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Mass-Spectrometry Phytochemical Profiling of *Etlingera coccinea* (Blume) S. Sakai & Nagam from Sabah

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ABSTRACT

Etlingera coccinea (Blume) S. Sakai & Nagam is classified in the genus of the *Etlingera* (Zingiberaceae). Locally known as 'tuhau' in Sabah, it is used as traditional remedy such as to cure food poisoning, stomachache as well as condiment in cooking. Previous studies on the genus *Etlingera* have focused on its phytochemical compounds and biological activities. However, mass-spectrometric profiling of crude extracts has not been reported. In this research, *E. coccinea* stalks were taken from two different localities: Ranau and Tambunan areas to determine their chemical profiles. Each of locations have three types of crude fractions: n-hexane, chloroform and methanol. The phytochemical screening for both locations shows the presence of flavonoids, terpenoids and tannins. Through detailed chemical profiling, the library of Liquid Chromatography Mass-Spectrometry (LC-MS) analysis results showing 10 and 8 known metabolites found in Ranau methanolic, and chloroform extracts respectively. While for Tambunan methanolic and chloroform extracts, 8 and 4 known metabolites found. Additionally, Gas Chromatography Mass-Spectrometry (GC-MS) analysis reported 15 and 9 known metabolites respectively from Ranau and Tambunan n-hexane extracts. All identified phytochemical compounds have their own potential application such as antibacterial, anti-fungal, antioxidants, anti-inflammatory, and anti-cancer. The roles could serve as a basis for further exploration of the chemical composition as well as the biological activities of *E. coccinea*.

INTRODUCTION

The Zingiberaceae, or ginger family, is made up of around 13,000 species divided into 52 genera that are mostly found in tropical and subtropical climates, particularly in Asia [1, 2]. Most Zingiberaceae species are rhizomatous or perennial herbs that sprout from underground stems [3]. *Etlingera* is the largest genus

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for Zingiberaceae. *E. coccinea* is the native to Sumatra, Java, The Malay Peninsula and Brunei. In Sabah, it is called 'tuhau' by the Dusun and Murut natives. It is also called 'tubu nanung' by the Kelabits in Sarawak and 'tepus' by the Ibans in Brunei and Sarawak. Traditionally, it is used as a remedy such as curing food poisoning, stomach-aches, and gastric problems. It is easily distinguishable by its strong and pungent aroma, the inflorescence which appears at ground level, consists of a long red flower stalk with a yellow border at the center. The fruit is edible and used by the locals as food. The center of leafy shoot is used as condiments in Borneo and Java. Its young shoots are collected as vegetables and for pickles.

Figure 1. *Etlingera coccinea* flower. Source: [4]

The *Etlingera* genus is well known as aromatic plants as they have their unique scent. As a result, studies on *E. coccinea* have primarily focused on tis essential oil composition. The interest in *E. coccinea* has grown because for its beneficial compounds that has been found, which are related to the well-known *E. elatior* (kantan). Previous studies on *E. coccinea* focused on its volatile compounds profiling, particularly essential oils and hexane leaf extract, due to its strong, pungent scent that attracts insects and humans. Aside from that, they have focused on biological and chemical activities, and species comparisons. The class of compounds of *E. coccinea* were discovered to have sesquiterpene hydrocarbon, oxygenated diterpenes, monoterpenes, alkanes and alkenes, aldehydes, ketones, fatty acid derivatives, esters and amine [5, 6]. It was also reported that in *E. coccinea* they found several compounds such as borneol, *L*-calamene, 1-dodecanol, lauryl aldehyde, aromadendrene oxide, elemicin, camphor as well as 5-decen-1-ol [5, 7]. Trans-2-dodecenal was from the hexane extract of *E. coccinea* leaves, which was the first chemical identified from this plant [8]. As of today, the non-volatile compound of *E. coccinea* is yet to be investigated. Most of the crude extracts were assessed primarily on phytochemical screening stage as well as biological activities. In our record, a further analysis on these non-volatile compounds have not conducted yet.

