was used as the carrier gas with a constant flow rate at 1 mL min^{-1} and the total analysis time was 57 min. Electron ionization at 70 eV determined the mass spectra in the *m/z* range between 50 - 500 Da. The chromatogram percentage area was measured and identified using the MassHunter Qualitative Analysis software (Agilent Technologies, Santa Clara, California, United States). A series of mixture of *n*-alkanes standard (C_9 - C_{30}) was analysed at similar program temperature to the sample above mentioned. The retention time

of each standard peak was recorded and used for the calculation of Kovats retention (KI) indices. The KI value for each peak were calculated based on the following formula:

$$KI = 100n + 100\frac{Tr_x - Tr_n}{Tr_{n+1} - Tr_n} \quad \text{(Equation 3.5)}$$

where Tr_n and Tr_{n+1} are the retention times of the *n*-alkane standard eluting before and after compound X, and Tr_x is the retention time of component of interest (Minaie & Minaie, 2020). The characterization of isolated compounds and the chemical compositions of essential oil were identified by comparing their Kovats retention indices with literature values (Adams, 2017; Babushok *et al*., 2011) and their mass spectral data with those from NIST mass spectral database (National Institute of Standards and Technology, 2017). Kovats retention indices have been effectively identified thousands of compounds across various stationary phases by providing precise dataset to identify and compare compounds based on their retention time on different phases and depending on various temperature programming of the gas chromatography - mass spectrometry relative to a series of standard reference compounds (Zhang & Lu, 1996; Goodner, 2008).

3.4.2 Fourier Transform Infrared (FTIR)

The FTIR analysis was carried out on a Shimadzu Fourier transforms infrared spectroscopy ATR - FTIR equipped with diamond crystal to determine the functional groups present in isolated compound(s). The background spectra and the sample spectra were collected under similar conditions at 12 scans within the range of 400 - 4 000 cm^{-1}. OMNIC software (Thermo Nicolet Analytical Instruments, Madison, Wisconsin, United States) was used for the spectra processing and interpretation (Umaru *et al*., 2019).

3.4.3 Nuclear Magnetic Resonance (NMR)

Following the successful purification of the target compound from the ethyl acetate extract of *Etlingera nasuta*. Approximately, 10 mg of the purified compounds was dissolved in 600 μL of deuterated chloroform ($CDCl_3$) served as both the solvent and deuterium lock into the 5 mm NMR tube for spectral analysis (Umaru *et al*., 2019). The ^{1}H NMR analysis were recorded on a JEOL JNM-ECA 500 Spectrometer NMR spectrometer at 500 MHz (^{1}H NMR). The signal was reported as chemical shift (δ, ppm) and relative to the reference standard of Tetramethylsilane (TMS). The analysis provided key information regarding the number, type and environment of hydrogen atoms within the molecule, including multiplicities, coupling constants (*J*) and integration corresponding to the number of protons contributing to each signal. The integration of the data was performed on JEOL's DELTA version 5.0.4 software which facilitated peak integration, signal assignment and structural interpretation.

3.5 Biological Activities

Antioxidant activity of *Etlingera* spp. was evaluated against 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals at different concentrations of 1, 10, 100 and 1000 μL/mL according to the method developed by Chan *et al*. in 2007. Meanwhile, antirepellent activity was investigated against rice pest of *Sitophilus oryzae* at various concentrations of 2, 4 and 8 μL/mL up to 5 hours of treatment (Khani *et al*., 2007). This repellency test was carried out based on the filter paper impregnation method.

3.5.1 Antioxidant Activity

Exactly 5.9 mg DPPH (2, 2-diphenyl-1-picrylhydrazy) radical was dissolved in 100 mL methanol to prepare 0.6 M of methanolic DPPH solution. 2 mL of methanolic DPPH solution was added to 1 mL of sample at different concentrations (1, 10, 100 and 1000 μL/mL). The mixture was incubated for 30 minutes in a dark ambiance at room temperature. The absorbance of the samples, standards, control and blanks were analyzed at 517 nm using Shimadzu Cary 60 UV-Visible spectrophotometer. Ascorbic acid and butylated hydroxytoluene (BHT) were used as standards at similar concentrations (Ghasemzadeh *et al*., 2015). All tests were run in triplicates. The IC_{50} value, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50 % was calculated and used for comparison. A low IC_{50} indicates strong antioxidant activity in a sample test. The value of IC_{50} was expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg ascorbic acid/100 g.

The percentage (%) of DPPH radical scavenging activity of sample was calculated using the following equations:

Scavenging activity (%) = (Equation 3.6)

$$1-\frac{\text{Absorbance}_{\text{sample 517 nm}} - \text{Absorbance}_{\text{blank 517 nm}}}{\text{Absorbance}_{\text{control 517 nm}}} \text{ x } 100\ \%$$

The data was analysed using Microsoft Excel. Data represents mean ± standard deviation (n=3).

3.5.2 Repellent Activity

Adults of *Sitophilus oryzae* were collected from infested rice purchased from a local market. The stock culture of rice weevil was further reared in the same commercial rice brand of Royal Chrysanthemum Thai Fragrant Rice in the laboratory at 28 ℃ and 70 - 75 % relative humidity with a 12 L: 12 D photoperiod for 27 days. Females' rice weevils deposited eggs then developed into larvae and pupa and emerged adults. The weevils were reared for about two generations. Approximately, 50 - 100 of *S. oryzae* adults were introduced into a 0.5 L plastic container covered with a muslin cloth containing 200 g of rice. Figure 3.4 showed the experimental setup according to study conducted by Aimad et al. (2022).

The repellent test was carried out based on the method developed by Murugesan *et al*. (2021) with a slight modification. This test was performed using filter paper impregnation method with a 9 cm diameter petri dish. Filter paper (Whatman no. 5) was cut into two halves. Test solutions were prepared at 2, 4 and 8 μL/mL respectively in 1 mL of acetone. 1 mL of pure acetone was used as a control and sprinkled on half of the filter paper. Another half of the filter paper was sprinkled with the sample. Both treated and control sides of filter paper were air-dried for about 20 minutes. Ten adults of rice weevils were released at the centre of the petri dish. The number of rice weevils presented on each side of the filter paper was recorded hourly up to 5 hours. Negative percentage repellency is referred as 0. All tests were done in triplicates. The following classification is based on the percentage of repellency, Class 0 = 0 % - 0.1 %, Class I = 0.1 % - 20 %, Class II = 20.1 % - 40 %, Class III = 40.1 % - 60 %, Class IV = 60.1 % - 80 %, Class V = 80.1 - 100 %.

The percentage of repellency was calculated as follows (Murugesan *et al*., 2021):

$$\text{Percentage repellency (PC)} = \frac{N_c - N_t}{N_c + N_t} \times 100 \qquad \text{(Equation 3.8)}$$

where, N_c = Number of insects presents on the control area and N_t = Number of insects presents on the treated area. The mean and standard error of repellent activity crude extracts were calculated.

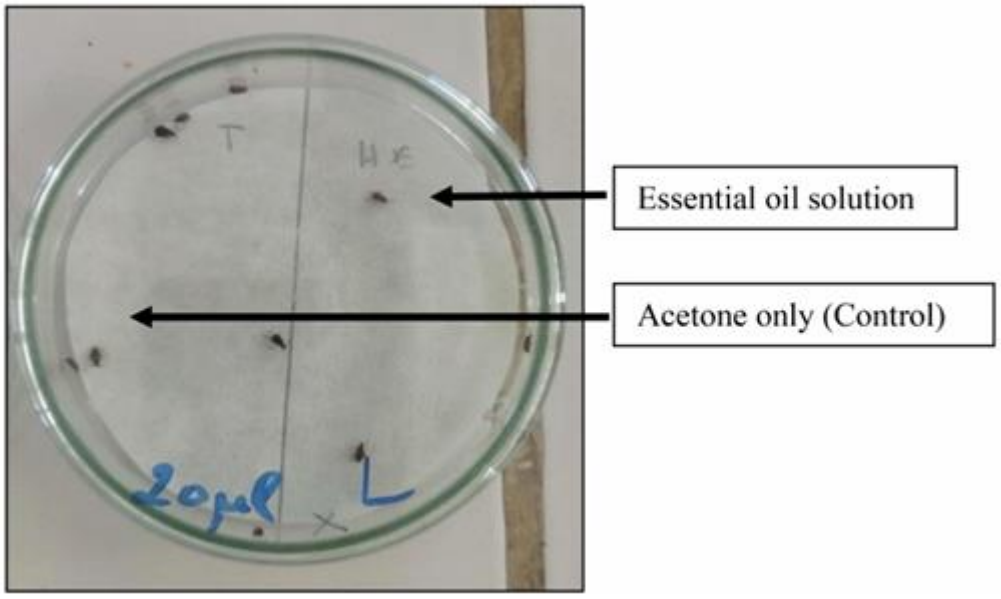


Figure 3.4: Experimental setup for adapted for repellency assay from the study of *Origanum compactum* essential oil against *Callosobruchus maculatus* (Aimad *et al*., 2022).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Yield of Crude Extracts and Essential Oils

Figures 4.1 and 4.2 illustrate the percentage yields of crude extracts and essential oils, respectively, obtained in this study. These yields were a crucial parameter because it reflected the efficiency of the extraction process and the potential of the plant material as a source of bioactive compounds. In this study, the yield of essential oils and crude extracts varied on different plant parts such as leaves, rhizomes and stems. Other factors that might contribute also from the extraction methods and environmental factors.

Different parts of *Etlingera* species, such as leaves, rhizomes and stems produce different amount of essential oil due to their anatomical and biochemical compositions. In most studies, rhizomes proven to produce highest essential oil yield. Norhazlina *et al*. (2017) reported that the essential oil yield from *Etlingera elatior* was significantly higher from leaves. In contrast to the previous study, this study demonstrated a notably high essential oil yield from the leaves using fresh sample of all studied species. This unexpected result suggests that the leaves of the studied *Etlingera* species may serve as an alternative and sustainable source of essential oils, potentially reducing the need for rhizome harvesting and supporting conservation efforts. The plant part used for essential extraction significantly affects both the yield and composition of the oil. According to Figuiredo *et al*. (2008) ifferent parts of a plant such as leaves flowers, stems, roots, bark and fruits produce distinct concentrations and types of secondary metabolites. Leaves often rich in monoterpends and phenolic compounds. Flowers typically produced complex, aromatic compounds that attract pollinators. Many floral oils contain alcohols and esters that contribute to their fragrance.

Stems and bark may contain higher concentrations of sesquiterpenes giving woody and earthy aroma. Roots store heavier, less volatile compounds such as certiver and ginger oils which more to deep and earthy tones. Fruits and seeds often rich in limonene and other tepenoids.

Major impact on percentage yield affected based on the choice of extraction method. This study focused on the sequential solvent extraction and essential oil hydrodistillation using Clevenger-type apparatus. Sequential solvent extractions based on increasing polarity were used to obtain a wider range of phytochemical as plant compounds differ in their solubility and polarity. Meanwhile, essential oil extraction targeting volatile compounds and results in low yields. In this study, sequential solvent extraction using solvents of increasing polarity was employed to maximize the range of phytochemicals extracted from the plant material. This method was chosen because plant secondary metabolites differ in polarity and solubility, and using a series of solvents from non-polar to polar allows for the efficient extraction of both lipophilic and hydrophilic compounds. Non-polar solvents such as hexane are effective in extracting less polar compounds like essential oils and terpenoids, while moderately polar and polar solvents such as dichloromethane and ethyl acetate target more polar constituents, including flavonoids, phenolics, and other bioactive compounds (Sashidaran *et al*., 2011). By applying this approach, the study aimed to improve the overall yield and diversity of phytochemicals, thereby enhancing the potential for identifying bioactive compounds with antioxidant, antimicrobial, or repellent activities.

Additionally, the selection of different solvents for extraction aimed to obtain hydrophilic and more lipophilic compounds in different crude extracts to facilitate the purification process. In the extraction, polar and moderate polarity of solvents are used to obtain hydrophilic and more lipophilic compounds, respectively (Sashidaran et *al.*, 2011).

In this study, it was obviously seen *E. foetens* were rich in hydrophilic, lipophilic and less polar compounds as given by the highest percentage yield of hexane (14.49%), dichloromethane (9.20%) and ethyl acetate (3.91%) crude extracts, respectively compared to other species (Figure 4.2). Meanwhile, *E. coccinea* and *E. nasuta* contained comparable amount of less polar and lipophilic compounds with the percentage yield in the range of 2.40 - 3.05 % (hexane) and 2.30 - 2.68 % (dicholoromethane). However, *E. coccinea* was found to contain the least number of hydrophilic compounds with the percentage of less than 1 % recorded in ethyl acetate crude extract.

Several examples of hydrophilic and lipophilic compounds that have been isolated from polar solvents of *E. elatior* were 1,7-bis(4-hydroxyphenyl)-2,4,6-heptatrienone, demethoxycurcumin, 1,7-*bis*(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, 16-hydroxylabda-8(17),11,13-trien-16,15-olide, stigmast-4-en-3-one, stigmast-4-ene-3,6-dione, stigmast-4-en-6β-ol-3-one, and 5α,8α-epidioxyergosta-6,22-dien-3β-ol (Habsah *et al*., 2005) . Among these compounds, stigmast-4-en-3-one and stigmast-4-en-6b-ol-3-one exhibited notable antitumor-promoting activity. In addition, Chan *et al*. (2009) isolated other hydrophilic compounds such as 3-O-caffeoylquinic acid, 5-O-caffeoylquinic acid (chlorogenic acid), and 5-O-caffeoylquinic acid methyl ester from *E. elatior* leaves using methanol crude extract (polar solvent). Chlorogenic acid has valuable applications in cosmetics, food and medicine.

In this study, dried plant material was chosen because it was generally used by complementary and alternative medicine practioners. Precautions were taken during preparation of crude extracts particularly the temperature used for concentration to assure the potential active constituents are not lost or destroyed. As highlighted by Nawaz et al., 2020, the phytochemical content and the quality of plant extracts were affected by the time

of extraction, duration of extraction, polarity and nature of the solvent used were being considered to ensure optimal yield and compound integrity.

According to Tongnuanchan & Benjakul (2014), environmental factors significantly influenced crude extract and essential oil yield. Environmental factors are known to play a crucial role in influencing the yield and quality of essential oils. In this study, variations in chemical composition and yield may be partially attributed to such factors. Elevated temperatures generally enhance essential oil biosynthesis; however, exposure to extreme heat can lead to the degradation of volatile constituents. Increased light exposure is beneficial, as it stimulates photosynthesis and promotes the synthesis of secondary metabolites, which are key components of essential oils. Water availability also influences yield, where moderate water stress has been shown to enhance oil concentration, although prolonged drought conditions typically result in reduced production. The influence of light on secondary metabolite production has direct relevance to the potential antioxidant and repellent activities of *Etlingera* species. Like the diverse plant taxa discussed such as *Arabidopsis thaliana*, *Artemisia annua*, and *Salix myrsinifolia*. *Etlingera* species also synthesize a wide array of secondary metabolites, particularly phenolic compounds, flavonoids, and terpenoids, many of which are known for their strong antioxidant and insect-repellent properties.

Light exposure plays a key role in enhancing the biosynthesis of bioactive compounds in *Etlingera*. Increased sunlight or UV radiation can boost flavonoid and phenolic acid levels, strengthening antioxidant activity and plant defense. Similarly, light stimulates terpenoid production, especially in the essential oils, contributing to *Etlingera*'s mosquito-repellent properties. This mirrors patterns seen in species like *Sambucus nigra*, *Betula* spp., and *Pinus taeda*. Thus, optimizing light conditions may improve the antioxidant

and repellent potential of *Etlingera* extracts for natural product applications (Ncube *et al*., 2012). According to Ncube *et al.* (2012), moisture availability also plays a key role in secondary metabolite production in *Etlingera* species. Similar to *Arnica montana* and *Hypericum perforatum*, moderate water stress may enhance the accumulation of phenolic compounds and flavonoids in *Etlingera*, strengthening its antioxidant properties. As observed in *Quercus* spp. and *Pachypodium saundersii*, reduced moisture can trigger the production of defense-related metabolites, potentially increasing the levels of tannins and terpenoids in *Etlingera* essential oils. This may also improve its repellent activity. Therefore, controlled moisture conditions could optimize the bioactive potential of *Etlingera* extracts.

In conclusion, the variations in crude extract and essential oil yields across different *Etlingera* species and plant parts were influenced by anatomical, biochemical, environmental, and methodological factors, highlighting the leaves as a promising and sustainable source of bioactive compounds for natural product applications.

4.1.1 Yield of Crude Extracts

The crude extracts of *Etlingera* spp. have been prepared by maceration of dried powdered plant materials in sequential organic solvents according to polarity (hexane, dichloromethane, ethyl acetate). The mass and percentage yield of crude extracts of *Etlingera coccinea*, *Etlingera foetens* and *Etlingera nasuta* were recorded in Table 4.1. The percentage yield of crude extracts from the leaves of three *Etlingera* species: *E. coccinea*, *E. foetens* and *E. nasuta* were calculated based on the dry weight of plant material used. The yield varied significantly depending on the species and the solvent used. Among all the species tested, *E. foetens* consistently produced the highest extract yields across all solvents. The hexane extract of *E. foetens* yielded the highest percentage (14.19 %), followed by dichloromethane (9.20 %) and ethyl acetate (3.91 %). This indicates that *E. foetens* leaves contain a higher concentration of non-polar to moderately polar secondary metabolites compared to the other species. *E. nasuta* showed moderate extractive efficiency, with yield 3.05 %, 2.68 % and 1.75 % for hexane, dichloromethane and ethyl acetate respectively. In contrast, *E. coccinea* produced the lowest overall yields, particularly with ethyl acetate (0.56 %). Its hexane and dichloromethane extracts yielded 2.40 % and 2.30 %, respectively. The trend observed that hexane extracts provided the highest yield for each species, followed by dichloromethane and ethyl acetate. This suggests that the majority of extractable compounds in the leaves of *Etlingera* spp. were predominantly non-polar or low polarity compounds such as lipids, terpenes and other hydrocarbons. The relatively lower yields obtained from ethyl acetate imply a lesser abundance of intermediate polarity compounds like certain flavonoids or phenolics. The variation in extractive yields among the three species could be attributed to differences in their phytochemical's composition, leaf structure or environmental conditions affecting metabolite production. These findings support the

selection of *E. foetens* for further phytochemical and bioactivity studies due to its higher yield potential. However, the chemical composition of the essential oils from *E. foetens* provides strong evidence of it potential for futher exploration with the high concentration of octanal acetate (85.63 %) *α*-gurjunene (47.75 %) and *E*-piperitol (31.03%) with notable bioactivities such as anti-inflammatory and antifungal activities (Nguyen *et al*., 2004; Flach *et al*., 2002).

