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Research Article

A Preliminary Survey and Chemical Profiling of Wild Ginger Species in Kadamaian, Kota Belud, Sabah

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Abstract
A preliminary survey of the diversity of gingers (Zingiberaceae) was conducted in Kadamaian, Kota Belud from 14th to 19th October, 2019. Wild ginger species is utilized widely as one of the most important material in traditional medicine among indigenous people of Sabah. However, few of these plant species have been studied for their chemical constituents and beneficial properties. In order to investigate the compound composition, the essential oil from *Etlingera brevilabrum*, *Alpinia nieuwenhuizii* and *Hornstedtia havilandii* were screened. The essential oil was obtained from leaves, stems and rhizomes of the plant through hydro-distillation and analysed for their chemical composition through Gas Chromatography-Mass Spectrometry (GC-MS). The result of this study indicated that the chemical constituents of all three parts for all species are similar; all have terpenoids (monoterpene and sesquiterpene), aldehyde, hydrocarbon, ketone and alcohol in the essential oil extracts. GC-MS analyses of the oils led to the identification of 35 compound constituents from the leaves, stems and rhizomes of *E. brevilabrum*, which is the highest. Meanwhile, *A. nieuwenhuizii* displayed 34 chemical compositions from all parts (leaf, stem and rhizome) of the plant. *H. havilandii* showed the lowest number of volatiles from all plant parts (24 compounds). Monoterpene is dominant in all wild ginger studied, except for rhizome of *E. brevilabrum*. On the contrary, *E. brevilabrum* showed sesquiterpene as the most abundant compound in its composition. This shows that the volatile oil composition of wild ginger species is extremely variable. This study provides preliminary key chemical information for evaluating the quality of local wild gingers in Kadamaian, Kota Belud, Sabah.

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Introduction

The ginger family, Zingiberaceae, is native to tropical and subtropical Southeast Asia and mainly distributed in Asia. The family consists of pseudo-stem and tuberous rhizomes, in which the latter are also known as ginger root or commonly known as ginger. Due to its strong odour and pungent taste, the most common custom usage of ginger is as a flavouring agent, ingredient for culinary and as traditional medicine (Mahomoodally et al., 2019). Zingiberaceae consists of 50 genera with approximately over 1,600 species (Christenhusz & Byng, 2016). Within the species itself, there are at least 172 species recorded from the Indo-Malayan region, with 80% found in Borneo (Poulsen, 2006).

As a natural product, ginger is a complex spice composed of standard food nutrients and volatile essential oils. Several terpene components that made up the compositions are bisabolene, farnesene, curcumene and quiphellandrene (Mahomoodally et al., 2019). The presence of high quality essential oils in many wild ginger species attributed its potential to pharmaceutical and nutraceutical values (Liu et al., 2012).

Due to the potential of wild gingers as an alternative source for health-related treatment, further scientific study has sought to reveal the chemical composition from different parts of ginger such as leaf, stems and rhizomes. Vairappan et al. (2012) has studied the chemical composition from the rhizomes of *Etlingera* species namely *E. pyramidosphaera*, *E. megalocheilos*and *E. brevilabrum* from Kimanis, Sabah, *E. coccinea* from Ranau, Sabah and *E. elatior* from Tambunan, Sabah. Four of the species, except for *E. pyramidosphaera* contained high concentrations of hydrocarbon sesquiterpenes that ranged from 21.4% to 50.0%. Oxygenated sesquiterpene contents ranged from 3.0% to 28.6% in *E. megalochelos, E. pyramidosphaera* and *E. coccinea*, while diterpene hydrocarbon were only detected in *E. elatior* (12.5%). Mahdavi et al. (2017) extracted essential oil through hydrodistillation from *Etlingera sayapensis* (leaf, stem and rhizome) collected from Kipandi, Sabah. The leaves exhibited high content of carveol (21.38%), the rhizome showed high linalool formate content (25.47%), while the stem was dominated by α-terpineol (39.86%). The essential oils of rhizomes from *etlingera* sp. were further studied by Nagapoan et al. (2017), and these include *E. pyramidosphaera*, *E. megalocheilos*, *E. coccinea* and *E. elatior* from Ranau, Sabah. A total of 39 volatile chemicals were detected from the said species consisting mixtures of oxygenated monoterpenes, sesquiterpenes, oxygenated diterpenes and diterpenes. Although all rhizomes revealed terpenoids as major compounds, only *E. coccinea* and *E. elatior* showed terpenoid at the highest abundance, with borneol from *E. coccinea* at 28.2% abundance and aromadendrene oxide from *E. elatior* at 46.2% abundance. Owing to the wide range of beneficial effects of this herb, studies are necessary to investigate the variation in the production of phytochemicals throughout the plant organs. It is important to gather relevant evidence regarding herbs with high level of potentially beneficial components (Ghasemzadeh et al., 2016). At the time when the present survey was conducted, there was no information available on ginger species from Kadamaian. Therefore, this study is important in providing valuable baseline data for conservation purposes through inventory, and information on the chemical properties of essential oils from different plant tissues of selected wild gingers through Gas Chromatography-Mass Spectrometry (GC-MS) technique.