Previous research was mainly focused on the leaves due to their strong smells. However, stalk parts are the part of *E. coccinea* that people always consume. There are many ways to have them, for example turning them into *jeruk tuhau'* (pickled), *serunding tuhau'* (floss), *sambal tuhau'* or being an additive in cooking to enhance the flavor. Therefore, more research on stalk metabolites and comprehensive chemical profiling are required. This study aims to broaden the chemical profile of *E. coccinea* stalks by examining extracts with varying polarities, methodology and sample collection site from two Sabah locations: Ranau and Tambunan. Samples collected from these many locations will

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provide a more complete picture of *E. coccinea*'s chemical profiles and yields. This study aims for phytochemical profiling of crude extracts of *E. coccinea* with the confirmation through LC-MS and GC-MS analysis.

EXPERIMENTAL

Plant Materials

The access license (JKM/MBS.1000-2/2 Jld 12(115)) was granted by Sabah Biodiversity Centre (SaBC), while a research permit was provided by Sabah Parks. The fresh plants of *E. coccinea* were collected from Poring Hotspring, Ranau (R) and Crocker Range Molunggong Substation, Tambunan (T) with the DMS coordinate of 6.0457° N, 116.7034° E and 5.533°N 116.100°E respectively. The samples were confirmed by Mr. Duni Molidin, the appointed ranger by Sabah Parks. The plant samples were washed under cold running tap water to remove any dirt. Only the stalk part of the plant was processed to be used in the experiments.

Chemical and Reagents

The lists of chemicals used were *n*-hexane, methanol, chloroform, Dragendorff's reagent, Mayer's reagent, hydrochloric acid, sulphuric acid, anhydrous sodium sulphate, magnesium powder, ferric chloride powder. All chemicals/reagents were of analytical or laboratory grade (unless stated as HPLC grade) purchased from certified manufacturers/companies.

Extractions Procedure

Fresh *E. coccinea* stalks (ECS) were cleaned, sliced into small pieces, ground using blender and left for air-dried before being macerated using 10% aqueous methanol for 72 hours at room temperature. The extracts were pooled, anhydrate and concentrated using Laborata 4000 – Eyela N-1100 rotary evaporator at 45 ˚C. The crude extracts then proceeded for solvent-solvent partitioning performed by dissolving methanol extracts with 10% aqueous methanol then poured into separating funnel [9]. The solvents used were n-hexane and chloroform which are non-polar and semi-polar solvents respectively. Since the study of metabolites in crude extracts of *E. coccinea* is still broad, these differences in the polarity of solvents could give a wide variation of compounds in the extracts.

About 150 mL of *n*-hexane was poured into the separating funnel and then pooled together leaving the methanol extract in the separating funnel. The step then repeated with 150 mL of chloroform. By adding anhydrous sodium sulphate to remove water in it. The extracts concentrated again until the extract concentrated in texture. The crude extract further concentrates by placing them inside a desiccator containing silica gel. Therefore, there were three fractions from each location: *n*-hexane (H), chloroform (C) and methanol (M) fractions obtained; ECSH-R, ECSH-T, ECSC-R, ECSC-T, ECSM-R and ECSM-T.

Phytochemical Screening

The methanolic and chloroform fractions from both locations were qualitatively tested for alkaloids, flavonoids, terpenoids, tannins, and saponins. Results are expressed as '+', '++' and '-' to indicate trace, presence and absence of phytochemicals respectively.

Test for Alkaloids (Mayer's Reagents)

Mayer's reagent was added about 1 mL onto the test tube containing fraction samples. The presence of alkaloids will give a formation of cream-colored precipitates [9].

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Test for flavonoids

About 1 mL of diluted extracts were added with mixture of 0.1g magnesium and 1mL hydrochloric acid. The presence of flavonoids was recorded as yellow or violet coloration disappears on standing [1].

Test for terpenoids

About 50mg of extracts were shaken with 1 mL of chloroform followed by the additions of 1mL of sulphuric acid, H_2SO_4. A reddish-brown coloration was recorded as the presence of terpenoids [10].

Test for tannins

About 0.5mL of extract was added with 1mL of distilled water. Then, 4 to 5 drops of 1% ferric chloride, $FeCl_3$ were added into it. Black or blue-green coloration was recorded as positive results for the presence of tannin [11].

Test for saponin (Froth Test)

1mL of extracts were added with 3mL of distilled water and proceeded with shaking for 5 minutes. A 1mL of foam which persists within 10 minutes indicates the presence of saponin [11].