Azmi *et al.* (2024) conducted a study on the phytochemical screening and antioxidant activity of the rhizome and leaves of *Tuhau* (*E. coccinea*). The fresh plant materials were initially dried to a moisture content of 10% before being finely ground. Subsequently, the powdered samples were extracted using 80% (v/v) methanol and left to macerate overnight. The resulting extracts were then filtered using filter paper and transferred into clean centrifuge tubes for further analysis. Results showed that the methanolic extracts of rhizomes and leaves of *E. coccinea* yielded 5.85 % and 8.15 %. In contrast, the present study recorded lower extraction for *E. coccinea* ranging from 0.56 % to 2.40 %, depending on the solvent used. Chan *et al*. (2011) examined the antioxidant and antibacterial properties of *Alpinia galangal, Curcuma longa,* and *Etlingera elatior*, using methanol extracts obtained from leaves, rhizomes, and flower strips. The extraction yields of *E. elatior* leaves and inflorescences were 4.1 % and 4.8 %, respectively. The results indicated that *E. elatior* had a lower extraction yield compared to *E. foetens*. However, no solvent-based extraction yield data have been reported for *E. foetens* and *E. nasuta*, highlighting a gap in the current understanding and emphasizing the need for further investigation

In conclusion, the lower extraction yields of *E. coccinea* observed in the present study compared to previous methanolic extractions suggest that solvent choice significantly influences the recovery of bioactive compounds. Notably, *E. foetens* exhibited higher yields

than *E. elatior*, indicating its potential as a richer source of bioactive constituents. These findings highlight the variability in phytochemical content among *Etlingera* species and emphasize the importance of targeted extraction strategies to maximize the recovery of bioactive compounds for further pharmacological evaluation.

Table 4.1: Mass and percentage yield of crude extract from *Etlingera* spp.

Species	Solvent	Physical appearance	Weight (g)	Percentage yield (w/w %)
E. coccinea	Hexane	Brown and oily	14.67	2.40
	Dichloromethane	Brown and oily	14.15	2.30
	Ethyl acetate	Brown and oily	4.42	0.56
E. foetens	Hexane	Brown and oily	84.63	14.49
	Dichloromethane	Brown and oily	52.18	9.20
	Ethyl acetate	Brown and oily	22.18	3.91
E. nasuta	Hexane	Brown and oily	10.93	3.05
	Dichloromethane	Brown and oily	9.58	2.68
	Ethyl acetate	Brown and oily	6.24	1.75

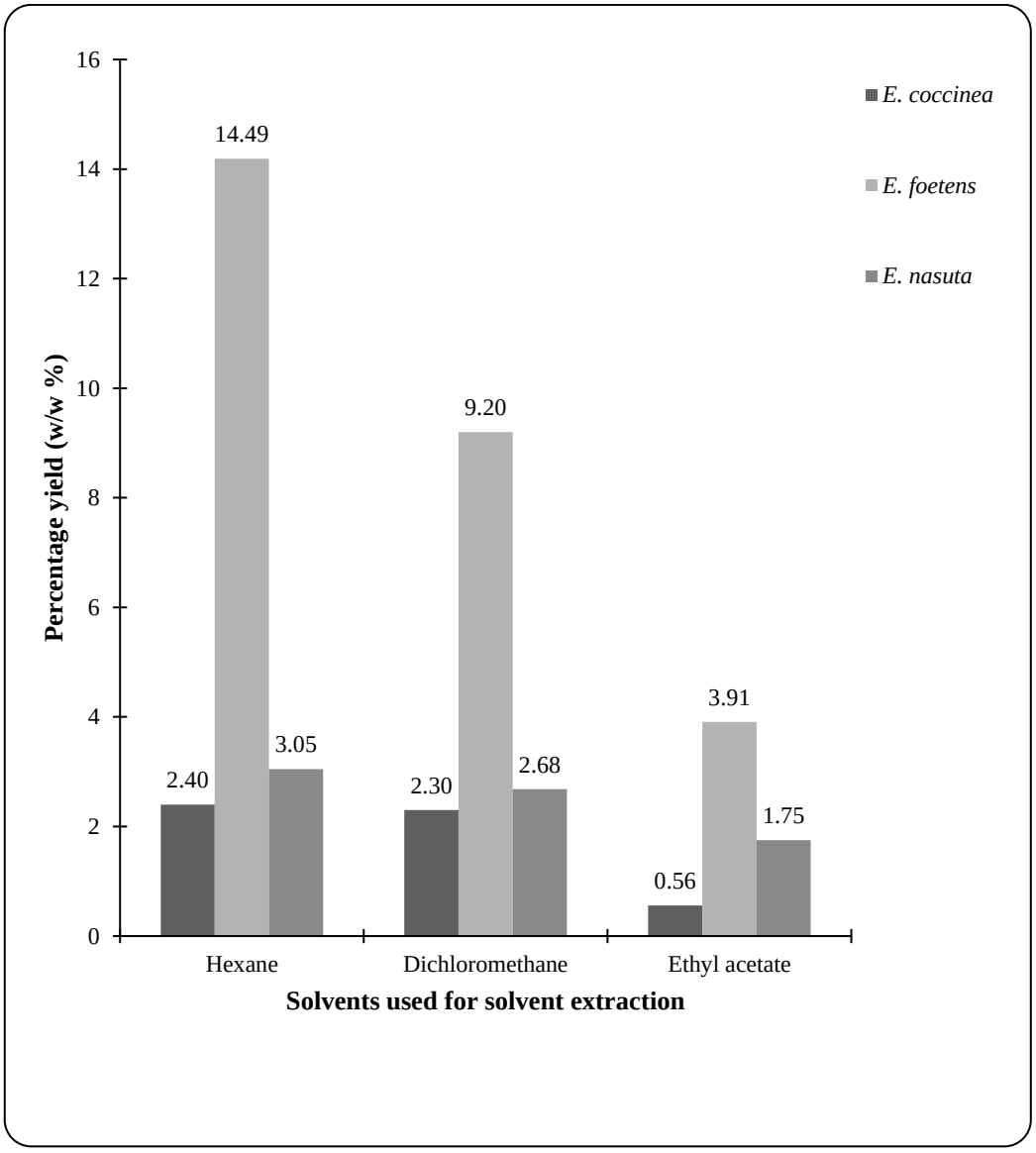


Figure 4.1: Percentage yield of crude extracts from several species of *Etlingera* spp.

4.1.2 Yield of Essential Oils

The EO was extracted using hydrodistillation using Clevenger-apparatus method from several parts of *Etlingera* spp. The volume and percentage yield of essential oils (EOs) are given in Table 4.2 and Figure 4.2. Hydrodistillation of the leaves, rhizomes and stems of *E.coccinea, E. foetens* and *E. nasuta* gave similar pale-yellow oils in respective yield ranges of 0.20 - 0.27, 0.12 - 0.42 and 0.08 - 0.42 % (v/w). Similarly, all the leaves parts of *Etlingera* spp. gave the highest yield compared to other parts. The highest percentage yields of EOs were obtained from *E. foetens* and *E. nasuta* proving the strong smell emitting by those plants when the leaves' part were crushed. However, the stem of *E. foetens* and *E. nasuta* contained poor yield of EO compared to *E.coccinea.* Different parts of *E.coccinea* are commonly used by native communities of Sarawak as food flavouring or aromatic enhancer due to its unique taste and aroma intensity (Yusli *et al,* 2021). Meanwhile, the essential oil percentage yield from rhizomes was comparable for all *Etlingera* spp. in the range of 0.20 - 0.24 %.

Jaafar *et al*. (2007) identified the essential oils of leaves, stems, flowers and rhizomes of *Etlingera elatior* using hydrodistillation method and analyzed the chemical composition on gas chromatography - mass spectrometry. Results showed that leaves yielding of 0.0735 % with major compounds including β-pinene (19.7 %), caryophyllene (15.4 %) and (E)-β-farnesene (27.9 %). Stems yielding of 0.0029 % dominated by 1,1-dodecanediol diacetate (34.3 %) and (E)-5-dodecane (27.0 %) and rhizomes yielding of 0.0021 % containing 1,1-dodecanediol diacetate (40.4 %) and cyclododecane (34.5 %). Among all plant parts, the leaves showed the highest essential oil yield in this study, consistent with previous findings but with a higher percentage yield, suggesting that leaves may be the most viable source for commercial extraction. Vairappan *et al*. (2012) studied the rhizomes essential oil

composition of five *Etingera* species from Borneo including *E. pyramidosphaera, E. elatior, E. coccinea, E. brevilabrum* and *E. megalocheilos* using hydrodistillation method and analyzed using gas chromatography-mass spectrometer. The highest yield was obtained from *E. pyramidosphaera* (0.45 %), followed by *E. elatior* (0.38 %), *E. coccinea* (0.30 %), *E. brevilabrum* (0.28 %), and *E. megalocheilos* (0.25 %). However, the rhizome yields obtained in this study were generally lower compared to those reported in previous studies, which ranged between 0.20 % and 0.24 %. Salasiah *et al*. (2022) studied the essential oil components of selected Alpinieae (Zingiberaceae) from Sarawak, Malaysia including *E. coccinea, E. nasuta* and other species. Essential oils were obtained from rhizomes of *E. coccinea* and *E. nasuta* using hydrodistillation using Clevenger-type apparatus and analysed using gas chromatography - mass spectrometry. Results revealed that E. coccinea and E. nasuta rhizomes essential oils yielding of 3.3 % and 2.9 %. In the present study, the rhizomes essential oils of both species recorded lower yield at only 0.20 %.

The hydrodistillation of various parts of *Etlingera* spp. revealed that the leaves consistently produced the highest percentage yields of essential oils (0.20 - 0.27 %), followed by the rhizomes (0.20 - 0.24 %) and stems (0.08 - 0.42 %). Among the species studied, *E. foetens* and *E. nasuta* exhibited the highest yields from leaves, aligning with their strong aromatic characteristics. While rhizome yields in this study were comparable across species, they were generally lower than those reported in previous research. The findings reaffirm that leaves are the most viable part for essential oil extraction in *Etlingera* spp., potentially offering greater yields for commercial applications.

Table 4.2: Percentage yield of three different species of *Etlingera* spp. essential oils

Etlingera spp.	Parts	Volume of oil collected (mL)	Weight of sample (g)	Percentage yield (%)
Etlingera coccinea	Leaves	0.50	185.78	0.27
	Rhizomes	0.60	300.00	0.20
	Stems	0.60	250.00	0.24
Etlingera foetens	Leaves	0.80	191.26	0.42
	Rhizomes	0.50	207.00	0.24
	Stems	0.50	401.17	0.12
Etlingera nasuta	Leaves	0.80	191.28	0.42
	Rhizomes	0.10	50.00	0.20
	Stems	0.20	235.74	0.08

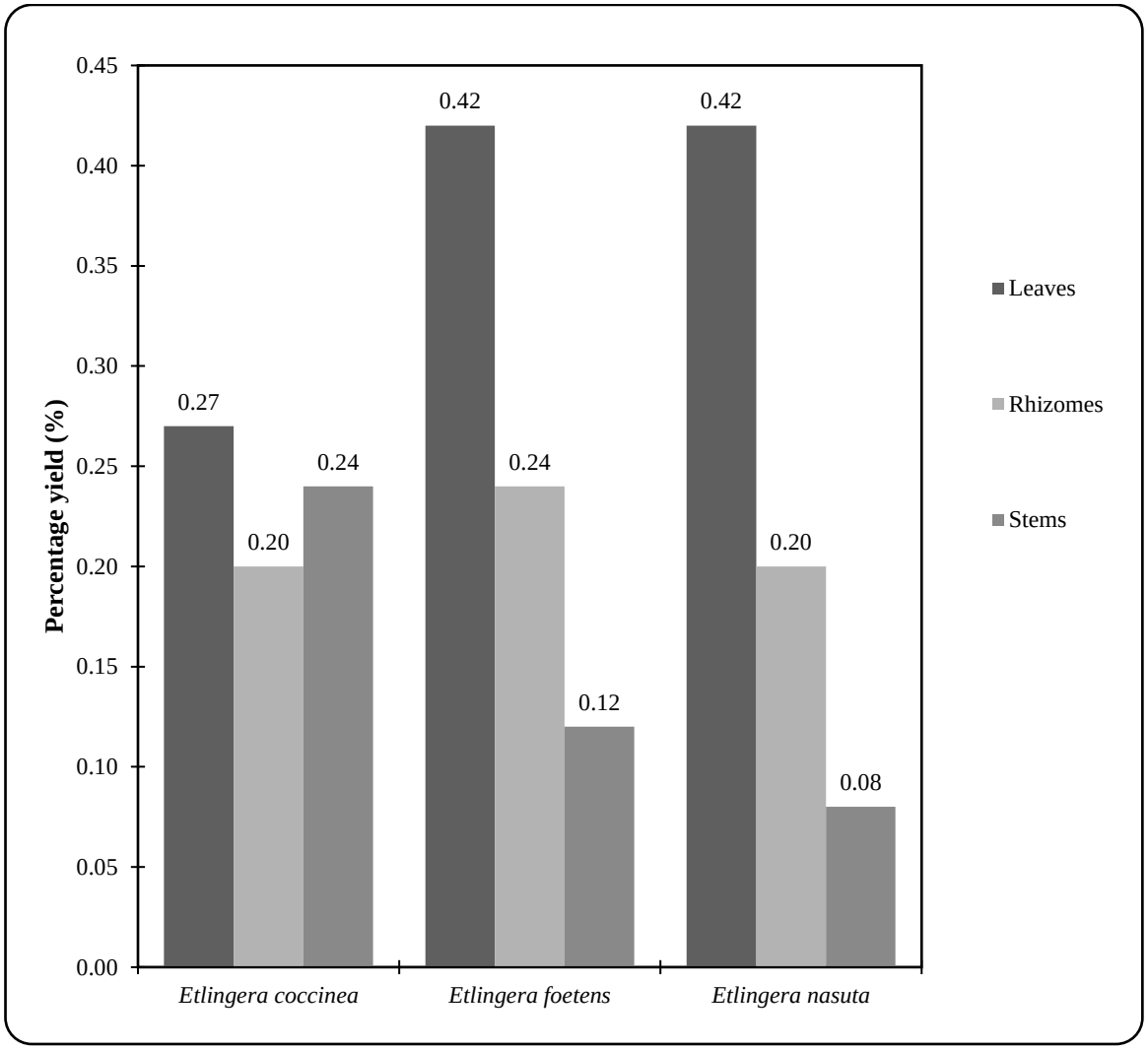


Figure 4.2: Percentage yield of essential oils from several species of *Etlingera* spp.

4.2 Isolation and Characterization of Compound 1

Compound 1 was identified as 5-methyl-2-1-methylethenyl acetate (1), which was obtained from the ethyl acetate crude extract of the leaves part of *Etlingera nasuta*. It was purified using column chromatography by eluting over silica gel 60 with the solvent system of hexane: ethyl acetate (9:1). A single spot was observed in fraction 1 - 15 containing compound 1 under UV light with the R_f value of 0.58. Fraction 1 - 15 yielded Compound 1 as brown oil (10 mg).

4.3 Structural elucidation of Compound 1

Fraction 1 - 15 was continued for gas chromatography - mass spectrometry (GCMS) analysis. The GCMS chromatogram of fraction 1 - 15 was given in Figure 4.3. One significant peak was observed at a retention time of 16.344 min evidenced to the purity of compound 1. Compound 1 was identified based on Kovats index (KI) using a series of *n*-alkane standards (C_9 - C_{30}) as described in Chapter 3 and referred to mass chromatogram from Adams (2007) in Figure 4.4. Based on KI value, compound 1 was identified as 5-methyl-2-1-methylethenyl acetate (1) with calculated KI of 1293 in which consistent to the reference KI for 5-methyl-2-1-methylethenyl acetate (lavandulyl acetate) of 1292 (Asuming *et al*., 2005). The molecular weight of compound 1 was 196.29 g/mol corresponding to the chemical formula of $C_{12}H_{20}O_2$ suggested the molecular structure of (1) to be shown in Figure 4.5.

The FTIR analysis was carried out to determine the functional group present in the structure of compound 1 (Figure 4.6, Table 4.3). The absorption at 1680.04 cm^{-1} in the IR spectrum signified the presence of carbonyl group in 1. This was further supported by the appearance of peak at 3034 cm^{-1} and 1631.27 cm^{-1} that could be assigned to alkene $RCH=CH_2$ and alkene C=C, respectively. Furthermore, the IR spectrum showed aliphatic C-H stretching at 2907.65 cm^{-1} and 2852.30 cm^{-1}, completing all the functional groups present in (1) in which consistent to 5-methyl-2-1-methylethenyl acetate. This FTIR characterization of (1) corroborating the findings from Lafhal *et al*. (2019) for the structure elucidation of (1). The alkene groups contribute to the compound's antioxidant activity by providing sites for radical stabilization through conjugation and resonance. This is a typical characteristic of unsaturated terpenoids, which can scavenge free radicals (Silva *et al*., 2014). Furthermore, the presence of a carbonyl group (C=O) from the ester moiety enhances lipophilicity and

membrane permeability, potentially improving bioavailability in antioxidant pathways (Bakkali et al., 2008).

In terms of repellent activity, the ester group enhances volatility and fragrance, traits critical for interactions with insect olfactory receptors. Volatile compounds with ester and alkene functionalities are common in essential oils with known repellent properties (Nerio *et al*., 2010). As a monoterpene ester, lavandulyl acetate is structurally similar to other compounds used in plant defense, thus supporting its role in repelling insects.

NMR analysis of (1) was performed for further supporting the elucidation of the chemical structure (Figure 4.7, Table 4.4). However, overlapped peaks, broad and weak signals appeared in the spectrum led to difficulty in the spectrum interpretation of splitting pattern. Overlapping absorptions of broad singlets and singlet signal observed at 5.20 ppm and 4.93 ppm, respectively signified the presence of alkene proton (H-1a, b, H-7). Methylene signal of H-6 absorbed more downfield compared to H-5 due to electronegativity of oxygen atom. Similarly, singlet signal of H-3 appeared slightly downfield than H-11 due to an effect from pi electrons. Meanwhile, three singlet signals observed at upfield area assigned to three methyl groups for H-4, H-9 and H-10. The compound 1 hydrogen atom with labelled was shown in Figure 4.8.

In conclusion, through the comprehensive analysis using GCMS, FTIR and ^{1}H-NMR techniques, along with comparisons to existing literature, compound 1 was identified as 5-methyl-2-1-methylethenyl acetate (lavandulyl acetate). This monoterpene is commonly found in lavender essential oils (Demissie *et al*., 2013) has significant ecological implications as highlighted by Govindarajan and Benelli (2016) who reported the effectiveness of lavandulyl acetate isolated from *Heracleum sprengelianum* (Apiaceae) leaves essential oil as a potent eco-friendly larvacide against mosquito larvae, including

Anopheles subpictus, *Aedes albopictus* and *Culex tritaeniorhynus*. These findings highlighted the potential of this compound in developing sustainable pest control solutions.