Materials and Methods

Sampling and Study Area

Twelve species of wild gingers from six different trails/site (Malangkap Noriou (a), Gensurai (b), Wasai waterfall (c), Basecamp (d), Ulu Malawa (e) and Pinolobu (not shown)) were collected in Kadamaian, Kota Belud, Sabah from 14th to 19th October, 2019 (Figure 1). The plants (with or without floral structures) were then brought back to Universiti Malaysia Sabah (UMS) for plant material preparation. The plants were preserved using a standard herbarium method.

Plant materials

Three selected fresh samples of *Etlingera brevilabrum*, *Alpinia nieuwenhuizii* and *Hornstedtia havilandii* were collected from three different trails in Kadamaian, Kota Belud, Sabah from 14th to 19th October, 2019. Samples were brought back to ITBC, cut separately (leaves, stems and rhizomes) and stored in -80°C prior to further use. The samples were authenticated by Mr. Johny Gisil during sampling collection. However, no voucher specimens were deposited due to limited availability of samples during collection.

Extraction of essential oil from plant

Briefly, about 250-300g of fresh leaves and stems were chopped into small pieces and crushed using a blender to increase the surface area. Then, 400mL of distilled water was added and placed into a 500-1000mL round flask. The 10mL of 99% (v/v) n-pentane (BDH, Germany) were added to trap the condensed oil, through the top of the condenser into the glass round flask containing samples of leaves and stems. Hydro-distillation of each sample was carried out in a