LC-MS Analysis

The methanol and chloroform fractions were subjected to LC-MS at the Institute of Biotechnology, UMS. The brand and model used for LC was Vanquish/Thermo Scientific and as for MS was Impact II/Bruker. The analysis was conducted by using C18 column (2.1 x 50 mm, 1.7-micron particle), with solvent system of; A – 0.10% formic acid, B – Acetonitrile with 0.10% formic acid; 40-100% B over 2 mins; 100% B for 0.5 min. The parameter is 0.5 mL/min of flow rate with 1mg/mL of concentrations and 5µL of injection volume. The method was using electrospray ionization (ESI) positive ion modes with the scan range of 50m/z until 1500 m/z. The components that identified as plant metabolites were compared by matching their mass spectra with those recorded in the mass spectral library database; Bruker Compass DataANalysis 4.3 and National Center for Biotechnology Information. The identified plant metabolites were also compared with previous studies of the genus as well as the benefits of the plant metabolites itself in plants. The LC-MS method referred to [8] with some modifications.

GC-MS Analysis

The *n*-hexane fractions were subjected to Gas Chromatography – Mass Spectrometry (GC-MS) at Pusat Instrumentasi dan Perkhidmatan Sains, UMS. The brand and model used for GC were Agilent 7890A, while MS detector was Agilent 5975C inert XL EI/CI MSD with Triple-Axis Detector. The analysis was done by using capillary column HP 1 (50 m long x 0.25 µm phase thickness 0.22 mm column diameter). The operating conditions for GC-MS column were as follows: start temperature 60 ºC, end temperature 220 ºC, head rate 2 k/min, end time 20 min. Helium gas was used as the carrier gas and the components were identified by matching their mass spectra with those recorded in the mass spectral library database; NIST 11. The method was referred to [1] with some modifications.

RESULTS AND DISCUSSION

Yield of Extractions

E. coccinea stalks from Ranau and Tambunan were extracted through cold maceration using 10% aqueous methanol, yielding 12.77% and 11.08% respectively. The crude extracts were then submitted to partitions using three different solvents: *n*-hexane, chloroform and methanol. For the Ranau sample, the fractions

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obtained 8.40%, 5.42% and 2.90% for ECSM, ECSC and ECSH respectively. As for Tambunan, the fractions were yielding 3.72%, 4.94% and 1.02%.

Phytochemical screening

The phytochemical classes test such as alkaloids, flavonoids, terpenoids, tannins and saponins performed would facilitate chemical profiling using LC-MS and GC-MS. A qualitative phytochemical screening was performed thrice to observe the changes in the respected fractions. Due to a lack of hexane extracts, screening was limited to methanol and chloroform fractions from Ranau and Tambunan. Referring to Table 1.0 below, terpenoids were present moderately '++' as well as flavonoids from Ranau and Tambunan chloroform fractions. The tannins and flavonoid's methanolic fractions were detected in trace amounts, while alkaloids and saponin were absent. Both locations yielded positive results for flavonoids, tannins, and terpenoids. Yet, alkaloids were not present, as evidenced by the lack of orange-red and cream-coloured precipitates in Dragendorff's and Mayer's assays, respectively. Saponins were likewise absent, with no persistent foam detected after 10 minutes.

Table 1. Phytochemical screening tests

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

Note: Qualitative approximation scale: '+' trace, '++' presence, '-' absence

LC-MS ANALYSIS

Four fractions (ECSM-R, ECSM-T, ECSC-R, and ECSC-T) were analysed using LC-MS, yielding 11 plant metabolites. The majority were discovered in the fractions, but not all metabolites previously reported from the Zingiberaceae family, the *Etlingera* genus, or *E. coccinea* species were detected. Only a few were reported, such as phthalic acid, phytosphingosine and 2-phenylacetamide. Phthalic acid had previously discovered in the rhizomes of *Kaempferia galanga L*. [12, 13]. It is a naturally occurring metabolic byproduct widely employed in industry. It also has been reported to possessed anti-bacterial, anti-fouling and anti-fungal activities [14, 15]. Phytosphingosine was discovered in the rhizomes of *Amomum subulatum*, a Zingiberaceae plant with implications for cell signalling and lipid metabolism [18]. 2-Phenylacetamide was recently discovered in the Zingiberaceae family among *E. elatior* flowers using 80% aqueous ethanol extracts [13]. This compound may play a key role in antibacterial activity [17, 18].