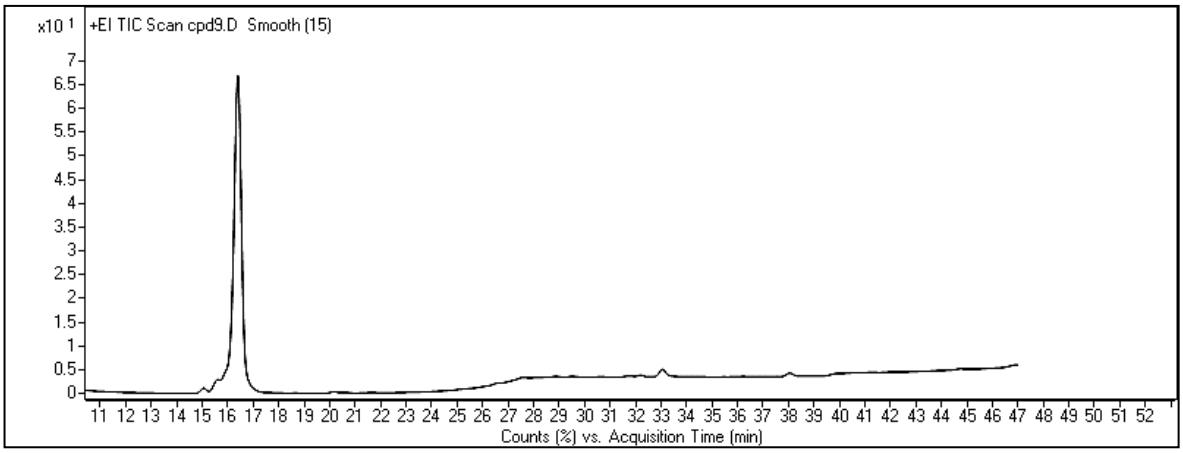


Figure 4.3: Chromatogram of (1)

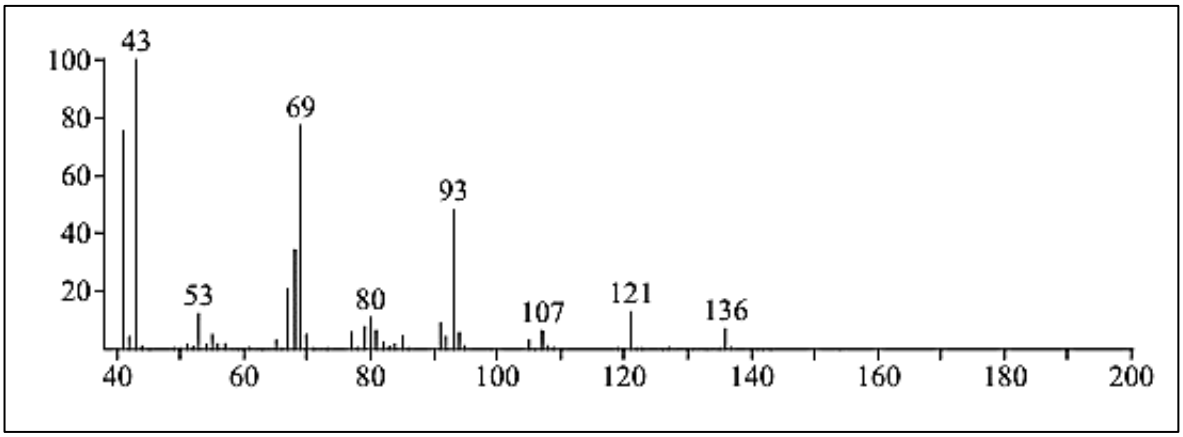


Figure 4.4: Mass chromatogram of (1) (Adams, 2007)

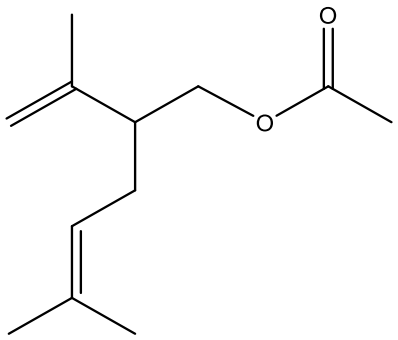


Figure 4.5: The molecular structure of (1)

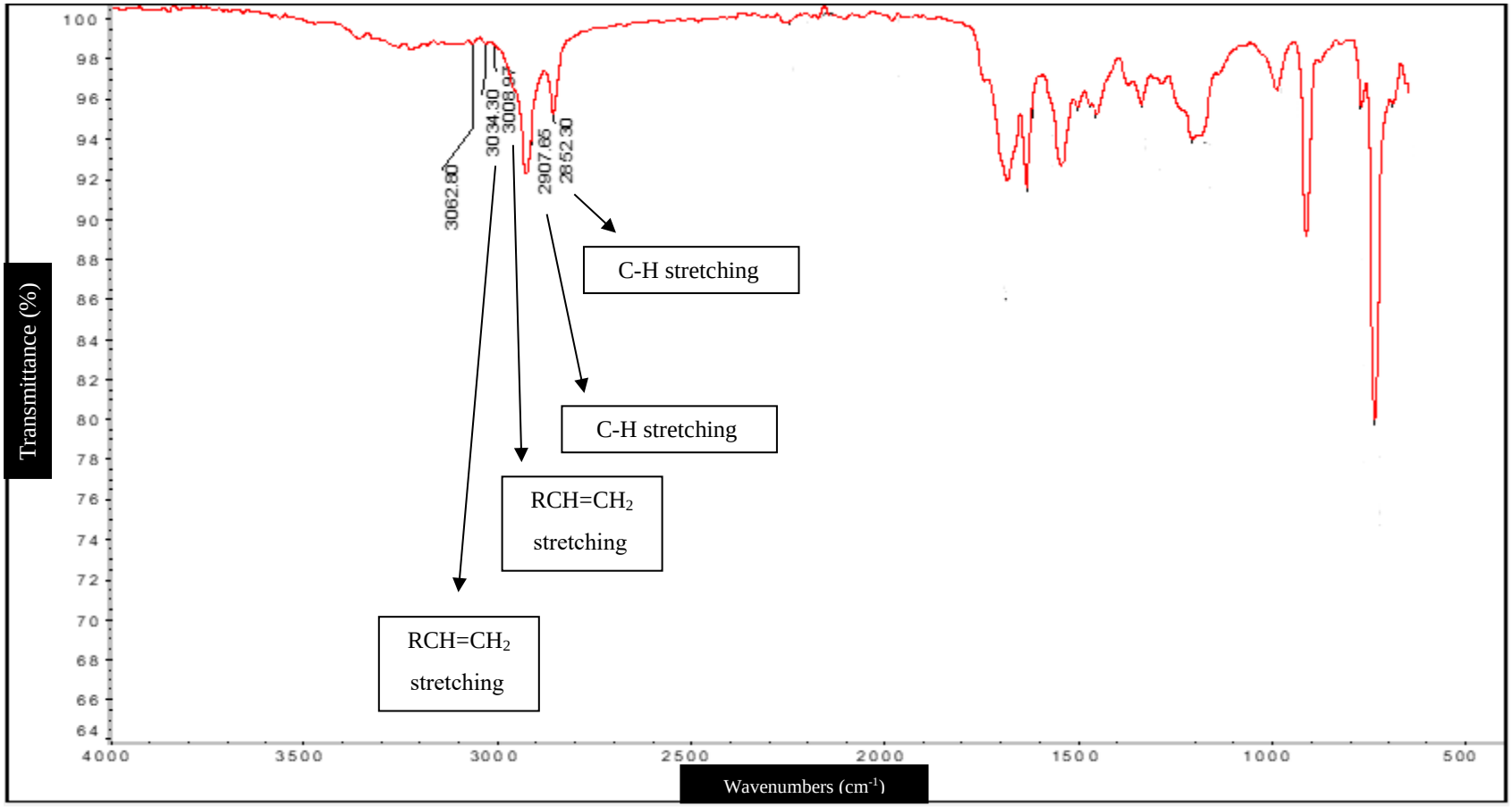
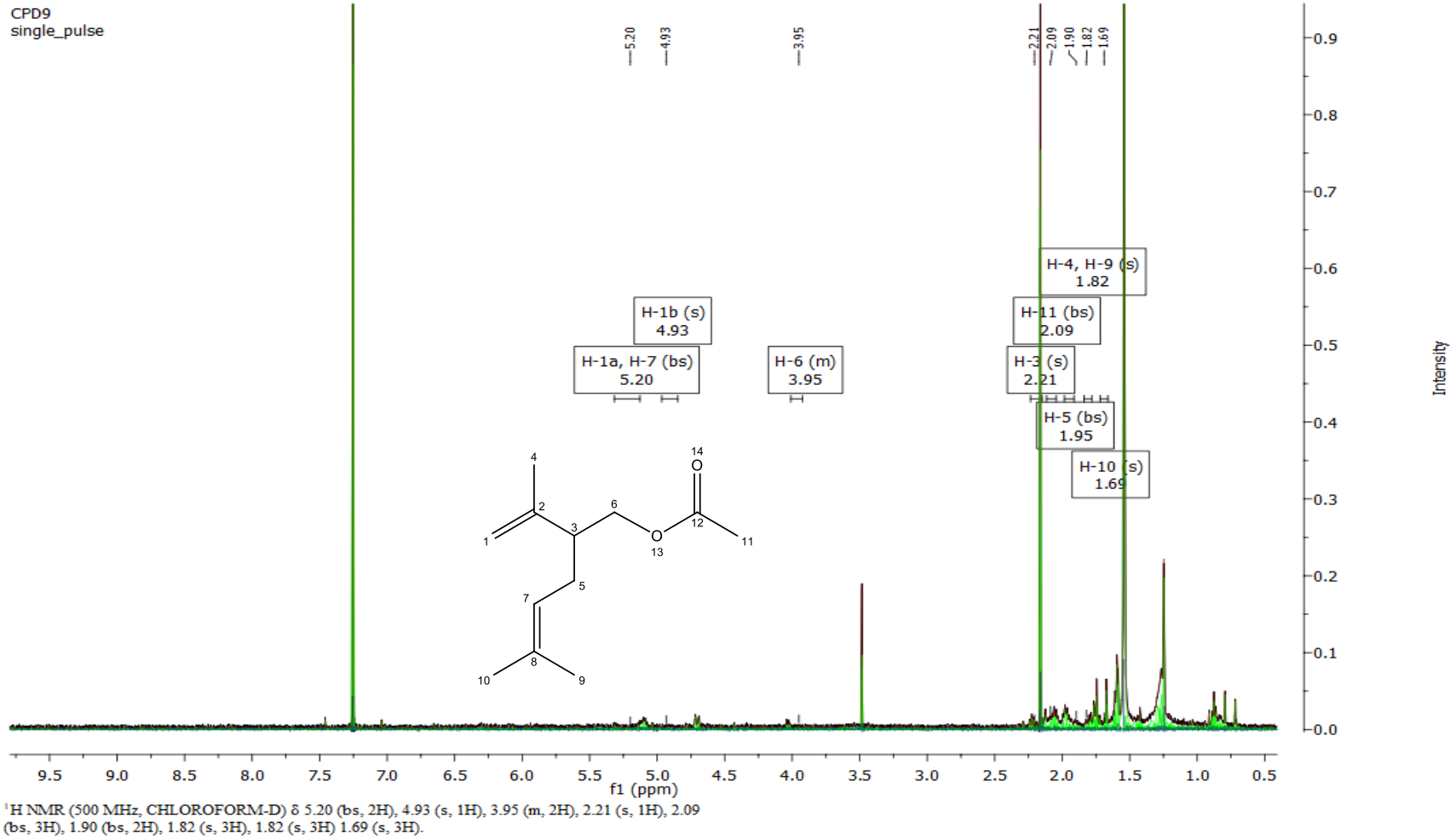


Figure 4.6: FTIR spectrum of (1)

Table 4.3: FTIR analysis of (1)

Type of Absorption Band	Frequency (cm^{-1})	Frequency Range (cm^{-1}) (Lafhal *et al.*, 2019)
$RCH=CH_2$ stretching	3034.30	3100 – 3000
$RCH=CH_2$ stretching	3008.87	3100 – 3000
C-H stretching	2907.65	2960 – 2850
C-H stretching	2852.30	2960 – 2850
C=O	1680.04	1755 – 1650
C=C	1631.27	1640 - 1680



^{1}H NMR (500 MHz, CHLOROFORM-D) δ 5.20 (bs, 2H), 4.93 (s, 1H), 3.95 (m, 2H), 2.21 (s, 1H), 2.09 (bs, 3H), 1.90 (bs, 2H), 1.82 (s, 3H), 1.82 (s, 3H) 1.69 (s, 3H).

Figure 4.7: ^{1}H NMR analysis of (1)

Table 4.4: Signal, chemical shifts and integration of (1)

Signal	**^{1}H chemical shift** (δ, ppm, multiplicity, J in Hz)	**Integration**
H-1a, H-7	5.20 (bs)	2H
H-1b	4.93 (s)	1H
H-6	3.95 (m)	2H
H-3	2.21 (s)	1H
H-11	2.09 (bs)	3H
H-5	1.9(bs)	2H
H-4	1.82 (s)	3H
H-9	1.82 (s)	3H
H-10	1.70 (s)	3H

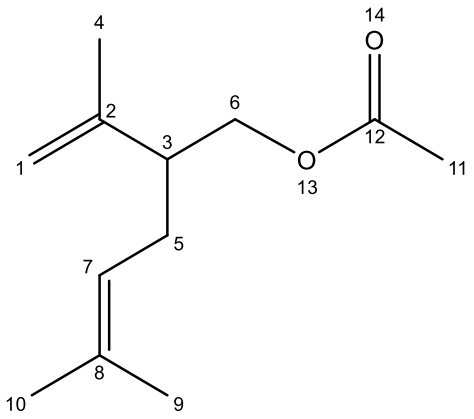


Figure 4.8: (1) with atom label

4.4 Chemical Composition of EO

The chemical composition of the essential oils from different parts of the plants was reported in Table 4.5. The Kovats index was calculated based on standard reference in Figure 4.9. In the leaves, *E. coccinea* was rich in *γ*-elemene (31.91 %), *α*-copaene (28.62 %), and 2-methyldodecane (16.38 %). In the rhizomes, *E. coccinea* featured high levels of *α*-copaene (51.63 %) and cis-chrysanthenyl acetate (39.86 %). As for the stems, *E. coccinea* had high contents of *β*-chamigrene (57.41 %) and cis-chrysanthenyl acetate (26.96 %).

E. foetens leaves showed dominance of *β*-cubebene (14.69 %), *β*-caryophyllene (13.99 %), *E*-piperitol (11.86 %), (Z)-hex-3-enyl-butanoate (11.98 %), and limonene (9.03 %). *E. foetens* rhizomes essential oil was characterized by octanal acetate (85.63 %) and limonene (4.98 %). *E. foetens* stems was rich in *α*-gurjunene (47.75 %), *E*-piperitol (31.03%), cryptone (8.38 %), and *α*-methylnaphthalene (4.21 %).

For *E. nasuta*, the major leaf constituents were bicyclogermacrene (27.31 %), *α*-muurolene (27.82 %), and bulnesol (18.79 %). *E. nasuta* rhizomes were dominated by neo-intermedeol (49.89 %), borneol (16.15 %), and *α*-terpineol (15.66 %). In *E. nasuta*, the stems contained α-muurolene (20.06 %), *β*-longipinene (19.99 %), and bicyclogermacrene (18.73%). These results indicate that not only species but also plant parts strongly influence the essential oil profile, with some constituents being dominant in only specific organs.

Differences in the chemical components of the plant essential oils attributed to the exogenous (climate, weather, light, precipitation, soil, composition, pH, habitat elevation or recent attack by herbivores) and endogenous factors (plant age or maturity or varied plant parts used) (Padalia *et al*., 2010). There was limited report available regarding the chemical components of *E. coccinea*, *E. foetens* and *E. nasuta* from Sarawak because most of the

reports were from Peninsular Malaysia, Sabah (Malaysia), Indonesia and several parts of the world. One of the reports by Salasiah *et al*. (2022) studied the essential oil in selected Alpineae (Zingiberaceae) from Sarawak using hydroditillation method and analyzed the chemical composition using gas chromatography - mass spectrometry, found higher percentage of β-bisabolene (4.42 %) in *E. coccinea* rhizomes essential oil and caryophyllene oxide (8.75 %) in *E. nausta* rhizomes essential oil compared to current study with lower percentage of β-bisabolene (0.85 %) in *E. coccinea* rhizomes essential oil and caryophyllene oxide (1.16 %) in *E. coccinea* stems essential oils. Tuan *et al*., (2022), identified the chemical composition of essential oils extracted from the rhizomes and leaves of *Zingiber monophyllum*, a species within the Zingiberaceae family found in Vietnam extracted by hydrodistillation using Clevenger type apparatus and analyzed the chemical composition usng gas chromatography-mass spectrometry. It was found abundant of α-copaene (6.5 %) *Z. monophyllum* leaves oil which lower percentage area than this *E. coccinea* leaves essential oil (26.82 %) and *E. coccinea* rhizome essential oil (51.63 %). Swor *et al*., (2023) reported the essential oil composition of *Anthriscus caucalis* M. Bieb (Apiaceae) growing wild in Southwestern Idaho obtained by hydrodistillation and analyzed by gas chromatography - mass spectrometry and gas chromatography flame - ionization detection. *cis*-chrysanthenyl acetate was found in big amount of 42.3 % compared to *E. coccinea* leaves and rhizomes essential oils ranging from 26.96 - 39.86 %. Zoghbi & Andrade (2005) investigated the volatiles of the *Etligera elatior* and *Zingiber spectabile* Griff, two Zingiberaceae cultivated in the Amazon. The essential oils were obtained from inflorescence and inflorescence axis of both species using hydrodistillation equipped with Clevenger apparatus and analyzed using gas chromatography and gas chromatography - mass spectrometry. It was found that the inflorescence essential oil of *E. elatior* contained lower β-caryophyllene (1.3 %)

compared to *E. foetens* leaves essential oil (13.99 %). Huong *et al*. (2022) identified the chemical compositions and antimicrobial activity of essential oils from the leaves of *Wurfbainia tenella*, *Hedychium villosum* var. *tenuiflorum*, *Meistera sudea* and *Alpinia hongiaoensis* obtained using hydrodistillation method and analyzed by gas chromatography-flame ionization detection / mass spectrometry. Limonene (12.1%) was identified as the predominant constituent in *H. villosum* var. *tenuiflorum*, with a higher concentration compared to the major constituents present in the essential oils of *E. foetens* leaves and rhizomes, which ranged from 4.98% to 9.03%. Dai *et al*. (2020) evaluated the chemical composition of essential oil from the leaves of *Alpinia globasa* and *Alpinia tonkinensis* obtained from hydrodistillation using Clevenger type apparatus and identified their chemical composition by gas chromatography - mass spectrometry. α-Gurjunene (10.5%) was identified as the major component in *A. globasa*, but its concentration was lower compared to the 47.75 % found in the stem essential oil of *E. foetens*. Ngoc Dai *et al*. (2024) investigated chemical compositions and antimicrobial and mosquito larvcidal activities of the leaf essential oils of *Goniothalamus yunnanensis* and *Goniothalamus touranensis*. The leaves essential oils of both species were extracted using hydrodistillation and analyzed using gas chromatography - flame ionization detection / mass spectrometry. Bicyclogermacrene was the main compound in *G. yunnanensis* (31.03 %), which was higher than in *E. nasuta* leaves essential oil (27.31 %). Le *et al*. (2016) determined the borneol and other chemical compounds of essential oil of *Dryobalanops aromatic* exudate from Malaysia by fractional distillation of exudates and analyzed the chemical composition using gas chromatography – mass spectrometry. Borneol was present at 0.74 %, which is much lower than the 16.15 % found in *E. nasuta* rhizomes essential oil. Kumar *et al*. (2024) identified the chemical composition of essential oil of *Globba sessiliflora* by hydrositillation using

Clevenger-type apparatus and analyzed the essential oil using gas chromatography - mass spectrometry. *β*-longipinene (10.9 %) was slightly lower than the amount present in *E. nasuta* stems (19.99 %). In conclusion, the variation in chemical compositions of essential oils among *Etlingera* species and other related Zingiberaceae and plant families is influenced by both environmental and intrinsic factors. The findings highlight notable differences in the concentration of key compounds such as *β*-bisabolene, *α*-copaene, and *β*-longipinene when compared to other studies, emphasizing the uniqueness of essential oil profiles from *E. coccinea*, *E. foetens*, and *E. nasuta* collected in Sarawak.