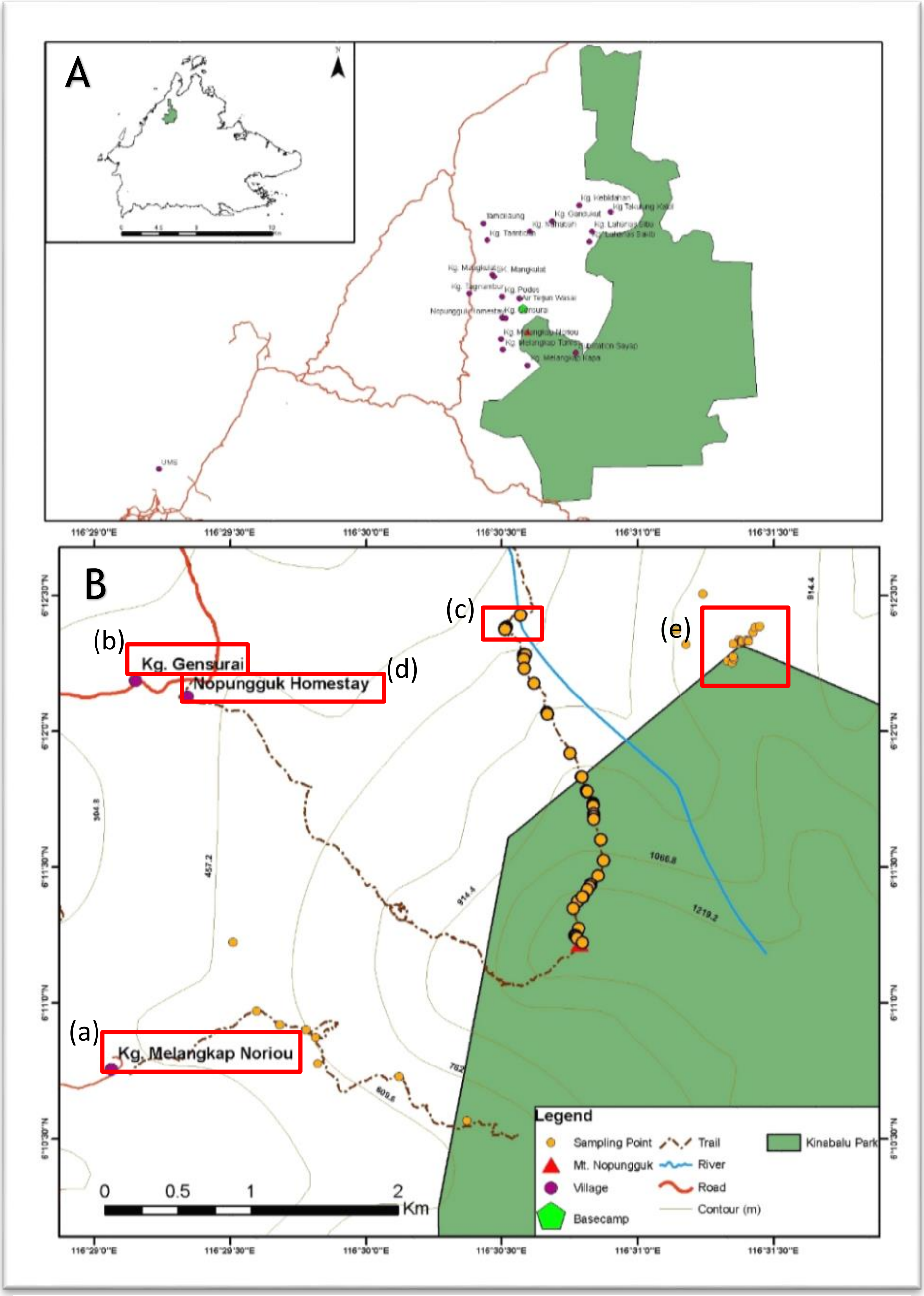

Figure 1. The location of the study area in Kadamaian, Kota Belud District in Sabah (A) and locations of the study plots (B). shown in the map are sampling sites/trails of Malangkap Noriou (a), Gensurai (b), Wasai waterfall (c), Basecamp (d) and Ulu Malawa (e).

modified Clevenger apparatus separately with a water-cooled oil receiver to reduce the potential of hydro-distillation over-heating artifacts. The extraction was carried out at 100ºC for 6-7 hours. The light-yellow colour with pleasant aroma was obtained, then separated and dried over with 10g of anhydrous sodium sulphate (Na_2SO_4) overnight, and then filtered. Finally, the essential oil was concentrated by blowing with pure nitrogen gas. The oils were then stored in sealed vials at 4ºC prior to further use (GC-MS analysis). The yields were calculated based on dry weight of plant materials (Tajidin et al., 2012).

Identification of volatile composition of essential oils

The GC-MS analysis was carried out using Shimadzu QP-2010 gas chromatograph equipped with SH-Stabil wax-DA capillary column (30m x 0.25mm x 0.25µm) coupled with mass chromatography (MS) detector. The initial oven temperature was programmed from 60°C to 250°C at a rate of 5°C/min and was held at 250°C for 5 min. The injections of an ion source were adjusted to 270°C and 280°C, respectively. Helium was used as a carrier gas at 1mL/min. The detector interface temperature was set to 280°C, with the actual temperature in the MS source reaching approximately 230°C, and the ionization energy was 70eV. A 1 µL volume of the essential oil extract mixed with hexane at 1:1 ratio was injected in spitless mode. Acquisition mass range was set to 39-600 amu. Total volatile production was estimated by summing all the GC peak areas in the chromatogram and individual compounds were quantified as relative percent area. Chemical composition was identified by comparing the mass spectra of the samples with the data system (NIST 08 and Flavor and Fragrance 2.0).