This research also discovered some undocumented chemicals in the *Etlingera* genus or Zingiberaceae family demonstrating the diversity of plant metabolites. Betaine aldehyde, a

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tetraalkylammonium salt present in all plants and humans, is required for amino acid metabolism [19]. The enzyme BADH converts it into glycine betaine, which helps with aroma synthesis, stress response, and antibiotic-free selection in plants. Glycine betaine also helps plants cope with environmental stress [20, 21]. Phloridzin, which is widely present in apple trees, has also been discovered in *Curculigo latifolia* roots, indicating that it has a broader role in plant species [22, 23]. 4-hydroxyhippuric acid was generated from hydroxybenzoic acids, in coffee (*Coffea spp.*), which may influence its flavour and health benefits [24]. Then, 2-phenylbutyric acid in sunflower (*Helianthus annuus*) roots, which linked to plant defence and stress responses, highlighting sunflowers' chemical complexity and recommending additional research into *E. coccinea*'s ecological and physiological effects [25].

Phenylacetic acid is highly linked to polyphenols, as evidenced by research on plant-based supplements that found excellent absorption of various polyphenolic components and metabolites in plasma [26]. Plant products are rich in phytochemicals, with polyphenols being the most prevalent and intensively investigated [26, 27, 28]. A detailed characterisation of 3-methoxytyramine may increase the awareness of brain illnesses associated with aberrant dopamine transmission, such as Parkinson's disease, dyskinesia, and schizophrenia. Additionally, 3-methoxytyramine inhibits catecholaminergic action in the brain [29].

Table 2. Identified compounds in LC-MS analysis of both methanolic and chloroform fractions from Ranau and Tambunan

No.	Identified compound	ECSM		ECSC		Molecular formula	RT (min)	m/z	Calculated mass	Class of compound
		R	T	R	T					
1	Betaine Aldehyde	/	/			$C_5H_{12}NO^+$	0.8	120.10	102.15	Quartenary ammonium ion
2	Phloridzin	/				$C_{21}H_{24}O_{10}$	1.1	275.09	436.40	Phloretin
3	Salicyl amide	/		/	/	$C_9H_{11}NO_2$	15.6	138.06	165.19	Amide
4	4-hydroxyhippuric acid	/		/	/	$C_9H_9NO_4$	15.6	196.00	195.17	Hippuric acid
5	Phthalic acid	/	/	/	/	$C_8H_6O_4$	15.6	167.03	166.13	Benzenedicarboxylic acid
6	3-hydroxy-2-methylbutyric acid	/	/		/	$C_5H_{10}O_3$	19.7	NA	118.13	Butyric acid
7	Phytosphingosine	/	/	/		$C_{18}H_{39}NO_3$	20.8	318.30	317.50	Amino alcohol
8	2-phenylbutyric acid	/	/	/		$C_{10}H_{12}O_2$	26.4	119.08	164.20	Monocarboxylic acid
9	Phenylacetic acid	/	/	/		$C_8H_8O_2$	26.4	137.06	136.15	Monocarboxylic acid
10	2-phenylacetamide	/	/	/		C_8H_9NO	26.4	136.08	135.16	Acetamide
11	3-methoxytyramine		/	/		$C_9H_{13}NO_2$	26.4	151.08	167.20	Amine

GC-MS Analysis

The analysis of ECSH-R and ECSH-T resulting a complex chromatogram with 98 and 75 peaks respectively. Many plant metabolites were discovered using spectrum libraries, demonstrating the chemical diversity of the fractions. Due to a scarcity of research conducted on *E. coccinea* metabolites,

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these compounds were compared with previous studies on the Zingiberaceae family, *Etlingera* genus, and *E. coccinea* species.

The GC-MS study of ECSH-R revealed 98 peaks, and 15 compounds from it which had previously been reported on the Zingiberaceae family, *Etlingera* genus, and *E. coccinea* species. The analysis identified four compounds: cyclopentane, oxirane, dodecane, and heptadecane. Cyclopentane was discovered in cardamom stems [30], oxirane and dodecane in *E. elatior* flowers [1], and heptadecane in *Kaempfria galanga L*. essential oils [31]. All these plants are from the Zingiberaceae family, with *E. coccinea* and *E. elatior* sharing the same genus. Two aldehydes were discovered: decanal, the main component of *E. fimbriobracteata* stem oils [32], and 2-octenal from *E. coccinea* [33]. Three alcohols were also discovered: 1-decanol and 2-cyclohexen-1-ol, which were investigated by [33] and [30] respectively, and 1,2-cyclohexanediol, which was discovered in *Curcuma longa L*., another Zingiberaceae member [34]. Zingiberaceae metabolites discovered six organic acids, including hexadecanoic acid, n-hexadecanoic acid, pentadecanoic acid, and 9,12-octadecadienoic acid [1, 2]. Oxalic acid was discovered in *E. elatior* rhizomes [30] and pentanoic acid in *E. megalocheilos* [35].

