

Short Communications

Essential Oils Composition of Kaffir Lime (*Citrus hystrix* DC.) Collection of Bogor Botanic Gardens from Central Java and East Sumba

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ABSTRACT

The Bogor Botanical Gardens' *Citrus hystrix* collections from East Sumba and Central Java differ in morphology and fruit aroma compared to the common *C. hystrix*. Hence, this study aimed to determine the essential oils' compositions of *C. hystrix* originated from Central Java and East Sumba to further clarify these differences. Extraction of essential oils were done using hydro-distillation, and the chemical compositions were investigated using GC-MS. The main compound of the leaf oil from East Sumba and Central Java was Linalool and Citronellal, respectively. Meanwhile, the main constituents were almost identical for the fruit oils, namely L- β -Pinene, D-Limonene, and L- α -Terpineol.

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Citrus hystrix DC. (kaffir lime) or "jeruk purut" in Indonesia, "limau purut" in Malaysia, and "som makrut" in Thailand, belongs to the Rutaceae family, which is cultivated mainly in Asian countries (Omar 1999; Ngan et al. 2019). This plant has been widely known and used by the community as a flavoring agent and to cure various diseases (Mustafa 2015). According to previous studies, *C. hystrix* has antioxidant, antibacterial, antimicrobial activity (Warsito et al. 2017; Ngan et al. 2019; Tunjung et al. 2018), anticancer (Ampasavate 2010; Tunjung et al. 2014), and antiinflammatory effects (Kidarn et al. 2018). Furthermore, this plant is also used as a natural insecticide (botanical insecticide) (Ikawati et al. 2017; Wikandari & Surati 2018) and also as antidandruff (Tanzil et al. 2017).

The Bogor Botanical Gardens (BBG) has several *C. hystrix* that originated from Central Java and East Sumba. Astuti (2011) stated that both *C. hystrix* plants in the BBG collection need to be restudied due to the differences in morphology and also in aromas produced by the fruits of these BBG collections in comparison to the common *C. hystrix*. Compared to com-

mon *C. hystrix*, *C. hystrix* fruits from Central Java are more oval, smaller in size, and have smoother peels. On the other hand, *C. hystrix* from East Sumba has thorns that are stronger and longer than the thorns of the common *C. hystrix* and also the *C. Hystrix* from Central Java (Astuti 2011). Common *C. hystrix* is kaffir lime which is commonly known, used, and cultivated by people in Asian countries. Generally, the fruit of *C. hystrix* are globose, ovoid to elliptic, green and turns yellowish-green when ripe, 5-7 cm in diameter, the pulp is yellowish, the rind is thick, and taste very acid and bitter (Lim 2012).

According to Astuti and Ajiningrum's (2019) observations on the leaves, fruits, flowers, and seed morphological characters, *C. hystrix* from Central Java differed from *C. hystrix* from East Sumba and common *C. hystrix*. However, the results from anatomical structure observations of leaves and petioles of *C. hystrix* from Central Java, common *C. hystrix*, and *C. hystrix* from East Sumba showed a quantitative similarity. Therefore, Astuti and Ajiningrum (2019) proposed that *C. hystrix* from Central Java and *C. hystrix* from East Sumba are only varieties from common *C. hystrix*. However, the study of the morphology and anatomy cannot be used to reveal the species diversity of *C. hystrix* from Central Java and East Sumba. Further study is still needed as supporting data. One of them is the study of the essential oil composition of each *C. hystrix*, considering that *C. hystrix* is a member of the *Citrus* genus that contains many essential oils (Mustafa 2015; Ngan et al. 2019).

Citrus plants are one of the primary essential oil sources (Mustafa 2015). Research Amaral et al. (2012) stated that the essential oil of *Neomirantes obscura* fruit with different fruit morphology produced various types of compounds. Ebrahimi et al. (2010) stated that the difference in accessions of *Coriandrum sativum* indicated varieties of the essential oil. Moreover, both *C. hystrix* collections from BBG have differences in the aroma of their fruits compared to the *C. hystrix* commonly known by the public. Based on Omar (1999), each volatile compound has a distinctive aroma. Therefore, the different scents in *C. hystrix* collections of BBG can be distinguished by differences in the essential oil composition. The characterization of essential oil composition of each *C. hystrix* provides information not only about the metabolism-related research but also for chemotaxonomy and aromatic compound diversity (Baccati et al. 2021). Hence, this study aimed to determine the composition of the essential oils of *C. hystrix* leaf and fruit from Central Java and East Sumba collection of BBG.