Results and Discussions

The diversity of wild gingers

A total of 12 Zingerberaceae species were collected and identified up to species level (Table 1). The survey revealed that there are at least 12 species from six genus representing three tribes. The species commonly believed to be indicators of disturbed forests such as *Etlingera coccinea* were the most abundant, implying that the trails surveyed had been disturbed (Larsen et al., 1999). According to Larsen et al. (1999) some species of *Etlingera* rapidly inhabit disturbed secondary forest or newly opened areas and subsequently spread like weed. From the 12 species documented in this report, most of them have high potential to be developed into ornamental plants such as in the genera of *Boesenbergia* and *Ammomum* (Lamb et al., 2013).

Table 1. A checklist of wild gingers collected from Kadamaian, Kota Belud.

Site	Tribe	Genera	Species name	GPS	Elevation (m)
Malangkap Noriou	Alpiniae	*Honstedtia*	*havilanddi*	N: 06 10.696; E: 116 29.899	578
Gansurai	Zingiberaceae	*Zingiber*	N/A	N: 06 11.231; E: 116 30.332	901
	Alpinioideae	*Alpinia*	*nieuwenhuizii*	N: 06 11.479; E: 116 29.975	767
	Alpiniae	*Etlingera*	*probescence*	N: 06 11.348; E: 116 30.137	682
	Zingiberaceae	*Zingiber*	N/A	N: 06 11.348; E: 116 30.137	682
Wasai Waterfall	Alpiniae	*Etlingera*	*brevilabrum*	N: 06 11.319; E: 116 30.147	290
Basecamp	Alpiniae	*Etlingera*	N/A	N: 06 15.217; E: 116 30.382	245
Pinolobu	Alpiniae	*Etlingera*	N/A	N: 06 15.238; E: 116 30 314	207
	Alpiniae	*Etlingera*	N/A	N: 06 15. 341; E: 116 30 169	163
Ulu Maluwa	Globbeae	*Globba*	N/A	N: 06 12. 164; E: 116 31.212	933
	Alpiniae	*Ammomum*	*kinabaluensis*	N: 06 12.159; E: 116 81.213	934
	Zingibereae	*Boesenbergia*	N/A	N: 06 12. 192; E: 116 31.211	982

Chemical composition of selected wild gingers

Of the 12 species of gingers reported in this study, not much work has been done to explore their bioactive and antioxidant potential. Due to limited number of samples, only three selected gingers were submitted to chemical profiling study. The identification of *Hornstedtia havilandii* (Figure 2a), *Alpinia nieuwenhuizii* (Figure 2b) and *Etlingera brevilabrum* (Figure 2c) were based on their morphology during sample collection. In the present study, the GC-MS analysis of the essential oil extracts from leaves, stems and rhizomes of selected wild gingers resulted in the identification of 52 chemical compounds of which a majority belonged to the terpenoid group (Table 2). These included monoterpenes such as α-pinene, α-myrcene, borneol, camphene and camphor, and sesquiterpenes such as α-caryophyllene, alloaromadendrene, copaene, cubenene and nerolidol. The essential oil also showed the presence of other volatile groups such as phenylpropanoids methyleugenol and asarone, and fatty acid derivative, benzeneacetyldehyde. In terms of yield, the leaf of *Alpinia nieuwenhuizii* contained the highest oil yield (0.21%), followed by the rhizome of *A. nieuwenhuizii* (0.19%) and rhizome of *Hornstedtia havilandii* (0.18%). Synthesis and accumulation of essential oils can accumulate in all plant organs, but in varying amounts. This is contributed to the distribution secretory structures such as glandular trichomes outside the plant and secretory cells and intercellular spaces inside the plant (Sarac & Butnariu, 2018). The findings from Dodoš et al. (2021) revealed variation of essential oil accumulation across plant organs (leaf, calyx, corolla and herba) of *Satureja* montana, *S. subspicata* and *S. Kitaibelii* contributed by peltate and capitate glandular trichomes.