The GC-MS study of ECSH-T revealed 75 peaks, 9 compounds of which had previously been reported. Two alkanes, heptadecane [31] and oxirane, were discovered. The aldehyde 2-dodecenal, was found as well in 2017 [8]. Oxalic acid [36] was also discovered. Four compounds—hexadecanoic acid, *n*-hexadecanoic acid, tetradecanoic acid, and 9,12-octadecadienoic acid—have been described in Zingiberaceae investigations, with the latter being discovered [1]. Octadecanoic acid was discovered in *Kaempferia galanga L.* by [23]. Among compounds identified below, there were several compounds that came with benefits. Heptadecane was previously reported to possessed anti-microbial, antioxidant and anti-cancer properties [14]. From a GC-MS analysis of roselle calyx, some of the compounds being discovered were the same as *E. coccinea*'s. It was reported that hexadecenoic acid has anti-inflammatory property and anti-cancer [15]. Hexadecanoic acid previously reported to have anti-androgenic, and hemolytic 5-alpha reductase inhibitor properties. While n-hexadecanoic acid possessed the same properties with addition of antioxidant and hypo-cholesterolemic properties, as well as controlling the swelling in the human body [37]. 9,12-octadecadienoic acid also has the properties mentioned before as well as anti-histamic, insectifuge, anti-eczemic and anti-acne properties. Previously, pentadecanoic acid were reported to have antioxidant properties [14]. Octadecanoic acid has anti-tumor property [15].

Hence, the presence of these classes of compounds in this analysis can be considered as unique to *E. coccinea*. It is an added value to the plant's fraction extracts which some of the metabolites have not yet been reported in any past studies. While some of them were mentioned before, which confirmed the compounds' existence circulated whether in *E. coccinea* or Zingiberaceae family. Plant metabolites that are mapped within the Zingiberaceae family are highly recommended to further structural elucidation in future works. The data shown will work as primary data to pursue elucidation.

Table 3. Identified compounds in GC-MS analysis of hexane fractions from Ranau (R) and Tambunan (T).

No.	Identified Compounds	ECSH-R	ECSH-T	Chemical Formula	RT (min)	Area (%)	Class of Compounds
12	cyclopentane	/		C_5H_{10}	14.841	2.05	Alkane
13	oxirane	/	/	C_2H_4O	18.366	0.04	Alkane
14	dodecane	/		$C_{12}H_{26}$	19.734	0.54	Alkane
15	heptadecane	/	/	$C_{17}H_{36}$	23.636	0.27	Alkane

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16	decanal	/		$C_{10}H_{20}O$	13.72	0.05	Aldehyde
17	2-octenal	/		$C_8H_{14}O$	20.174	0.03	Aldehyde
18	2-dodecenal		/	$C_{12}H_{22}O$	19.625	0.22	Aldehyde
19	1-decanol	/		$C_{10}H_{22}O$	14.538	0.02	Alcohol
20	2-cyclohexen-1-ol	/		C_6H_10O	19.384	1.55	Alcohol
21	1,2-cyclohexanediol	/		$C_6H_{12}O_2$	23.304	0.02	Alcohol
22	hexadecanoic acids	/	/	$C_{16}H_{32}O_2$	27.481	0.01	Carboxylic acid
23	n-hexadecanoic acid	/	/	$C_{16}H_{32}O_2$	28.231	30.47	Carboxylic acid
24	pentadecanoic acid	/		$C_{15}H_{30}O_2$	29.547	0.23	Carboxylic acid
25	9,12-octadecadienoic acid	/	/	$C_{18}H_{32}O_2$	30.966	27.77	Carboxylic acid
26	pentanoic acids	/		$C_5H_{10}O_2$	25.204	0.01	Carboxylic acid
27	oxalic acid	/	/	$C_2H_2O_4$	19.173	0.01	Carboxylic acid
28	tetradecanoic acid		/	$C_{14}H_{28}O_2$	28.253	31.97	Carboxylic acid
29	octadecanoic acid		/	$C_{18}H_{36}O_2$	28.499	2.21	Carboxylic acid