Dominant compounds in the leaves, rhizomes and stems of *E. coccinea*, *E. foetens* and *E. nasuta* have demonstrated various bioactivities in previous studies (Table 4.6, Figure 4.10). *γ*-elemene, α-copaene, and 2-methyldodecane were the major constituents in the leaves. *γ*-Elemene is widely recognized for its anticancer properties, particularly against lung cancer (Song *et al*., 2023), while *α*-copaene possesses insecticidal to control medfly for pest control management (Lull *et al*., 2023). Although 2-methyldodecane lacks extensive pharmacological data, it may influence biological interactions through hydrophobic mechanisms because it was a phytotoxic compound in inhibit germination and radical growth of *Raphanus sativus* (Maradino *et al*., 2011). The rhizomes of *E. coccinea* were rich in α-copaene and cis-chrysanthenyl acetate, the latter of which demonstrated antimicrobial activity (Ivashchenko, 2017). In the stems, *β*-chamigrene and cis-chrysanthenyl acetate were predominant, with *β*-chamigrene associated with antibacterial and antioxidant effects (Rana *et al*., 2022).

In *E. foetens*, the leaves contained *β*-cubebene, *β*-caryophyllene, *E*-piperitol, (Z)-hex-3-enyl-butanoate, and limonene. *β*-Cubebene and *β*-caryophyllene are known for their anti-inflammatory, antioxidant, and anti-tumour (Zhu *et al*., 2013), while limonene is well-

established for its anticancer, neuroprotection, antioxidant, antibacterial, analgesic, immune regulation and analgesic properties (Chen *et al*., 2024). *E*-piperitol contributes additional anti-inflammatory activity (Nguyen *et al*., 2004). Rhizomes were dominated by octanal acetate and limonene, both of which exhibit antimicrobial and insect-repellent properties (Geraci *et al*., 2017; Heibish *et al*., 2008). The stems featured α-gurjunene, *E*-piperitol, cryptone, and methylnaphthalene, with *α*-gurjunene and cryptone known for their, antifungal and insecticidal effects (Muzzazinah *et al*., 2024; Isman, 2000).

E. nasuta exhibited a strong presence of sesquiterpenes across all plant parts. The leaves contained bicyclogermacrene, α-muurolene, and bulnesol, which have been linked to antioxidant, anti-inflammatory, and anti-acetylcholinesterase activities (Formagio *et al*., 2022; Schepetkin *et al*., 2022; Thusoo *et al*., 2014). The rhizomes were dominated by neo-intermedeol, borneol, and *α*-terpineol compounds known for their antimicrobial, anti-nociceptive, anti-bronchitis, antimicrobial, and anti-inflammatory effects (Poudel *et al*., 2025; Ha et al., 2021; Almeida *et al*., 2013). In the stems, α-muurolene, *β*-longipinene, and bicyclogermacrene were abundant, all contributing to potential antioxidant, antimicrobial, enzyme inhibitory and antiproliferative and anti-inflammatory activities (Zhao *et al*., 2024; Kumar *et al*., 2012).

In comparison, all three *Etlingera* species demonstrated strong antioxidant and anti-inflammatory potentials due to their rich sesquiterpene content. *E. nasuta* showed consistent bioactivity profiles across all plant parts. *E. foetens* stood out for its insecticidal and antimicrobial potential, attributed to compounds like cryptone, methylnaphthalene, and octanal acetate. Meanwhile, *E. coccinea* displayed notable anticancer-related activity due to its high levels of *γ*-elemene and *α*-copaene. These findings underscore the pharmacological

potential of each species and highlight the unique compound profiles that could be further explored for medicinal and pesticidal applications.

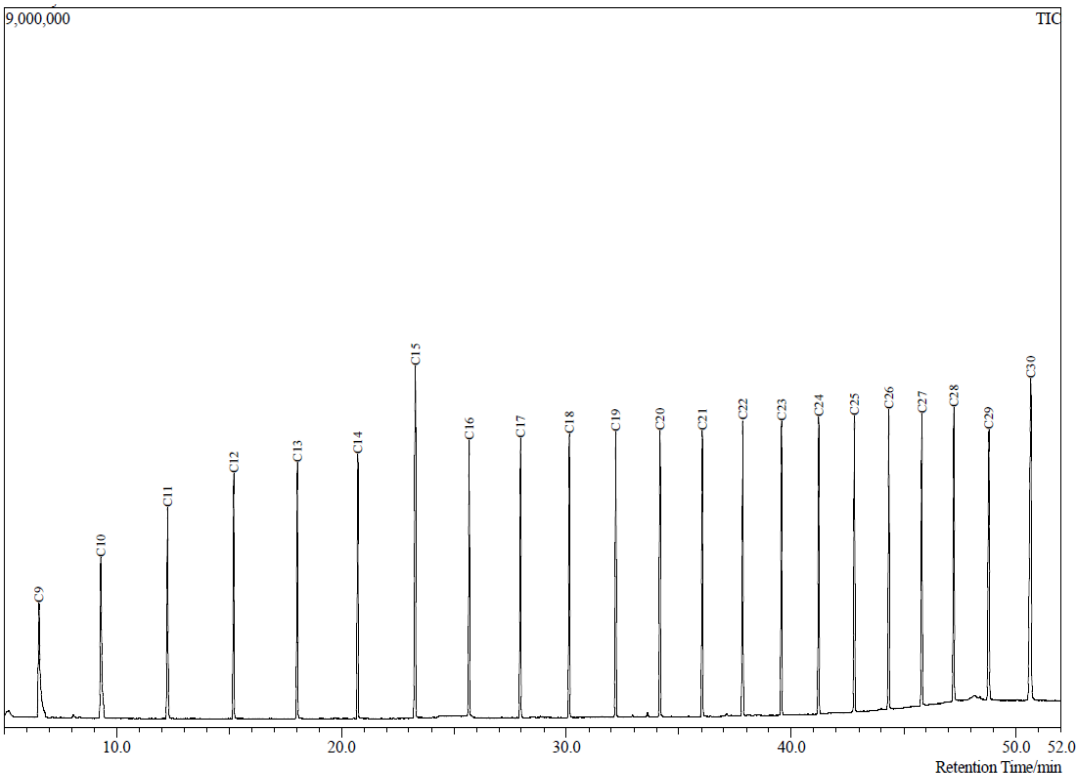


Figure 4.9: GCMS Chromatogram of *n*-alkane standards (C_9 - C_{30})

Table 4.5: Chemical composition of several parts of *E. coccinea*, *E. foetens* and *E. nasuta*

Compounds	KI[a]	KI[b]	Percentage Composition of Essential Oils (%)								
			ECL	ECR	ECS	EFL	EFR	EFS	ENL	ENR	ENS
Monoterpene hydrocarbons											
α-phellandrene	1006	1007	-	-	-	0.39	-	-	-	-	-
Limonene	1029	1030	-	-	-	9.03	4.98	-	-	-	-
D-limonene	1032	1033	-	-	-	4.51	-	-	-	-	-
Terpinolene	1090	1090	-	-	-	0.24	-	-	-	-	-
(4*E*,6*Z*)-allo-Ocimene	1131	1131	-	-	-	-	0.53	-	-	-	-
Oxygenated monoterpene derivatives											
Camphor	1147	1147	-	-	-	1.41	-	2.23	3.54	9.75	-
Isoborneol	1163	1160	-	-	-	-	0.76	3.71	-	-	-
Isomenthone	1164	1162	-	-	-	0.95	-	-	-	-	-
Borneol	1173	1173	-	-	-	-	-	-	-	16.15	-
Terpinen-4-ol	1179	1179	-	-	-	2.14	-	-	-	-	-

E-piperitol	1208	1208	-	1.34	1.67	11.86	-	31.03	-	-	-
(*Z*)-hex-3-enyl-butanoate	1211	1212	-	-	-	11.98	-	-	-	-	-
Geraniol	1251	1253	-	-	0.59	-	-	-	-	-	-
Thymol	1295	1296	-	0.63	-	-	-	-	-	-	-
α-terpineol	1438	1438	-	-	-	-	-	-	-	15.66	-
Sesquiterpene hydrocarbons											
α-copaene	1380	1380	-	-	-	0.48	0.89	-	-	-	-
6-*Epi*-*β*-cubebene	1389	1388	-	-	-	-	-	-	-	-	2.02
β-elemene	1396	1397	-	-	-	-	-	-	4.44	-	-
β-longipinene	1398	1398	-	-	-	-	-	-	-	-	19.99
α-gurjunene	1407	1409	-	-	-	-	1.39	47.75	-	-	-
α-cedrene	1411	1409	-	-	2.31	-	-	-	-	-	-
β-cubebene	1420	1419	-	0.32	-	14.69	-	-	-	-	-
Isocaryophyllene	1421	1422	-	2.88	-	-	-	-	-	-	-
β-caryophyllene	1424	1425	-	-	-	13.99	-	-	1.48	-	0.89
γ-elemene	1425	1425	31.91	-	-	-	-	-	-	-	-

β-copaene	1428	1428	-	-	-	0.89	-	-	-	-	-
Aromadendrene	1439	1439	-	-	-	-	-	-	3.02	8.56	-
Seychellene	1458	1458	3.95	-	-	-	-	-	-	-	-
Alloaromadendrene	1460	1460	-	-	-	0.32	-	-	-	-	3.36
α-copaene	1471	1471	28.62	51.63	-	-	-	-	-	-	-
β-chamigrene	1475	1475	-	-	57.41	-	-	-	-	-	-
Germacrene D	1481	1483	-	-	-	-	-	-	6.46	-	4.38
α-zingiberene	1489	1487	6.02	-	-	-	-	-	-	-	-
Bicyclogermacrene	1494	1494	-	-	-	-	-	-	27.31	-	18.73
B-bisabolene	1499	1499	-	0.85	2.81	-	0.59	-	-	-	-
α-muurolene	1503	1504	-	-	-	0.23	-	-	27.82	-	20.06
Cubenene	1512	1512	-	-	-	-	-	-	-	-	1.61
β-sesquiphellandrene	1524	1524	-	-	-	-	-	-	1.33	-	1.14
E-calamenene	1528	1529	-	-	-	0.36	0.58	2.68	-	-	-
δ-cadinene	1539	1537	-	0.33	-	-	-	-	-	-	-
β-bisabolol	1673	1672	-	-	2.32	-	-	-	-	-	-

Dehydroaromadendrene	1699	1699	1.61	0.95	-	-	-	-	-	-	-
Oxygenated Sesquiterpene derivatives											
Globulol	1582	1582	0.15	-	-	-	-	-	-	-	-
Viridiflorol	1591	1590	4.53	1.18	1.65	-	-	-	-	-	-
Caryophyllene oxide	1612	1613	-	-	1.16	-	-	-	-	-	-
Epoxyallo-aromadendrene	1639	1639	1.86	-	-	-	-	-	-	-	-
α-muurolol	1643	1644	1.88	-	-	-	-	-	-	-	-
β-eudesmol	1658	1659	-	0.02	-	-	-	-	-	-	-
Neo-intermedeol	1661	1661	-	-	-	-	-	-	-	49.89	-
Bulnesol	1665	1666	-	-	-	-	-	-	18.79	-	6.99
Selin-7(11)-4α-ol	1690	1690	-	-	-	-	-	-	-	-	6.99
Non-terpenoid compounds											
α-tolualdehyde	1048	1048	-	-	-	0.09	1.43	-	-	-	-
4-methyldecane	1060	1060	-	-	-	0.13	-	-	-	-	-
Nopol	1211	1212	-	-	-	0.83	-	-	-	-	-

Octanal acetate	1213	1214	-	-	-	-	85.63	-	-	-	-
Dodecane, 2-methyl	1264	1265	16.38	-	-	-	-	-	-	-	-
Cis-chrysanthenyl acetate	1266	1266	-	39.86	26.96	-	-	-	-	-	-
4-thujen-2-*α*-yl acetate	1289	1288	-	-	-	-	1.46	-	-	-	-
α-methylnaphthalene	1300	1299	-	-	-	0.32	-	-	-	-	-
Geranyl acetone	1454	1454	-	-	1.35	-	-	-	-	-	-
Dodecyl acetate	1602	1603	-	-	-	-	0.92	-	-	-	-
Dill apiole	1621	1620	-	-	-	-	-	-	5.79	-	2.78
(*Z*)-6-Dodecenyl-y-lactone	1661	1661	-	-	-	-	0.84	-	-	-	-
7-Ethyl-1,4-dimethyl-azulene	1733	1734	-	-	1.75	-	-	-	-	-	-
Ambroxide	1749	1749	3.09	-	-	-	-	-	-	-	-
Total of monoterpene hydrocarbons			0.00	0.00	0.00	14.17	5.51	0.00	0.00	0.00	0.00

Total of oxygenated monoterpene derivatives	0.00	1.97	2.26	28.07	0.76	37.24	3.54	41.56	0.00
Total of sesquiterpene hydrocarbons	72.11	56.95	64.85	30.96	3.45	50.43	71.86	8.56	72.18
Total of oxygenated sesquiterpene hydrocarbons	8.42	1.20	2.81	0.00	0.00	0.00	18.79	49.89	13.98
Total of non-terpenoid compounds	19.47	39.86	30.06	1.37	90.28	0.00	5.79	0.00	2.78
Total abundance	100	99.99	99.98	74.57	100	87.67	99.98	99.99	88.94

KI^a = Kovats index experimentally determined using homologous series of C_9-C_{30} alkanes from HP - 5 MS for all EOs and DB - 5 for *E. nasuta* rhizomes EO. KI^b = Kovats index taken from NIST 17 for HP - 5 MS or Adams (2007) for DB - 5.

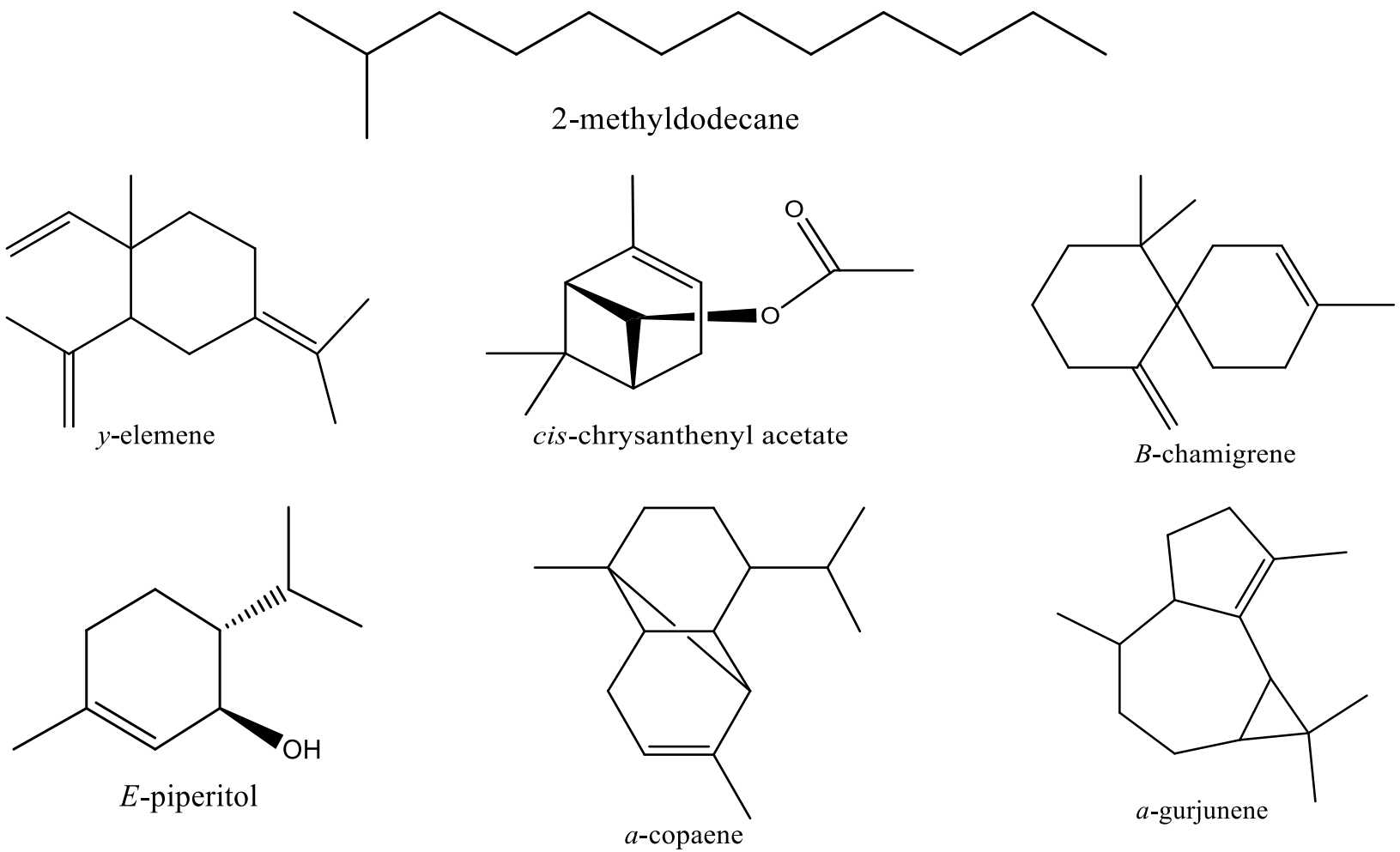


Figure 4.10: Several chemical structures of major components identified from present study of *Etlingera* spp.

Table 4.6: Dominant compounds in *Etlingera* spp.

Species	Plant parts	Dominant compounds
Etlingera coccinea	Leaves	*γ*-elemene (31.91 %) *α*-copaene (28.62 %) 2-methyldodecane (16.38 %).
	Rhizomes	*α*-copaene (51.63 %) cis-chrysanthenyl acetate (39.86 %).
	Stems	*β*-chamigrene (57.41 %) cis-chrysanthenyl acetate (26.96 %)
Etlingera foetens	Leaves	*β*-cubebene (14.69 %) *β*-caryophyllene (13.99 %) *E*-piperitol (11.86 %) (Z)-hex-3-enyl-butanoate (11.98 %) Limonene (9.03 %)
	Rhizomes	octanal acetate (85.63 %) limonene (4.98 %)
	Stems	*α*-gurjunene (47.75 %) *E*-piperitol (31.03%) cryptone (8.38 %) methylnaphthalene (4.21 %).
Etlingera nasuta	Leaves	bicyclogermacrene (27.31 %) *α*-muurolene (27.82 %) bulnesol (18.79 %
	Rhizomes	neo-intermedeol (49.89 %) borneol (16.15 %) *α*-terpineol (15.66 %)
	Stems	α-muurolene (20.06 %) *β*-longipinene (19.99%) bicyclogermacrene (18.73%)

4.5 Antioxidant Activity of *Etlingera* spp.

The antioxidant activity of *Etlingera* spp. was evaluated based on DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity at concentrations of 1, 10, 100 and 1000 ppm. The graphs were plotted between DPPH inhibitions (%) versus sample concentrations (log) (Figure 4.11 - 4.12) compared to the ascorbic acid as standard reference. Based on the graphs, all tested extracts and essential oils of *Etlingera* spp. exhibited scavenging activity against DPPH radicals with concentrations. Generally, at high concentration, the possibility of having secondary metabolites possessing antioxidant capacity that capable to protect plant against oxidative damage by free radicals could increase. Interestingly, most of the *Etlingera* spp. extracts and essential oils (EOs) even started exhibiting the scavenging activity with more than 10 % at the lowest concentration (1 ppm) except dichloromethane and ethyl acetate (EtOAC) extracts of *E. nasuta* and *E. coccinea*, repectively, as well as the rhizomes EO of *E. nasuta*. However, those samples started exhibiting scavenging activity at slightly higher concentration of 10 ppm with inhibition percentage of more than 10 % and continue to increase with concentration.