Figure. 2 Selected wild ginger species that were used in this study. (a) *Etlingera brevilabrum*; (b) *Alpinia nieuwenhuizii*; (c) *Hornstedtia havilandii*

GC-MS analyses of the oils extracted from the stem, leaves and rhizomes combined of *Etlingera brevilabrum* resulted in the identification of 35 different chemical constituents, the highest compared to *A. nieuwenhuizii*. and *H. havilandii*. The stem of *E. brevilabrum* recorded the highest number of chemical constituents (25 compounds), whereas, the leaves and rhizomes comprised the same number of chemical compositions (18 compounds). On the other hand, *A. nieuwenhuizii* exhibited a total of 34 compound constituents, in which the rhizome displayed the highest number of chemical compositions (21 compounds), followed by its leaf (17 compounds) and stem (15 compounds). *H. havilandii* and *A. nieuwenhuizii* leaves showed similar number of volatile constituents, while displaying as the highest among all parts of *H. havilandii*. The rhizome of *H. havilandii* contained 13 compounds, whereas the stem of *H. havilandii* displayed the lowest number of volatiles (10 compounds).

Monoterpenes dominated all plant parts of *H. havilandii*, *A. nieuwenhuizii*, and *E*. brevilabrum, except for the rhizome of *E. brevilabrum* which exhibited sesquiterpenes as the major compounds (Figure 3). This was followed by alcohol, aldehyde, hydrocarbons and ketones. The stem and leaf of *E. brevilabrum* revealed eucalyptol as the most abundant chemical compound (18.59% and 16.63%, respectively). Meanwhile, the rhizome of *E. brevilabrum*, leaves of *A. nieuwenhuizii* and *H. havilandii* recorded sesquiterpene caryophyllene as the compound with highest abundance at 16.23%, 34.53% and 22.54% respectively. On the other hand, the rhizome of *A. nieuwenhuizii* and stem of *H. havilandii* showed (-)-ß-pinene as their highest abundant volatiles, at 28.07% and 46.68%.

Cis-sabinene is recorded at highest abundance (28.81%) in the stem of A. *nieuwenhuizii*. Finally, the rhizome of *H. havilanddi* recorded sabinene as the most abundant constituent at 46.30%. Apart from monoterpenes and sesquiterpenes, other notable volatiles were also detected, such as phenylpropanoid methyleugenol in the stems and leaf of *E. brevilabrum* at 4.83% and 1.07%, and benzeneacetyldehyde in the leaf of *A. nieuwenhuizii* (0.04%).

Similar variations of chemical constituents across plant organs were also observed. Feng et al. (2021) observed the accumulation of terpenoids as the major compound in stems, leaves, flowers and fruits of *Alpinia zerumbet*. While monoterpene eucalyptol dominated the constituents in leaves, stems and fruits, camphor exist as the most abundant compound in the flowers. On the other hand, Jusoh et al. (2020) reported the constituents of essential oils extracted from the leaf and pseudo-stems of *Alpinia malaccensis*. As the leaf revealed ß-pinene, 1,8-cineol, trans-caryophyllene and α-pinene as the major compounds, while 1,8-cineol, ß-pinene, α-terpineol, trans-caryophyllene and α-terpinolene as the major components in the pseudo stems. Ramos et al. (2020) suggested that the variations in volatile compositions and abundance were affected by the structure development of secretory structures within the plant organs and the gene expression profile across plant organs and species for the particular compound synthesis.

Many of the chemical compounds detected in wild gingers of this study were observed to exhibit various pharmaceutical potential. The high abundance of sabinene produced from *H. havilandii* was reported to exhibit anti-fungal and anti-inflammatory properties (Cao et al., 2017). The monoterpene eucalyptol was also known to display anti-inflammatory, anti-nociceptive and reduce the interferon-gamma levels in mice (Júnior et al., 2017). Besides, α-pinene that can be found in all three wild ginger species in this research were also known to exhibit neuroprotective effect by attenuating neuroinflammation and inhibit apoptosis (Khoshnazar et al., 2020).

Table 2. Volatile profiling and percentage yield of stems, leaves and rhizomes of three selected wild gingers species by using Gas Chromatography-Mass Spectrometry (GC-MS).