Based on the compounds from Table 2 and Table 3 above, the class was significantly significant with the phytochemical screening results. Most of them were from the classes of terpenoids, followed by flavonoids and tannins. Phloretin, a glucoside derivative from the subclasses of chalcone, was mapped to the positive results of flavonoids existence. As chalcone itself is the derivatives of flavonoids [22]. In addition, phloretin itself inhibits pathogenicity and virulence factors [38]. Hippuric acid is a glycine-conjugated metabolite of polyphenolic compounds. Polyphenol refers to a variety of chemical structures, including flavonoids and related substances, as well as hydroxycinnamates and phenolic acids with a single phenolic ring [26]. Many alkanes were classified as monoterpene compounds [30]. Flavonoids, tannins, saponins, alkaloids, and terpenoids are phytochemicals having antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anti-immunomodulatory properties [15]. Phenolic compounds contribute to antioxidant action by arranging functional groups (hydroxyl) around their nuclear structure for hydrogen donation, which helps to stabilise radical molecules [39].

The presence of tannins has the potential for medical uses, particularly in anticancer treatments, due to their high antioxidant content [40]. Therefore, it can be said that the results observed through phytochemical screening were confirmed from the classes of identified compounds. This aligns with the traditional usage by the indigenous people who often treats as a curing food poisoning, stomach-aches, and gastric problems remedies. Fig. 1 and 2 below shows the structure of identified compounds through LC-MS and GC-MS analysis. These structures obtained were referred to and confirmed through the National Centre for Biotechnology Information.

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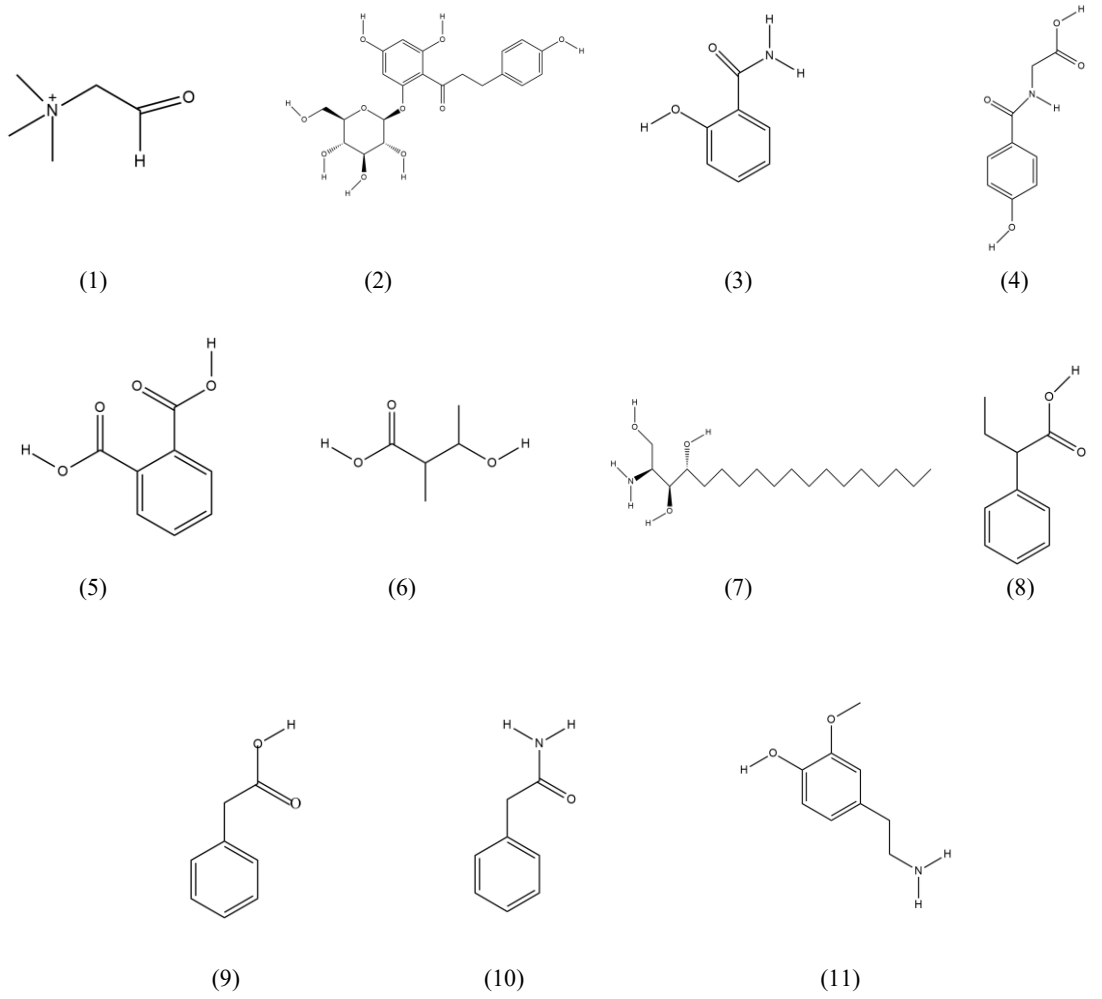