The half maximal inhibitory concentration (IC_{50}) values that refer to the amount of antioxidant necessary to decrease the initial DPPH concentrations by 50 % were obtained from the graphs and given in Table 4.7. It is important to have positive control for the study of complex mixture like plant extracts alongside with sample (Singh *et al*., 2007). Analysis of antioxidant activity showed that *E. foetens* and *E. nasuta* hexane extracts and leaves EO displayed high antioxidant activity at IC_{50} range of 21.95 - 25.12 ppm and 20.95 - 22.59 ppm, respectively. Similarly, rhizomes EO of *E. foetens* as well as hexane and ethyl acetate (EtOAc) extracts of *E. coccinea* also displayed high antioxidant activity, lie within the same range of IC_{50} value. A low IC_{50} value indicates strong antioxidant activity in a sample test.

Eventhough ascorbic acid is still the best antioxidant with IC_{50} value of 5.01 ppm, however, this compound is known to be very sensitive to light, heat, oxygen and easily destroyed during processing and storage (Tewari *et al*., 2017). One of the biggest challenges in the application of ascorbic acid as antioxidant is maintaining its stability.

Meanwhile, dichloromethane (DCM) and EtOAC extracts of *E. foetens* and EtOAC extract of *E. nasuta* demonstrated moderate antioxidant activity with IC_{50} values range of 39.81 - 63.10 ppm. Result revealed the DCM and EtOAc extracts of *E. nasuta* and *E. coccinea*, respectively showed low radical scavenging activity with IC_{50} values of more than 100 ppm. These findings are similar to *E. coccinea* EOs from all parts of plant, rhizomes and stems EO of *E. nasuta*, and stem EO of *E. foetens*.

Plants expose too much sunlight especially in tropical rain forest produces more antioxidant and claimed to exhibit effective antioxidant properties to counter oxidative damage (Masuda *et al*, 1999). These findings might explain high radical scavenging activity of *Etlingera spp*. in the present study and this was agreed by Chan *et al*. (2008) as well. The genus *Etlingera* is one of the largest gingers in Zingiberaceae that can grow up to 10 m in height. By having this advantage, it allows this plant to receive continuous exposure to direct sunlight leading to more antioxidant production. The present findings on high antioxidant activity demonstrated by *E. coccinea, E. foetens* and *E. nasuta* are consistent with other studies on different species of *Etlingera* that include *E. sayapensis, E. elatior, E. rubrostriata, E. littoralis, E. fulgens* and *E. maingayi* (Mahdavi *et al*., 2017; Chan *et al*., 2008). The present findings suggest the leaves extract of these three species of *Etlingera* and their EOs except *E. coccinea* and rhizomes EO of *E. foetens* have high potential to be developed as antioxidant products for the application in food, cosmetic or pharmaceutical industry.

In this study, the antioxidant activity of *Etlingera* spp. might be also attributed by the presence of several compounds including thymol, terpinen-4-ol, geraniol, D-limonene, limonene, *β*-caryophyllene, isoborneol, and borneol (Zhao *et al*., 2024; Ijinu *et al*., 2023). The presence of hydroxyl group in the compound structure was reported could enhance and promote the antioxidant activity as exemplified in the structure of thymol, terpinen-4-ol, geraniol, isoborneol, and borneol. The antioxidant activity was associated with free radicals such as reactive oxygen species and reactive nitrogen species. The hydroxyl radical was categorized as radical species that able to react with biomolecules (Al-Mamary & Moussa, 2021). The effectiveness of the antioxidant to neutralize the free radicals depends on the concentration of phenolic compounds, donor-proton capacity, position of hydroxyl groups, presence of glycosylation and electron - delocalization of the aromatic nucleus (Ilie *et al*., 2024). This suggests that the hydroxyl groups in these compounds is one of the factors contributing to the antioxidant activity of the essential oils derived from *Etlingera* spp..

Table 4.7: The IC_{50} (ppm) values of *Etlingera* spp. extracts and essential oils against DPPH radicals

Samples	Extracts			Essential Oils		
	Hexane	DCM	EtOAc	Leaves	Rhizomes	Stems
E. coccinea	22.95	20.85	398.11	255.19	530.96	141.25
E. foetens	21.95	63.10	63.10	20.95	20.95	251.19
E. nasuta	25.12	199.53	39.81	22.59	316.23	100.00

IC_{50} of Ascorbic acid: 5.01 ppm.

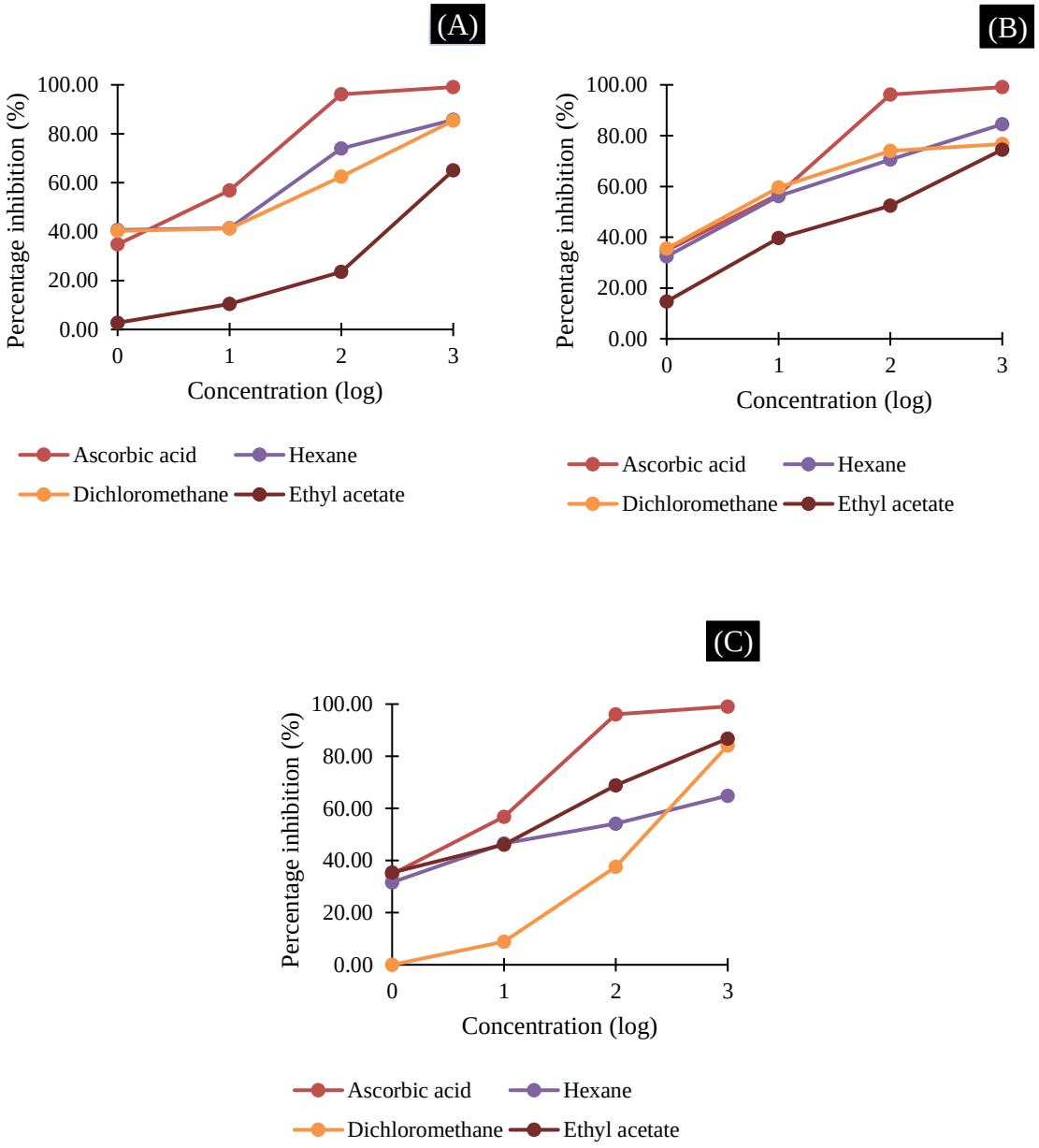


Figure 4.11: Percentage inhibition of *Etlingera* spp.: (A) *E. coccinea*, (B) *E. foetens* and (C) *E. nasuta* extracts against DPPH radicals

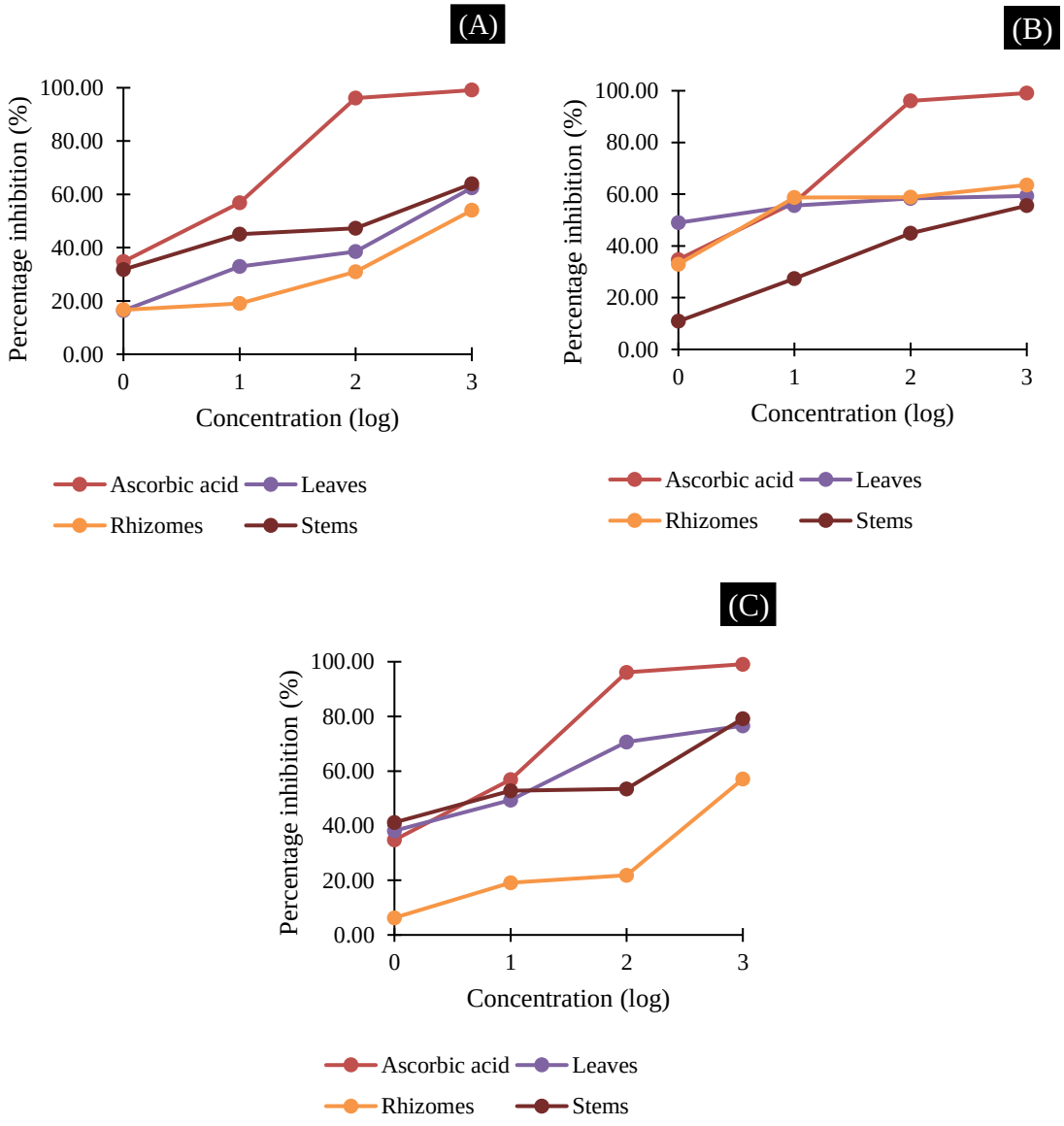


Figure 4.12: Percentage inhibition of Etlingera spp.: (A) *E. coccinea*, (B) *E. foetens* and (C) *E. nasuta* essential oils against DPPH radicals

4.6 Repellent Activity of *Etlingera* spp.

In this study, the repellency potential of *Etlingera* spp. crude extracts with different polarity of solvents (hexane, dichloromethane and ethyl acetate) were evaluated based on repellency test towards rice weevils of *S. oryzae*. This species is known as destructive insect pest in stored product leading to billion tonnes of food loss during postharvest stage. Data is shown in Table 4.8.

Overall, all of *E. coccinea* extracts showed repellent activity against *S. oryzae* at all time intervals from 1 to 5 hours of exposure between 2 - 8 μL/mL. Result revealed the dichloromethane extract showed the highest repellent activity at the concentration of 8 μL/mL with 75.00 % repellency after 5 hours of exposure (Figure 4.13). It is classified as class IV for repellency activity indicating strong repellency effect towards *S. oryzae* adults (Shahreesh *et al.*, 2023). The repellency effect of dichloromethane extract was clearly demonstrated even during first hour of exposure by repelling 50.00 % of pest population at the same concentration. At lower concentrations, it started to repel half of the population after 2 hours and 4 hours of exposure at 4 μL/mL and 2 μL/mL, respectively (Table 4.8). Likewise, the data indicated that the ethyl acetate extract possessed strong repellent activity after 5 hours of exposure at the highest concentration (8 μL/mL) as shown in Figure 4.13. The repellent activity was recorded slightly lower than the dichloromethane extract at 71.43 % and categorised in the same class of repellency, Class IV. However, the ethyl acetate extract showed weaker to moderate repellent activity at lower concentrations (2 and 4 μL/mL) with the repellency class ranging from Class I to Class III (Table 4.8). The hexane extract possessed moderate repellent activity with more than 50.00 % repellency after 4 hours of exposure only at the highest concentration (Figure 4.13). The repellency activity is classified as Class III. As predicted, the hexane extract showed weaker repellent activity at

lower concentrations (2 and 4 μL/mL) for all time intervals with maximum activity of 40.00 % (Table 4.8).

Similarly, all of *E. foetens* extracts showed repellent activity against *S. oryzae* at all concentrations up to 5 hours (Figure 4.14). The ethyl acetate extract exhibited high repellent activity at 8 μL/mL, achieving 71.43 % repellency after 5 hours of exposure, classified as Class IV. The dichloromethane extract followed suit by demonstrating high repellent activity at 8 μL/mL with 70.00 % repellency after 5 hours of exposure, also classified in Class IV. Surprisingly, the hexane extract possessed high repellent activity, even though slightly less than dichloromethane and ethyl acetate extracts but still categorised as Class IV repellent at 4 μL/mL and 8 μL/mL for 5 hours of exposure. The hexane extract at the lowest concentration (2 μL/mL) matched the expectation by exhibiting moderate repellent activity at the longest time of exposure (Table 4.8). This finding showed the hexane extract of *E. foetens* exhibited a better repellency potential against *S. oryzae* than the hexane extract of *E. coccinea* and *E. nasuta.*

E. nasuta crude extracts demonstrated repellent activity against *S. oryzae* with repellency percentage range between 11.11 - 71.43 % (Figure 4.15). Of all the crude extracts screened, ethyl acetate showed high repellency effect against *S. oryzae* at maximum concentration after 5 hours of exposure with 71.43 % repellency, categorised as class IV repellent. In comparison, dichloromethane extract demonstrated slightly less repellency against *S.oryzae* than ethyl acetate extract but still categorised in the same class of repellent (66.67 % repellency). However, hexane extract could only exhibit repellency against *S.oryzae* between 42.86 - 60.00 % at the longest time of exposure for all concentrations. This finding agreed with the study reported that the hexane extract of fruit and leaf of *Toddalia*

asiatica exhibited less than 50 % repellency against *S. oryzae* after 3 hours of exposure at concentrations between 5 - 20 μL/L (Nattudurai *et al*., 2015)

The high repellent activity of *Etlingera* spp. might be attributed by the presence of volatile compounds with the pungent smell. Several substances found in *Etlingera spp.* especially *E. coccinea* that reported to emit the pungent smell were saponins, flavonoids, tannins, alkaloids, terpenoids and other bioactive compounds with insecticidal activities (Ghasemzadeh *et al*., 2015). In addition, compounds of flavonoids, terpenoids, alkaloids, phenols and essential oils are the common class of repellent and insecticides (Ahmed *et al*., 2019). Shahid-Ud-Daula *et al.* (2015) revealed that the leaves of *E. coccinea* is rich in flavonoid. The presence of bioactive substances with repellent and insecticidal potential in *Etlingera spp.* causes the insect to make oriented movements away from the source of the substance. In other words, these secondary metabolites act as a prohibitor against stored product pests, preventing insect feeding and oviposition to reduce damage to stored product. (Stefanazzi *et al*., 2011).

Overall, all present study of *Etlingera spp.* exhibited repellency potential against rice weevils of *S. oryzae* particularly the dichloromethane and ethyl acetate extracts, classified as class IV repellent. Interestingly, only the hexane extract of *E. foetens* was found to exhibit promising repellent activity against *S. oryzae* in which classified as class 4 repellent in consistent to other extracts. To date, garlic essential oil is one of the best repellents against *S. oryzae,* categorised as class V repellent with the repellency of 88.89 % at a concentration of 6 μL after 4 hours of exposure (Kanimozhi *et al*., 2023). Nevertheless, this study provides the scientific evidence of *Etingera spp.* extracts in controlling the pest of *S. oryzae*. These species hold significant potential for the management of *S. oryzae* populations in storage products in a more environmentally friendly way. Further research and exploration are

necessary to assess the feasibility of integrating these natural extracts into pest control strategies.