No	Compound Name[a]	Compound group	*Etlingera brevilabrum*(%)[b]			*Alpinia nieuwenhuizii* (%)[b]			*Hornstedtia havilanddi* (%)[b]		
			leaf	stem	rhizome	leaf	stem	rhizome	leaf	stem	rhizome
1	δ-Cadinene	monoterpene	7.26	3.53	-	-	-	-	9.00	0.41	-
2	(-)-borneol	monoterpene	1.32	-	-	-	-	-	-	-	-
3	(-)-linalool	monoterpene	5.41	-	-	-	-	-	-	-	-
4	(-)-β-pinene	monoterpene	-	-	-	18.10	-	28.07	12.35	46.48	-
5	α-Caryophyllene	sesquiterpene	6.15	2.47	4.34	9.23	-	0.28	3.32	-	-
6	α-Phellandrene	monoterpene	0.91	-	-	-	-	-	0.59	-	-
7	α-Pinene	monoterpene	9.25	12.89	13.19	18.38	5.10	7.75	7.14	29.81	28.02
8	α-Myrcene	monoterpene	4.50	-	1.01	-	-	-	-	-	-
9	1-Decanol	alcohol	-	3.27	0.75	-	-	9.11	-	1.09	0.05
10	1-Hexanol	alcohol	-	-	-	0.10	-	-	-	-	-
11	1-Nonanol	alcohol	-	-	-	-	0.23	0.10	-	-	-
12	1-Octanol	alcohol	-	-	-	0.02	-	-	-	-	-
13	1-Undecanol	alcohol	-	-	-	0.13	-	-	-	-	-
14	2-Butanone	ketone	-	-	-	-	-	0.05	-	-	-
15	2-Nonanone	ketone	-	-	0.20	-	-	-	-	-	0.26
16	Alloaromadendrene	sesquiterpene	-	-	-	0.30	-	-	-	-	-
17	Asarone	Phenylpropanoid	-	7.10	15.03	-	22.02	-	7.53	-	-
18	Benzeneacetaldehyde	Fatty acid derivatives	-	-	-	0.04	-	-	-	-	-

(Continued on next page)

Table 2. (Continued)

19	Borneol	monoterpene	-	1.92	-	-	1.15	2.94	0.99	0.41	0.80
20	Camphene	monoterpene	10.76	7.61	14.06	0.38	3.71	3.65	8.06	-	4.45
21	Camphor	monoterpene	8.18	5.52	8.63	-	3.16	1.05	4.06	-	0.35
22	Caryophyllene	sesquiterpene	1.13	2.15	16.23	34.53	11.71	2.49	22.54	-	-
23	Caryophyllene oxide	Sesquiterpene	6.07	-	9.12	2.44	7.39	14.38	1.88	-	-
24	cis-sabinene	monoterpene	-	-	-	-	28.81	0.28	-	-	-
25	Copaene	sesquiterpene	5.60	1.14	2.12	-	-	1.25	1.95	-	3.45
26	Cubenene	sesquiterpene	-	-	0.85	-	-	-	-	-	-
27	Decanal	aldehyde	-	-	1.00	-	-	18.24	-	-	-
28	Eucalyptol	monoterpene	16.63	18.59	-	-	6.69	-	-	-	-
29	Fenchol	monoterpene	0.26	0.30	0.24	1.20	0.54	0.39	-	-	-
30	Fenchone	monoterpene	10.99	3.54	-	-	-	-	9.87	-	-
31	Gurjunene	sesquiterpene	-	-	-	-	1.56	-	-	-	-
32	Hexanal	aldehyde	0.26	0.10	-	0.79	-	-	0.21	-	0.12
33	L-4-terpineol	monoterpene	-	-	-	-	-	-	-	-	-
34	Limonene	monoterpene	-	3.96	-	6.64	2.01	2.20	-	4.88	5.15
35	Linalool	monoterpene	-	3.39	1.13	-	-	0.73	-	-	-
36	Linalyl alcohol	monoterpene	-	-	-	-	1.26	-	-	-	-
37	Methyleugenol	phenylpropanoid	4.83	1.07	-	-	-	-	-	-	-
38	Nerolidol	sesquiterpene	-	-	-	-	-	-	-	12.05	6.10
39	Nonane	hdrocarbon	-	-	-	-	-	0.12	-	-	-
40	Nopinone	monoterpene	-	0.19	-	0.86	-	-	-	-	-
41	Sabinene	monoterpene	-	-	-	-	-	-	10.17	-	46.30
42	Terpineol	monoterpene	-	-	-	-	4.66	-	-	-	-
43	Terpinolene	monoterpene	0.50	0.12	-	-	-	-	0.23	-	-