Figure 2. Chemical structures detected in LC-MS library (1-11).

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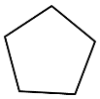

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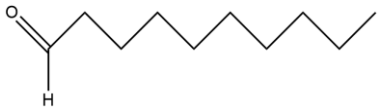

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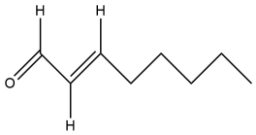

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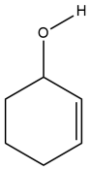

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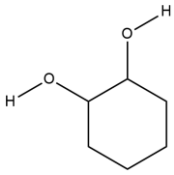

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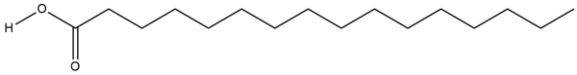

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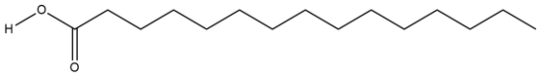

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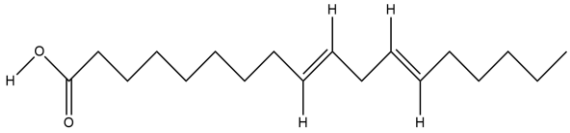

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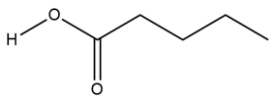

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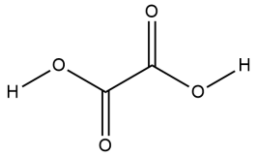

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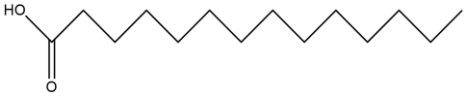

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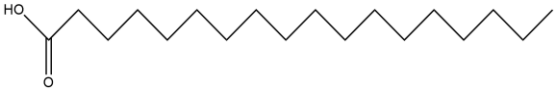

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Figure 3. Chemical structures detected in GC-MS library (12-29).

CONCLUSION

The phytochemical profile of *E. coccinea* suggests its potential for further investigation. The phytochemical screening mapped with the previous studies of *Etlingera* genus. Which they were rich in flavonoids, terpenoids found as well as tannins. A detailed analysis of major compounds using LC-MS and GC-MS analysis, there were 30 and 24 known compounds detected respectively. Part of them had been reported in their family and genus, and there were also compounds that have not been reported by researchers. Since, the study of *E. coccinea* is yet to be discovered more, the findings would serve as a basis for further explorations. It is highly recommended to perform specific analysis such Ultra High Performances Liquid Chromatography (UHPLC) and tandem mass spectrometry (MS/MS) to perform metabolomic fingerprinting for targeted metabolite quantification. It is also recommended that to do biological assay such as antimicrobial, antioxidant, cytotoxic activity, anti-inflammatory, anti-viral, or anti-cancers. Other than that, to do comparative profiling of other plant parts such as rhizomes and leaves for a comprehensive phytochemical understanding.

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AUTHOR'S CONTRIBUTION

Nurfateen Helmy Abdellah Nazeer carried out the research, analyzed the data, wrote and revised the article. Julenah Ag Nuddin conceptualized the central research idea, provided the theoretical framework and supervised the research progress assisted by Fatimah Salim.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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