Table 4.8: Repellent activity of Etlingera spp. extracts against *S. oryzae*

Species	Crude extracts	Concentration	Percentage repellency (Mean ± SE)				
			1 h	**2h**	**3h**	**4h**	**5h**
E. coccinea	Hexane	2 μL/mL	14.29± 0.57 **(RC I)**	25.00± 0.64 **(RC II)**	25.00± 0.94 **(RC II)**	33.33± 1.07 **(RC II)**	40.00± 1.26 **(RC II)**
		4 μL/mL	25.00± 0.77 **(RC II)**	33.33± 0.95 **(RC II)**	33.33± 0.95 **(RC III)**	33.33± 1.18 **(RC II)**	40.00± 1.26 **(RC II)**
		8 μL/mL	33.33± 0.64 **(RC II)**	42.86± 0.95 **(RC III)**	42.86± 0.95 **(RC III)**	55.56± 1.58 **(RC III)**	60.00± 1.90 **(RC III)**
	Dichloromethane	2 μL/mL	20.00± 0.32 **(RC I)**	33.33± 0.64 **(RC II)**	42.86± 0.95 **(RC III)**	50.00± 1.26 **(RC III)**	55.56± 1.58 **(RC III)**
		4 μL/mL	33.33± 0.32 **(RC II)**	50.00± 0.64 **(RC III)**	60.00± 0.95 **(RC II)**	66.67± 1.26 **(RC IV)**	66.67± 158 **(RC IV)**
		8 μL/mL	50.00± 0.64 **(RC III)**	60.00± 1.18 **(RC III)**	66.67± 1.26 **(RC IV)**	66.67± 1.26 **(RC IV)**	75.00± 1.90 **(RC IV)**
	Ethyl acetate	2 μL/mL	14.29± 0.32 **(RC I)**	25.00± 0.64 **(RC II)**	25.00± 0.94 **(RC II)**	33.33± 0.94 **(RC II)**	53.33± 1.71 **(RC III)**
		4 μL/mL	20.00± 0.32 **(RC I)**	33.33± 0.64 **(RC II)**	42.86± 0.94 **(RC III)**	50.00± 1.26 **(RC III)**	60.00± 1.52 **(RC III)**
		8 μL/mL	33.33± 0.32 **(RC II)**	50.00± 0.64 **(RC III)**	60.00± 0.94 **(RC III)**	66.67± 1.26 **(RC IV)**	71.43± 1.58 **(RC IV)**
E. foetens	Hexane	2 μL/mL	14.29± 0.32 **(RC I)**	25.00± 0.63 **(RC II)**	33.33± 0.95 **(RC II)**	42.86± 0.95 **(RC III)**	50.00± 1.26 **(RC III)**
		4 μL/mL	21.74± 0.57 **(RC II)**	35.71± 1.07 **(RC II)**	40.00± 0.87 **(RC II)**	42.86± 0.95 **(RC III)**	66.67± 1.26 **(RC IV)**
		8 μL/mL	33.33± 0.32 **(RC II)**	45.45± 0.57 **(RC III)**	50.00± 0.63 **(RC III)**	60.00± 0.95 **(RC III)**	68.42± 1.39 **(RC IV)**
	Dichloromethane	2 μL/mL	11.11± 0.32 **(RC I)**	14.29± 0.32 **(RC I)**	25.00± 0.63 **(RC II)**	33.33± 0.95 **(RC II)**	40.00± 1.26 **(RC II)**
		4 μL/mL	20.00± 0.32 **(RC II)**	33.33± 0.63 **(RC II)**	40.00± 1.26 **(RC II)**	55.56± 1.58 **(RC III)**	60.00± 1.90 **(RC III)**

		8 μL/mL	33.33± 0.22	50.00± 0.62	60.00± 0.95	66.67± 1.26	70.00± 1.49
			(RC II)	**(RC III)**	**(RC III)**	**(RC IV)**	**(RC IV)**
	Ethyl acetate	2 μL/mL	29.41± 0.67	36.84± 0.85	44.00± 1.18	50.00± 1.18	53.33± 1.71
			(RC II)	**(RC II)**	**(RC III)**	**(RC III)**	**(RC III)**
		4 μL/mL	30.77± 0.94	37.93± 1.18	45.45± 1.07	55.86± 1.52	60.00± 1.52
			(RC II)	**(RC II)**	**(RC III)**	**(RC III)**	**(RC III)**
		8 μL/mL	33.33± 0.32	42.86± 0.43	50.00± 0.63	60.00± 0.95	71.43± 1.58
			(RC II)	**(RC III)**	**(RC III)**	**(RC III)**	**(RC IV)**
	Hexane	2 μL/mL	11.11± 0.32	14.29± 0.32	20.00± 0.95	33.33± 1.26	42.86± 0.95
			(RC I)	**(RC I)**	**(RC II)**	**(RC II)**	**(RC III)**
		4 μL/mL	33.33± 0.64	42.86± 0.95	42.86± 0.95	50.00± 2.74	55.56± 1.58
			(RC II)	**(RC III)**	**(RC III)**	**(RC III)**	**(RC III)**
		8 μL/mL	42.86± 0.95	50.00± 1.26	55.56± 1.58	55.56± 1.58	60.00± 1.90
			(RC III)	**(RC III)**	**(RC III)**	**(RC III)**	**(RC III)**
	Dichloromethane	2 μL/mL	14.29± 0.32	20.00± 0.32	33.33± 0.64	42.86± 0.95	42.86± 0.95
			(RC I)	**(RC I)**	**(RC II)**	**(RC III)**	**(RC III)**
E. nasuta		4 μL/mL	33.33± 0.32	50.00± 0.64	50.00± 0.64	60.00± 0.95	60.00± 1.26
			(RC II)	**(RC III)**	**(RC III)**	**(RC III)**	**(RC III)**
		8 μL/mL	42.86± 0.95	50.00± 1.62	50.00± 1.26	66.67± 1.26	66.67± 1.26
			(RC III)	**(RC III)**	**(RC III)**	**(RC IV)**	**(RC IV)**
	Ethyl acetate	2 μL/mL	14.29± 0.32	25.00± 0.64	33.33± 0.95	40.00± 1.26	40.00± 1.26
			(RC I)	**(RC II)**	**(RC II)**	**(RC II)**	**(RC II)**
		4 μL/mL	20.00± 0.32	33.33± 0.64	42.86± 0.95	50.00± 1.26	55.56± 1.58
			(RC II)	**(RC II)**	**(RC III)**	**(RC III)**	**(RC III)**
		8 μL/mL	33.33± 0.32	50.00± 0.64	60.00± 0.95	66.67± 1.26	71.43± 1.58
			(RC II)	**(RC III)**	**(RC III)**	**(RC IV)**	**(RC IV)**

***RC=Repellency class**

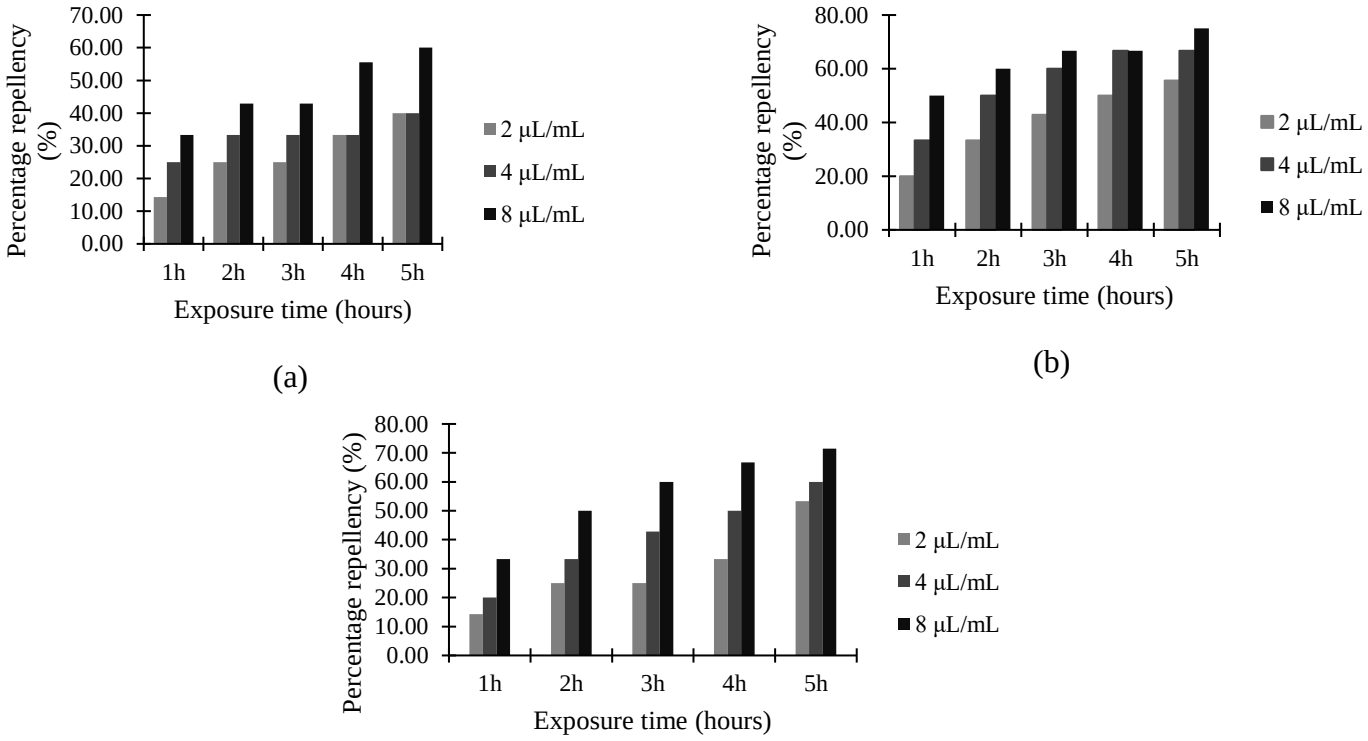


Figure 4.13: Percent repellency (%) of *E. coccinea's* extracts against *S. oryzae* (a) hexane (b) DCM (c) EtOAc

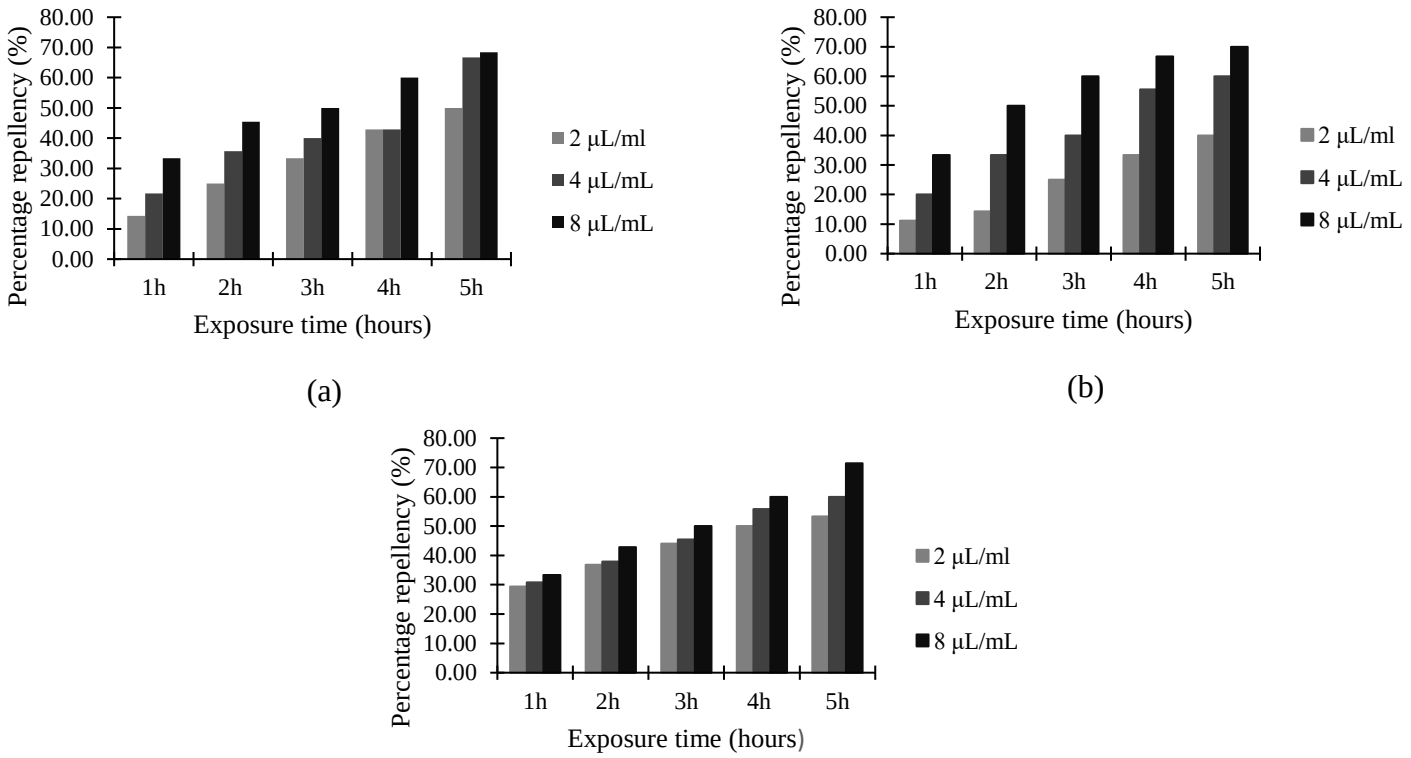
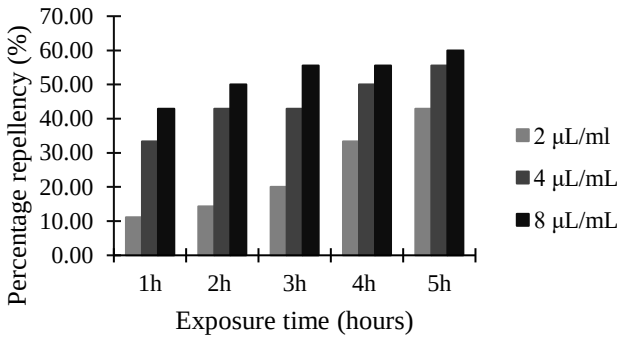
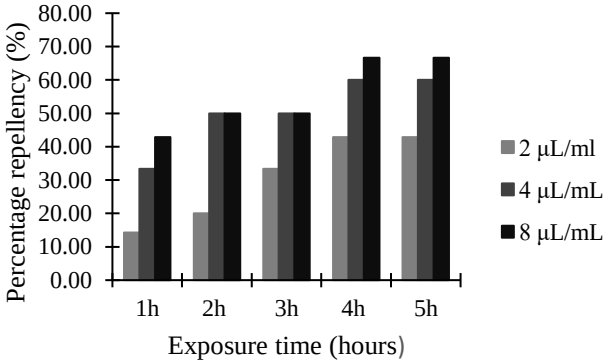


Figure 4.14: Percent repellency (%) of *E. foetens's* extracts against *S. oryzae* (a) hexane (b) DCM (c) EtOAc



(a)



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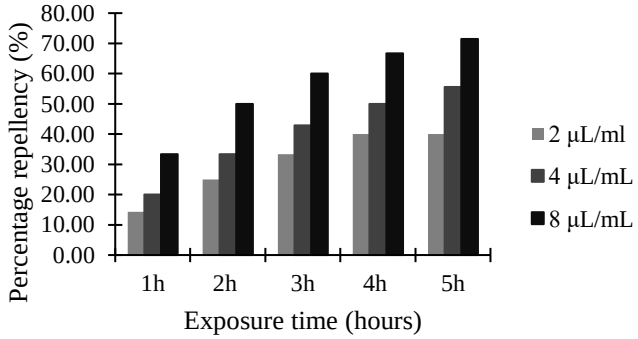

(c)

Figure 4.15: Percent repellency (%) of *E. nasuta's* extracts against *S. oryzae* (a) hexane (b) DCM (c) EtOAc

To sum up, the chemical composition and bioactivities of crude extracts and essential oils from *Etlingera coccinea*, *E. foetens* and *E. nasuta* varied significantly across plant parts, reflecting the influence of species-specidfic metabolic pathways and tissue-specific compound accumulation. Notably, sesquiterpene hydrocarbons were the predominant class across all species, particularly in leaves and rhizomes, which often correspond with higher bioactivity levels.

Antioxidant activity was closely associated with the presence of oxygenated sesquiterpenes and monoterpenes, such as neo-intermedeol, borneol, and α-terpineol in *E. nasuta* rhizomes, and α-copaene and cis-chrysanthenyl acetate in *E. coccinea*. These compounds have been previously reported to possess strong radical scavenging properties. The high antioxidant potential observed in *E. nasuta* and *E. foetens* hexane and ethyl acetate extracts, as well as in their leaves' essential oils (IC_{50}: 20.95 - 25.12 ppm), supports this correlation. Rhizome extracts and oils generally showed stronger antioxidant activity than stems and leaves, likely due to their richer content of oxygenated terpenoids.

In terms of repellency, dichloromethane and ethyl acetate extracts from all three species demonstrated high repellent activity against *Sitophilus oryzae*, with repellency percentages ranging from 71.43 % to 75 %, classified as Class IV repellents. This activity was particularly pronounced in extracts with high concentrations of volatile compounds such as octanal acetate (*E. foetens* rhizomes) and *β*-chamigrene (*E. coccinea* stems). These compounds have known insect-repelling properties, supporting their observed efficacy.

A comparative analysis across plant parts revealed that rhizomes consistently yielded higher amounts of essential oils and showed stronger bioactivities than leaves and stems. Rhizomes of *E. foetens* and *E. nasuta*, for example, contained octanal acetate and neo-intermedeol, respectively, which were strongly associated with both antioxidant and

repellent activities. In contrast, stems often exhibited lower oil yields and relatively fewer dominant bioactive compounds, suggesting their lower phytochemical richness. Nonetheless, in *E. coccinea*, stems were unexpectedly rich in *β*-chamigrene and cis-chrysanthenyl acetate, which may explain their notable repellent activity.

Compared to previous studies, the results align with findings that *Etlingera* species are a rich source of terpenoids and exhibit promising antioxidant and insect-repellent activities. However, the work extends current knowledge by providing a side-by-side chemical and biological comparison of multiple plant parts across three species. For instance, the high concentration of octanal acetate in *E. foetens* rhizomes and the identification of lavandulyl acetate in *E. nasuta* leaves represent novel findings not widely reported in the literature. These results emphasize the potential of underexplored *Etlingera* species, beyond the extensively studied *E. elatior*, for developing natural antioxidant and biopesticide agents.

In conclusion, the strong correlation between phytochemical content particularly terpenoids and bioactivities underscores the value of comprehensive profiling across different plant parts. These insights not only validate traditional uses of *Etlingera* spp. but also support their further development for natural product applications in food preservation, pharmaceuticals, and pest management.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Etlingera spp. is a promising alternative to synthetic antioxidant and insect repellent in stored products. The possible toxicity of synthetic antioxidants and resistance development of existing repellents against certain food storage pests requiring further research to develop new sustainable product from natural sources. This study highlighted the potential of crude extracts and essential oils from *Etlingera* spp along with the chemical identifications to provide scientific evidence in manifesting the antioxidant and repellent activity.

Based on solvent extraction techniques, the percentage yield of *Etlingera* spp. crude extracts was obtained in a range between 0.56 to 14.49 %. The highest percentage yield was obtained from *E. foetens* for different polarity of extracts. One compound was successfully isolated and purified from ethyl acetate extract of *E. nasuta* leaves and identified as lavandulyl acetate. The previous study reported the potential of this compound as eco-friendly larvicides against different species of mosquitoes.

The essential oil (EO) yield of *Etlingera* spp. ranged from 0.08 % to 0.42 %, with the highest yield obtained from *E. foetens* and *E. nasuta* leaves, while the lowest was recorded in *E. nasuta* stems. Chemical profiling revealed that *E. coccinea* EO was rich in γ-elemene (31.91 %) and α-copaene (28.62 %) in the leaves, α-copaene (51.63 %) and cis-chrysanthenyl acetate (39.86 %) in the rhizomes, and β-chamigrene (57.41 %) and cis-chrysanthenyl acetate (26.96 %) in the stems. In *E. foetens*, the leaves contained β-cubebene

(14.69 %), *β*-caryophyllene (13.99 %), and *E*-piperitol (11.86 %), the rhizomes were dominated by octanal acetate (85.63 %) and limonene (4.98 %), and the stems showed high levels of *α*-gurjunene (47.75 %) and *E*-piperitol (31.03 %). Meanwhile, *E. nasuta* leaves EO was characterized by neo-intermedeol (49.89 %), *α*-muurolene (27.82 %), and bicyclogermacrene (27.31 %), with *α*-terpineol (15.66 %) dominating the rhizomes, and *α*-muurolene (20.06 %), *β*-longipinene (19.99 %), and bicyclogermacrene (18.73 %) prevalent in the stems.