(Continued on next page)

Table 2. Continued

44	Trans-bornyl acetate	monoterpene	-	-	-	-	-	0.28	-	-	-
45	Tricyclene	monoterpene	-	0.13	0.42	-	-	-	0.11	0.34	-
46	Verbenone	monoterpene	-	0.18	-	-	-	-	-	-	-
47	α-gurjunene	sesquiterpene	-	-	11.16	-	-	4.78	-	-	0.85
48	α-sabinene	monoterpene	-	-	0.53	-	-	-	-	-	-
49	α-terpinene	monoterpene	-	0.10	-	0.75	-	-	-	0.41	-
50	α-terpineol	monoterpene	-	1.26	-	6.13	-	1.86	-	4.12	4.09
51	β-myrcene	monoterpene	-	1.07	-	-	-	-	-	-	-
52	β-pinene	monoterpene	-	18.41	-	-	-	-	-	-	-
	Terpene compound abundance (%)	Total monoterpene	68.70	79.17	39.21	52.43	57.09	76.65	53.57	86.45	89.43
		Total sesquiterpene	26.21	9.29	58.85	46.49	42.68	23.18	46.22	12.46	10.40
		Total terpenoid	99.74	88.46	98.05	98.93	99.77	99.83	99.79	98.91	99.83
		Total abundance	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
		Yield (%)	0.17	0.06	0.14	0.21	0.10	0.19	0.13	0.06	0.18

[a]*Volatile compounds were listed in elution time order from capillary VF-WAXms column*
[b]*Note*: (%) - Relative abundance

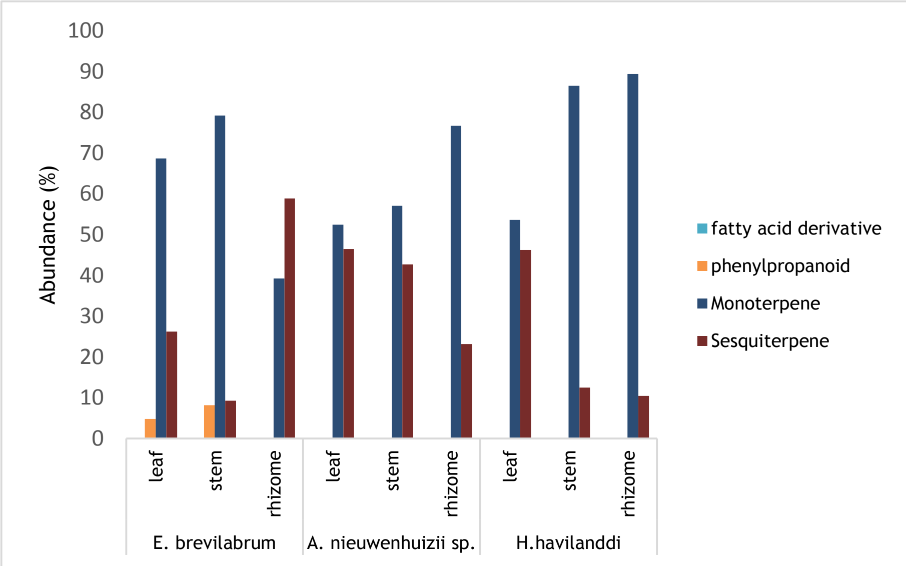

Figure. 3. Relative abundance of three major groups of volatiles (Terpenoids monoterpene and sesquiterpene, phenylpropanoid and fatty acid derivatives).

Conclusion

The chemical profile from the leaf, stem and rhizomes of selected wild gingers exhibited dissimilar volatile profile between and even within the species. There are still many wild ginger species in the forests of Sabah that require attention, waiting to be discovered and documented. It is of utmost importance to conserve Sabah's forests not only to protect the habitat, but also to maintain the existence of this plant family that serves as a potential reservoir for development into a variety of pharmaceutical and nutraceutical products.

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