Based on antioxidant activity, *E. foetens* and *E. nasuta* hexane extracts and leaves EO demonstrated high antioxidant activity with IC_{50} values ranging between 21.95 - 25.12 ppm and 20.95 - 22.59 ppm, respectively. Also, the rhizomes EO of *E. foetens* as well as hexane and EtOAc extract of *E. coccinea* exhibited high antioxidant activity with similar range of IC_{50} values. The antioxidant activity was influenced by the presence of hydroxyl group which acts as proton donors to neutralize the free radicals effectively. The key compounds contributed to the antioxidant potential in the present study of *Etlingera spp* were thymol, terpinen-4-ol, geraniol, D-limonene, limonene, *β*-caryophyllene, isoborneol, and borneol.

Assessment of repellency potential of *Etlingera* spp. showed all dichloromethane and ethyl acetate extracts exhibited high repellent activity against rice weevils of *S. oryzae* with repellency percentage in range of 71. 43 - 75.00 % and classified as class IV repellent. The findings showed the dichloromethane extract of *E. coccinea* recorded the highest repellency at 75.00 % followed by *E. foetens* and *E. nasuta* at 71.43 % after 5 hours of exposure at 8 *μ*L/mL. This study also highlighted the potential of only lipophilic extracts, *E. foetens* hexane extract exhibiting class IV repellent activity against *S. oryzae* in which consistent to other hydrophilic extracts. The high repellency activity of *Etlingera spp*. might be attributed

to the presence of saponins, flavonoids, tannins, alkaloids, terpenoids and phenols. These phytochemicals were previously reported to emit pungent smell and recognized for their repellent and insecticidal properties, making them effective in controlling insect populations in stored products.

5.2 Recommendations

With high antioxidant and repellency activity demonstrated by *Etlingera spp.*, it is hoped that this study would help in addressing the issue of synthetic antioxidant toxicity and insect resistance in stored product. Further research and developments are necessary to assess the feasibility of integrating the natural extracts and essential oils of *Etlingera spp.* into pest control strategies and as an agent of potential antioxidants. More study related to advance technique to sustain the repellency and antioxidant effect such as encapsulation technique are recommended to extend their application in many industries.

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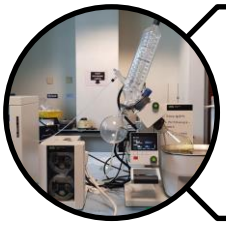
APPENDICES

Appendix 1. Flow process of solvent extraction

Air-dried leaves

Solvent extraction

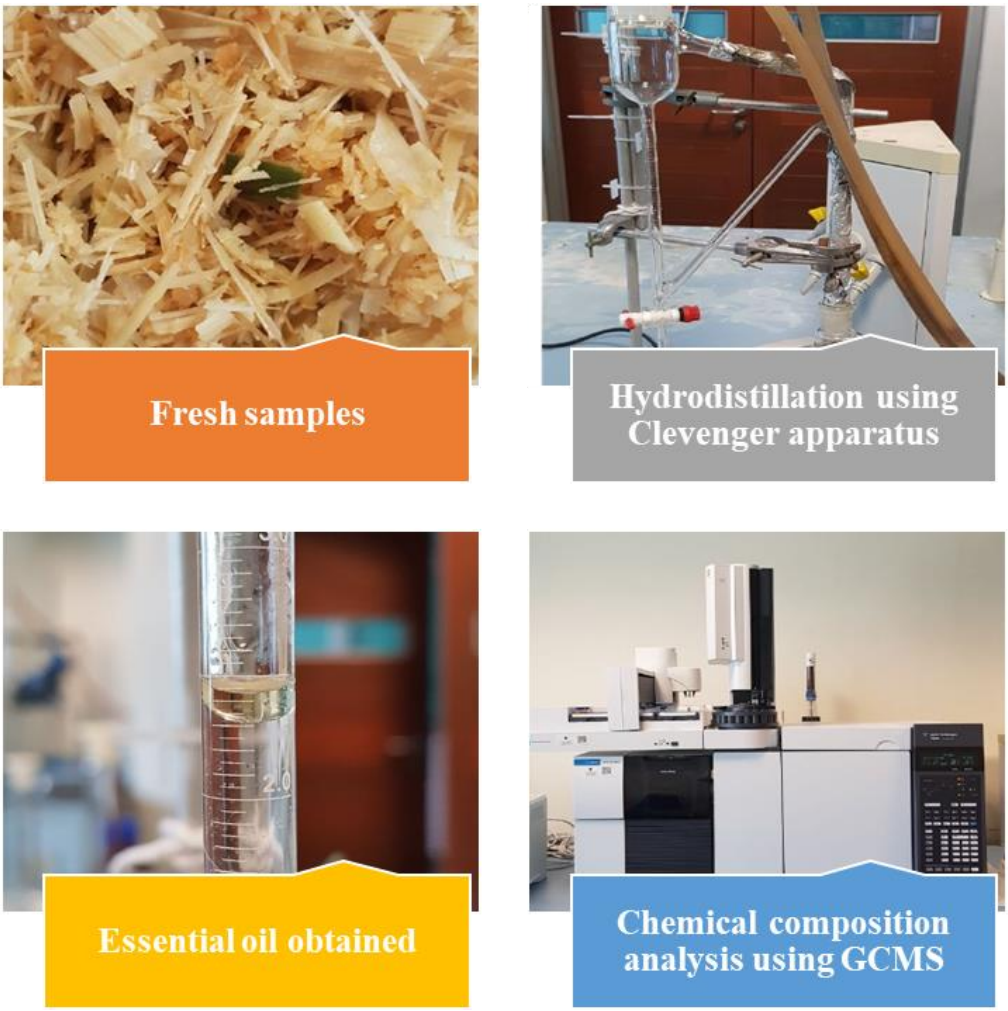
Filtered using filter paper



Concentrated using rotary evaporator

Crude extract obtained

Appendix 2. Flow process of hydrodistillation



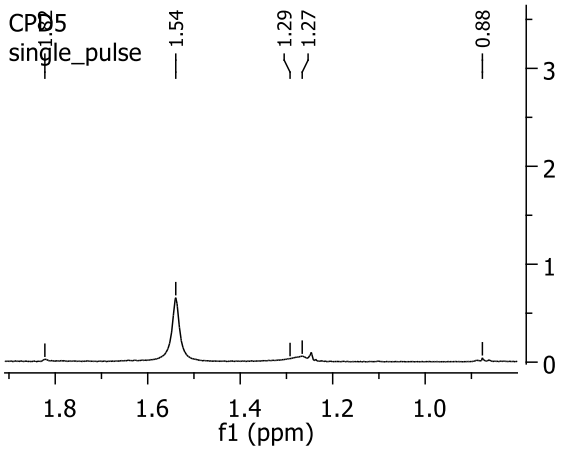
Appendix 3. Kovats indices calculation for Compound 1 and ^{1}H-NMR spectrum of Compound 1 with region at certain chemical shifts expanded

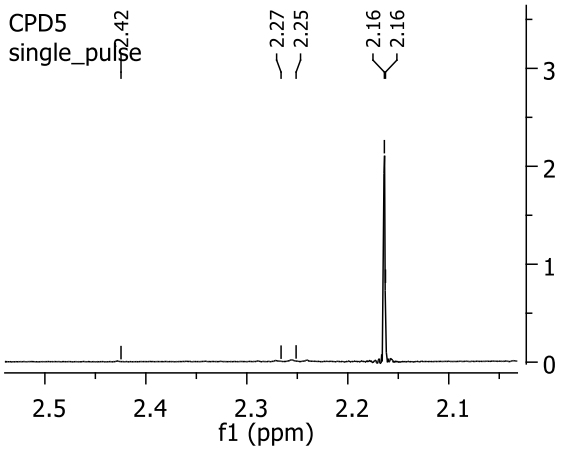
Table 3.1: Kovats indices calculation of the targeted compounds.

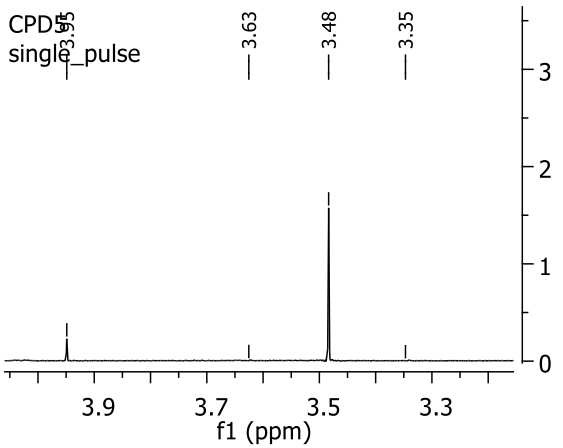
No	rt	C	100(n)	100(trx-trn/trn+1-trn)	trx-trn/ trn+1-trn)	KI[a]	KI[b]	Common name (nist)	common name (adams)	KI
1	18.341	13*14	1300	181.8	2.609	1370	1370	Ylangene	Carvacrol acetate	1370
2	16.334	12*13	1200	253.9	2.728	1293	1292	Lavandulyl acetate	Undecanone, Isomenthone, Methyl myrtenate, Undecanol	1293
3	16.438	12*13	1200	264.3	2.728	1297	1298	Carvacrol	Carvacrol, ethyl ether	1297

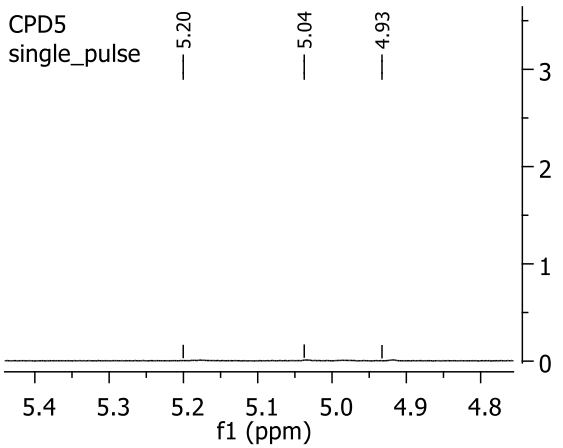
Table 3.2: Retention time of the Compound 1 and the standard reference retention time.

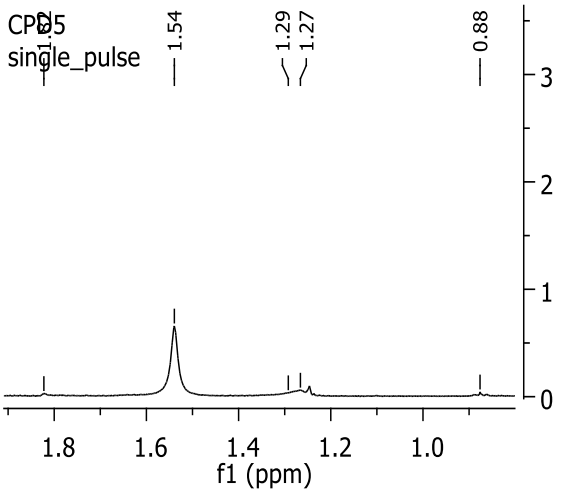
Compound	RT	Standard	RT
Compound 1	18.341	C10	8.255
Compound 2	16.334	C11	11.001
	16.438	C12	13.795
		C13	16.523
		C14	19.132
		C15	21.622
		C16	23.966
		C17	26.206
		C18	28.334
		C20	30.361
		C21	32.297



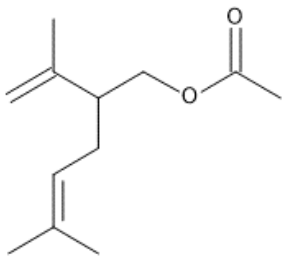








(A) Lavandulyl acetate



Appendix 4. Fragmentation calculation of structure confirmation

Lavandulyl cation ($C_{10}H_{15}^+$)	Acetyl ion ($C_2H_3O^+$)	Ethyl ion ($C_2H_5^+$)
CH_3 | CH_2-C_5H_9-C-C-H | CH3 ($C_{10}H_{15}$)	CH_3-C=O (C_2H_3O)	CH_3-CH_2 (C_2H_5)

1. *m/z* 43: This could correspond to an acetyl ion (C_2H_3O) or an ethyl cation ($C_2H_5^+$), which is common in ester fragmentation.
2. *m/z* 53: This is likely a fragment corresponding to an acetyl ion (C_2H_3O), which is common in ester compounds.
3. *m/z* 69: This might correspond to a fragment ion containing an ethyl group (C_2H_5) or the loss of a smaller fragment from the molecular ion.
4. *m/z* 80: This corresponds to a butyl ion ($C_4H_9^+$), which can be a product of the cleavage of the ester group or a fragmentation pathway involving a butyl group.
5. *m/z* 93: This is likely a fragment ion associated with the benzyl cation ($C7H7^+$) or $C9H11O^+$ resulting from the aromatic ring's rearrangement.
6. m/z 107: This could be a fragment involving an aromatic ion ($C9H11O^+$), possibly from the aromatic ring's partial cleavage or rearrangement.

7. *m/z* 121: This likely corresponds to a large fragment ion resulting from the aromatic structure of lavandulyl acetate or related rearrangement of the molecular structure.
8. *m/z* 136: This is likely related to a fragment derived from the lavandulyl structure, which is a portion of the molecule's alkyl or aromatic moiety.

The fragmentation pattern of lavandulyl acetate ($C_{12}H_{16}O_2$) in mass spectrometry is a well-defined sequence of bond cleavages that provides valuable information for structural identification. Upon ionization, the molecule undergoes the loss of the acetate group ($C_2H_3O_2$), leading to the formation of the lavandulyl cation ($C_{10}H_{15}^+$). This intermediate cation can then fragment further, with common pathways including the loss of alkyl groups such as CH_2**,** CH_3**,** or C_4H_9, as well as the formation of smaller ions like acetyl ions ($C_2H_3O^+$) or ethyl ions ($C_2H_5^+$)**.**

The resulting fragment ions generate characteristic peaks at *m/z* 43, *m/z* 53**,** *m/z* 69, *m/z* 93, *m/z* 107, and *m/z* 121, which can be used to confirm the identity of lavandulyl acetate. These patterns reflect the breakdown of the molecule's ester and terpenoid components, offering insights into the structural features of the compound. This fragmentation analysis is useful in interpreting the mass spectrum and validating the molecular structure of lavandulyl acetate.

Appendix 5. GCMS analysis of *Etlingera* spp. essential oils based on peaks

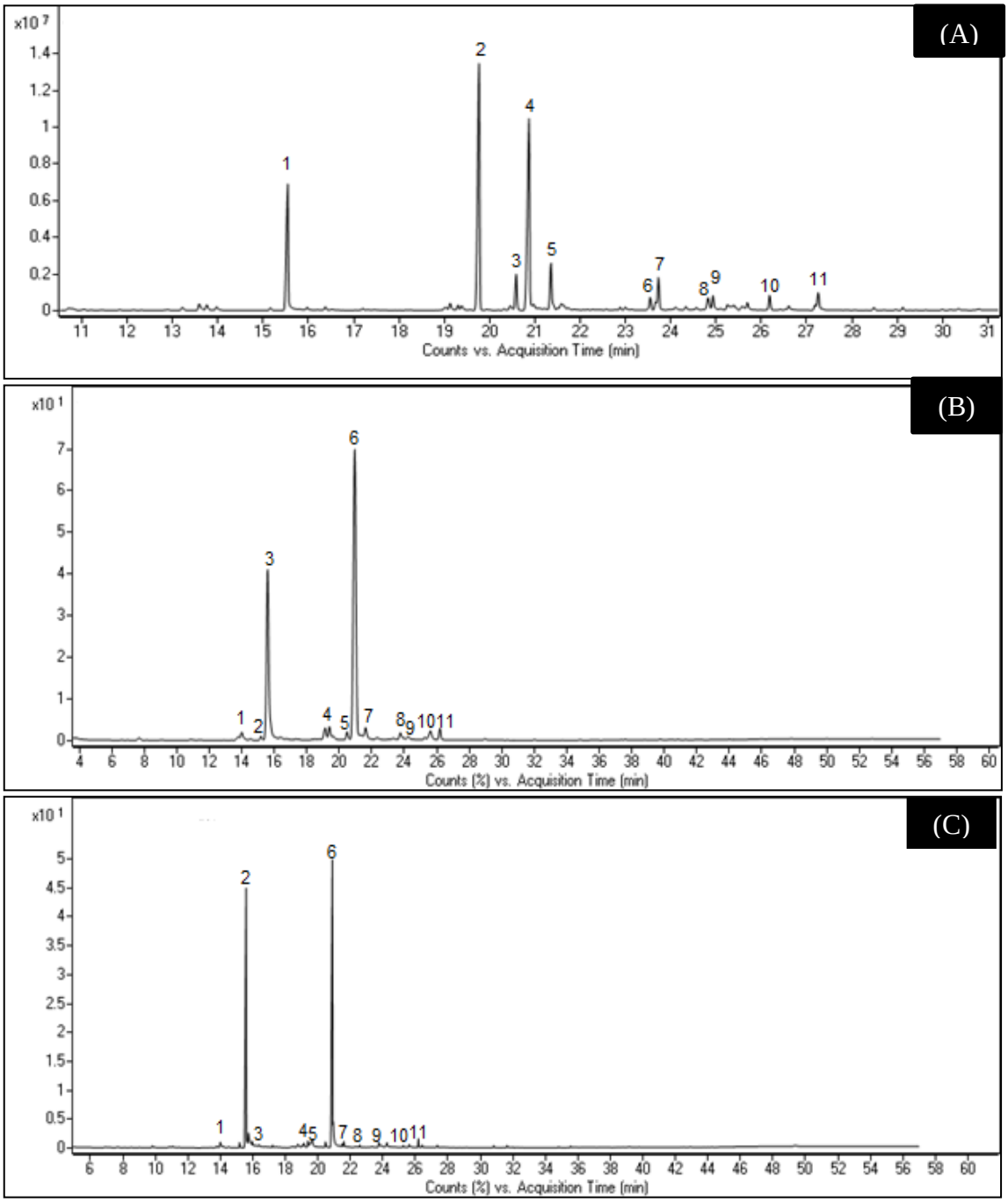


Figure 4.12: GC Chromatogram of *E. coccinea* EO: leaves (A), rhizomes (B) and stems (C)

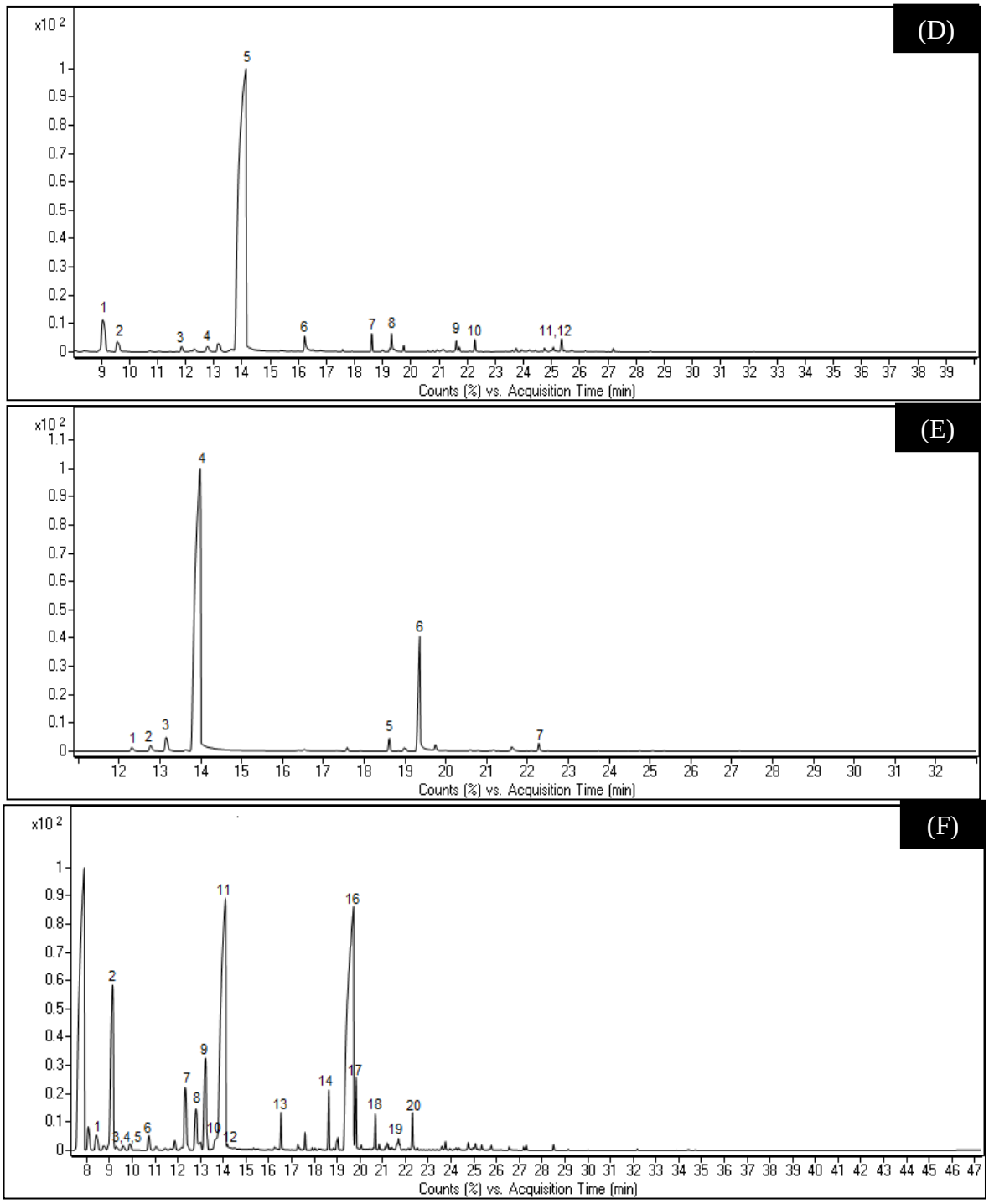


Figure 4.13: GC Chromatogram of *E. foetens* EO: leaves (D), rhizomes (E) and stems (F)

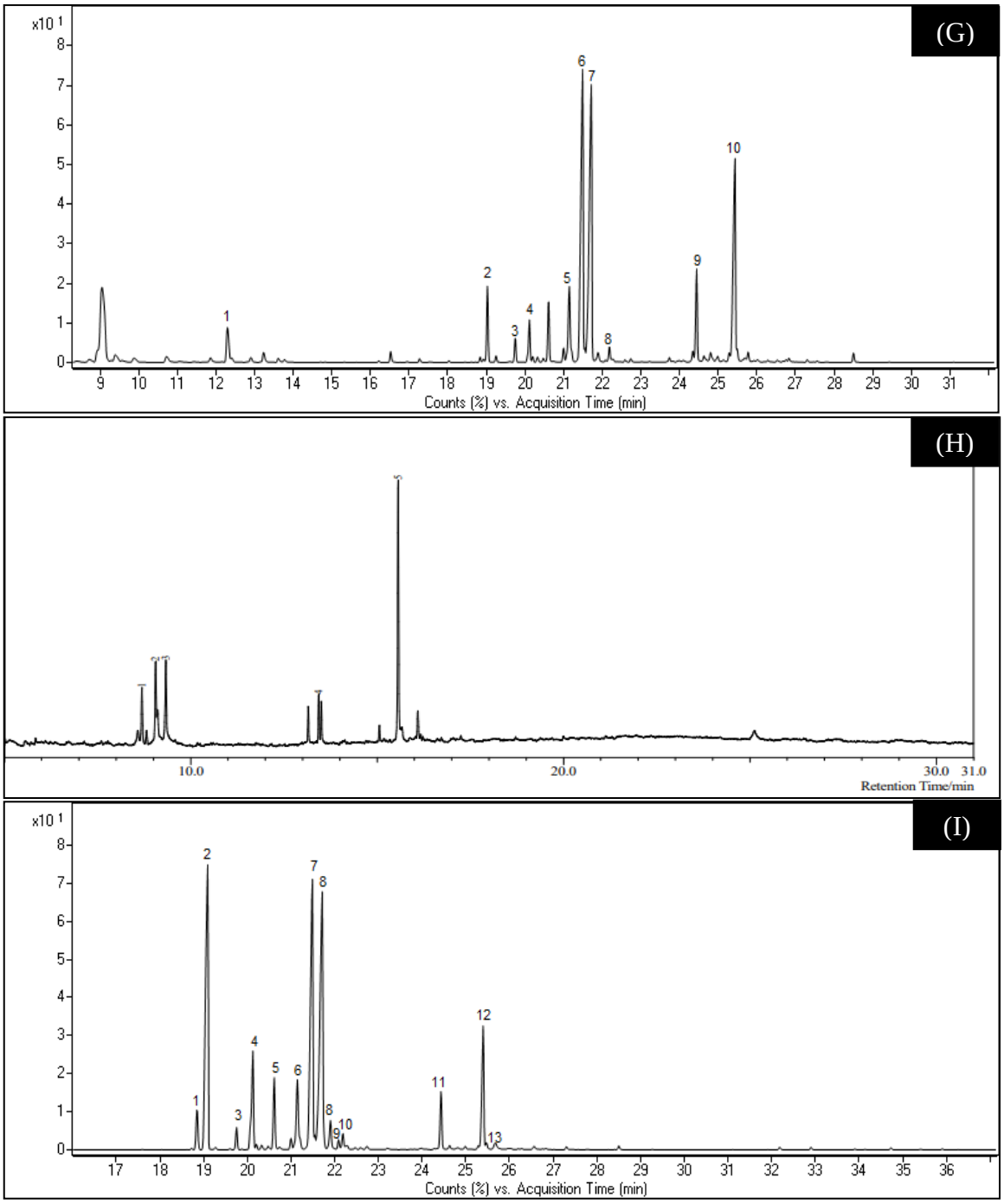


Figure 4.14: GC Chromatogram of *E. nasuta* EO: leaves (G), rhizomes (H) and stems (I)

(a) GCMS analysis of *E. coccinea* leaves oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	Dodecane, 2-methyl	$C_{13}H_{28}$	15.54	1264	1265	16.38
2	γ-Elemene	$C_{15}H_{24}$	19.76	1425	1425	31.91
3	Seychellene	$C_{15}H_{24}$	20.59	1458	1458	3.95
4	α-Copaene	$C_{15}H_{24}$	20.89	1471	1471	28.62
5	α-Zingiberene	$C_{15}H_{24}$	21.35	1489	1487	6.02
6	Globulol	$C_{15}H_{26}O$	23.54	1582	1582	0.15
7	Viridiflorol	$C_{13}H_{16}O_2$	23.73	1590	1589	4.53
8	Epoxyallo-aromadendrene	$C_{15}H_{24}O$	24.83	1639	1639	1.86
9	α-Muurolol	$C_{15}H_{26}O$	24.93	1643	1644	1.88
10	Dehydroaromadendrene	$C_{15}H_{24}$	26.18	1699	1699	1.61
					Total	99.99

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(b) GCMS analysis of *E. coccinea* rhizomes oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	*E*-Piperitol	$C_{10}H_{18}O$	13.99	1208	1208	1.34
2	*Cis*-Chrysanthenyl acetate	$C_{12}H_{18}O_2$	15.58	1266	1266	39.86
3	Thymol	$C_{10}H_{14}O$	16.39	1295	1296	0.63
4	*β*-Cubebene	$C_{15}H_{24}$	19.64	1420	1419	0.32
5	Isocaryophyllene	$C_{15}H_{24}$	19.66	1421	1422	2.88
6	*α*-Copaene	$C_{15}H_{24}$	20.89	1471	1471	51.63
7	*β*-Bisabolene	$C_{15}H_{24}$	21.6	1499	1499	0.85
8	*δ*-Cadinene	$C_{15}H_{24}$	22.59	1539	1537	0.33
9	Viridiflorol	$C_{15}H_{26}O$	23.75	1591	1590	1.18
10	*β*-Eudesmol	$C_{15}H_{26}O$	25.26	1658	1659	0.02
11	Dehydroaromadendrene	$C_{15}H_{24}$	26.19	1699	1699	0.95
					Total	96.89

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(c) GCMS analysis of *E. coccinea* stems oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	*E*-Piperitol	$C_{10}H_{18}O$	14.01	1208	1208	1.67
2	Geraniol	$C_{10}H_{18}O$	15.19	1251	1253	0.59
3	Cis-Chrysanthenyl acetate	$C_{12}H_{18}O_2$	15.64	1266	1266	26.96
4	α-Cedrene	$C_{15}H_{24}$	19.39	1411	1409	2.31
5	Geranyl acetone	$C_{13}H_{22}O$	20.48	1454	1454	1.35
6	β-Chamigrene	$C_{15}H_{24}$	20.99	1475	1475	57.41
7	β-Bisabolene	$C_{15}H_{24}$	21.62	1499	1499	2.81
8	Viridiflorol	$C_{15}H_{26}O$	23.75	1591	1590	1.65
9	Caryophyllene oxide	$C_{15}H_{24}O$	24.24	1612	1613	1.16
10	β-Bisabolol	$C_{15}H_{26}O$	25.61	1673	1672	2.32
11	7-Ethyl-1,4-dimethyl-azulene	$C_{14}H_{16}$	26.91	1733	1734	1.75
					Total	99.39

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(d) GCMS analysis of *E. foetens* leaves oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	*α*-Phellandrene	$C_{10}H_{16}$	8.42	1006	1007	0.39
2	Limonene	$C_{10}H_{16}$	9.11	1029	1030	9.03
3	D-Limonene	$C_{10}H_{16}$	9.12	1032	1033	4.51
4	*α*-Tolualdehyde	C_8H_8O	9.59	1048	1048	0.09
5	4-Methyldecane	$C_{11}H_{24}$	9.89	1060	1060	0.13
6	Terpinolene	$C_{10}H_{16}$	10.72	1090	1090	0.24
7	Camphor	$C_{10}H_{16}O$	12.33	1147	1147	1.41
8	Isomenthone	$C_{10}H_{18}O$	12.79	1164	1162	0.95
9	Terpinen-4-ol	$C_{10}H_{18}O$	13.21	1179	1179	2.14
10	*E*-Piperitol	$C_{10}H_{18}O$	14.02	1208	1208	11.86
11	(*Z*)-Hex-3-enyl-butanoate	$C_{10}H_{18}O_2$	14.08	1211	1212	11.98
12	Nopol	$C_{11}H_{18}O$	14.09	1211	1212	0.83
13	*α*-Methylnaphthalene	$C_{11}H_{10}$	16.53	1300	1299	0.32
14	*α*-Copaene	$C_{15}H_{24}$	18.63	1380	1380	0.48
15	*β*-Cubebene	$C_{15}H_{24}$	19.62	1420	1419	14.69
16	*β*-Caryophyllene	$C_{15}H_{24}$	19.72	1424	1425	13.99
17	*β*-Copaene	$C_{15}H_{24}$	19.82	1428	1428	0.89
18	Alloaromadendrene	$C_{15}H_{24}$	20.67	1462	1460	0.32
19	*α*-Muurolene	$C_{15}H_{24}$	21.69	1503	1504	0.23
20	*E*-Calamenene	$C_{15}H_{22}$	22.29	1528	1529	0.36
					Total	69.61

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, %: Percentage area

(e) GCMS analysis of *E. foetens* rhizomes oil

Peak no.	**Compounds**	**Molecular Formula**	**RT**	**KI[a]**	**KI[b]**	**%**
1	Limonene	$C_{10}H_{16}$	9.06	1029	1030	4.98
2	*α*-Tolualdehyde	C_8H_8O	9.57	1048	1048	1.43
3	(4*E*,6*Z*)-Allo-Ocimene	$C_{10}H_{16}$	11.86	1131	1131	0.53
4	Isoborneol	$C_{10}H_{18}O$	12.77	1163	1160	0.76
5	Octanal acetate	$C_{10}H_2O_2$	14.14	1213	1214	85.63
6	4-Thujen-2-*α*-yl acetate	$C_{12}H_{18}O_2$	16.23	1289	1288	1.46
7	α-Copaene	$C_{15}H_{24}$	18.61	1380	1380	0.89
8	*α*-Gurjunene	$C_{15}H_{24}$	19.31	1407	1409	1.39
9	*β*-Bisabolene	$C_{15}H_{24}$	21.61	1500	1499	0.59
10	*E*-Calamenene	$C_{15}H_{22}$	22.27	1528	1529	0.58
11	Dodecyl acetate	$C_{14}H_{26}O_2$	25.35	1602	1603	0.92
12	(*Z*)-6-Dodecenyl-y-lactone	$C_{12}H_{20}O_2$	25.34	1661	1661	0.84
					Total	94.89

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(f) GCMS analysis of *E. foetens* stems oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	Camphor	$C_{10}H_{16}O$	12.31	1147	1147	2.23
2	Isoborneol	$C_{10}H_{18}O$	12.77	1163	1160	3.71
3	Cryptone	$C_9H_{14}O$	13.15	1177	1178	8.39
4	*E*-Piperitol	$C_{10}H_{18}O$	13.98	1207	1208	31.03
5	*α*-Methylnaphthalene	$C_{11}H_{10}$	18.61	1300	1299	4.21
6	*α*-Gurjunene	$C_{15}H_{24}$	19.35	1407	1409	47.75
7	*E*-Calamenene	$C_{15}H_{22}$	22.27	1528	1529	2.68
					Total	99.99

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(g) GCMS analysis of *E. nasuta* leaves oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	Camphor	$C_{10}H_{16}O$	12.29	1147	1147	3.54
2	*β*-Elemene	$C_{15}H_{24}$	19.03	1396	1397	4.44
3	*β*-Caryophyllene	$C_{15}H_{24}$	19.75	1424	1425	1.48
4	Aromadendrene	$C_{15}H_{24}$	20.11	1439	1439	3.02
5	Germacrene D	$C_{15}H_{24}$	21.14	1481	1483	6.46
6	Bicyclogermacrene	$C_{15}H_{24}$	21.48	1494	1494	27.31
7	*α*-Muurolene	$C_{15}H_{24}$	21.71	1503	1504	27.82
8	*β*-Sesquiphellandrene	$C_{15}H_{24}$	22.19	1524	1524	1.33
9	Dill apiole	$C_{12}H_{14}O_4$	24.43	1621	1620	5.79
10	Bulnesol	$C_{15}H_{26}O$	25.43	1665	1666	18.79
					Total	99.98

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(h) GCMS analysis of *E. nasuta* rhizomes oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	Camphor	$C_{10}H_{16}0$	8.69	1149	1149	9.75
2	Borneol	$C_{10}H_{18}0$	9.06	1173	1173	16.15
3	α-Terpineol	$C_{10}H_{18}0$	9.33	1438	1438	15.66
4	Aromadendrene	$C_{15}H_{24}$	13.43	1195	1195	8.56
5	*Neo*-intermedeol	$C_{15}H_{26}O$	15.56	1661	1661	49.89
					Total	99.99

***RT:** Retention time, **KI[a]**: Retention index from DB-5 elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

(i) GCMS analysis of *E. nasuta* stems oil

Peak no.	Compounds	Molecular Formula	RT	KI[a]	KI[b]	%
1	6-*epi*-*β*-Cubebene	$C_{15}H_{24}$	18.84	1389	1388	2.02
2	*β*-Longipinene	$C_{15}H_{24}$	19.09	1398	1398	19.99
3	*β*-Caryophyllene	$C_{15}H_{24}$	19.75	1424	1425	0.89
4	Aromadendrene	$C_{15}H_{24}$	20.11	1439	1439	5.63
5	Alloaromadendrene	$C_{15}H_{24}$	20.62	1460	1460	3.36
6	Germacrene D	$C_{15}H_{24}$	21.14	1481	1483	4.38
7	Bicyclogermacrene	$C_{15}H_{24}$	21.48	1494	1494	18.73
8	*α*-Muurolene	$C_{15}H_{24}$	21.70	1503	1504	20.06
9	Cubenene	$C_{15}H_{24}$	21.90	1512	1512	1.61
10	*β*-Sesquiphellandrene	$C_{15}H_{24}$	22.19	1524	1524	1.14
11	Dill apiole	$C_{12}H_{14}O_4$	24.43	1621	1620	2.78
12	Bulnesol	$C_{15}H_{26}O$	25.40	1665	1666	6.99
13	Selin-7(11)-4α-ol	$C_{15}H_{26}O$	25.98	1690	1690	6.99
					Total	94.57

***RT:** Retention time, **KI[a]**: Retention index from HP-5 MS elution, **KI[b]**: Retention index from NIST 17 database, **%:** Percentage area.

Appendix 6. Antioxidant activity of *Etlingera* spp. extracts

Concentration (log)	Percentage inhibition of DPPH radical scavenging activity (%)										
	AA	**BHT**	**ECH**	**ECD**	**ECE**	**EFH**	**EFD**	**EFE**	**ENH**	**END**	**ENE**
0	34.76	5.42	40.68	40.26	2.73	32.44	35.52	14.70	31.64	0.00	35.37
1	56.84	30.21	41.44	41.27	10.46	56.14	59.65	39.63	46.57	8.86	46.10
2	96.11	93.34	73.98	62.41	23.52	70.59	73.95	52.45	54.16	37.58	68.86
3	99.10	96.88	85.70	85.36	65.00	84.48	76.67	74.50	64.88	84.13	86.77

*__AA__ = Ascorbic acid, __BHT__ = Butylated hydroxytoluene, __ECH__ = *E. coccinea* Hexane, __ECD__ = *E. coccinea* Dichloromethane, __ECE__ = *E. coccinea* Ethyl acetate, __EFH__ = *E. foetens* Hexane, EFD = *E. foetens* Dichloromethane, EFE = *E. foetens* Ethyl acetate, ENH = *E. nasuta* Hexane, END = *E. nasuta* Dichloromethane and ENE = *E. nasuta* Ethyl acetate.

Appendix 7. Antioxidant activity of *Etlingera* spp. essential oils

Concentration (log)	Percentage inhibition of DPPH radical scavenging activity (%)										
	AA	**BHT**	**ECL**	**ECR**	**ECS**	**EFL**	**EFR**	**EFS**	**ENL**	**ENR**	**ENS**
0	34.76	5.42	16.39	16.66	31.78	48.99	32.94	10.88	38.14	6.29	41.18
1	56.84	30.21	32.98	19.05	45.02	55.60	58.69	27.41	49.43	19.16	52.78
2	96.11	93.34	38.55	30.98	47.27	58.31	58.85	44.96	70.63	21.80	53.50
3	99.10	96.88	62.45	54.00	63.97	59.34	63.59	55.58	76.59	57.07	79.20

***AA:** Ascorbic acid, **BHT:** Butylated hydroxytoluene, **ECL:** *E. coccinea* leaves oil, **ECR:** *E. coccinea* rhizomes oil, **ECS:** *E. coccinea* stems oil, **EFL:** *E. foetens* leaves oil, **EFR:** *E. foetens* rhizomes oil, **EFS:** *E. foetens* stems oil, **ENL:** *E. nasuta* leaves oil, **ENR:** *E. nasuta* rhizomes oil, **ENS:** *E. nasuta* stems oil.