The leaves and fruits of *C. hystrix* from Central Java and East Sumba used in this study were gathered from the BBG collections. *C. hystrix* from Central Java was planted in area XXIV.A.49 in 1975 (46 years) and *C. hystrix* from East Sumba was planted in area XXIV.A.183 in 2002 (19 years). Both plants are planted in the same area with a spacing of about 5 meters without any other plant barriers (Figure 1). Fresh leaves and fruits with the same level

of maturity were collected from the plants, and the freshness was maintained for the essential oil isolation process.



Figure 1. *Citrus hystrix* (A) Central Java (B) East Sumba.

The collected leaves and fruits were cleaned from visible dust and other contaminants before extraction. Essential oils were isolated from 300 grams of clean, fresh leaves and 500 grams of whole fruits. Extraction was done using the hydro-distillation method using laboratory-scale hydro-distillation apparatus (Sibata, Japan) for approximately 5 hours for each sample. In the distillation result, two phases were observed, namely the aqueous phase and the organic phase or the essential oil phase. The essential oil was then separated from the aqueous phase, and the collected essential oil was then dried further using NaSO_4 anhydrite. The essential oil was stored at 4°C in sealed vials until further analysis.

The chemical compositions of essential oils were evaluated using gas chromatography coupled with mass spectrometry (GS-MS) (Shimadzu GCMS-QP Ultra, Japan). The GC-MS analysis was performed using the Rtx-5MS column (30 m x 0.25 mm) from Restek, US. Ultrahigh purity helium was used as carrier gas with the pressure set at 30.6 KPa. The injector and interface temperature were set at 150°C and 230°C , respectively, while the split ratio was programmed at 1:75. The column temperature was programmed at 35°C for 1 minute, then raised to 200°C at 10°C per minute, and maintained at 200°C for 10 minutes.

The identification of the different compounds was defined by comparing the mass spectra of the compounds within the sample with the data in the NIST library. The results of leaf and fruit essential oils composition analysis of *C. hystrix* from East Sumba and Central Java using GC-MS

indicated a variation of detected volatile compounds. Figure 2 and Table 1 provide the results for the volatile composition of the two leaf EOs from BBG as well as from a reference by Warsito et al. (2017) that used common kaffir lime leaves and fruit from East Java using the same extraction method as this study. A total of 26 compounds was identified in the leaf EO of *C. hystrix* from East Sumba whereas, 21 compounds were identified in the leaf EO of *C. hystrix* from Central Java. The major component in the leaf EO from East Sumba was Linalool, with a high percentage reaching up to 86.06%. In comparison, the primary compound in the leaf EO from Central Java and in the common *C. hystrix* was Citronellal (56.99% and 85.07%, respectively). Linalool belongs to the monoterpene group which is known to have biological activities such as antimicrobial, anti-inflammatory, anticancer, antioxidant properties, and several in vivo studies. Linalool also has a role as a key compound for the industrial production of fragrance chemicals and also as a lead compound in the synthesis of vitamins A and E (Kamatou & Viljoen 2008).

At the same time, Citronellal is a monoterpene present in the oil of several plants, such as *Cymbopogon winterianus* Jowitt (Poaceae) (Java citronella) and *C. citrates* (Lemongrass). Moreover, it has biological properties including antinociceptive and antiinflammatory effects (De Santana et al. 2013; Sudiarmanto et al. 2017). Besides Citronellal, Citronellol was also detected in the leaf EO from Central Java with a quite high amount (11.66%). In contrast, in the leaf EOs from East Sumba and the reference, this compound was not detected.

The observation by Omar (1999) revealed that *C. hystrix* leaf oil is dominated by Citronellal and Citronellol. Based on Loh et al. (2011), kaffir lime leaf oil from Selangor, Malaysia was dominated with β -Citronellal (66.85%), followed by β -Citronellol (6.59%) and Linalool(3.90%). In addition, differences were also exhibited from the minor compounds detected

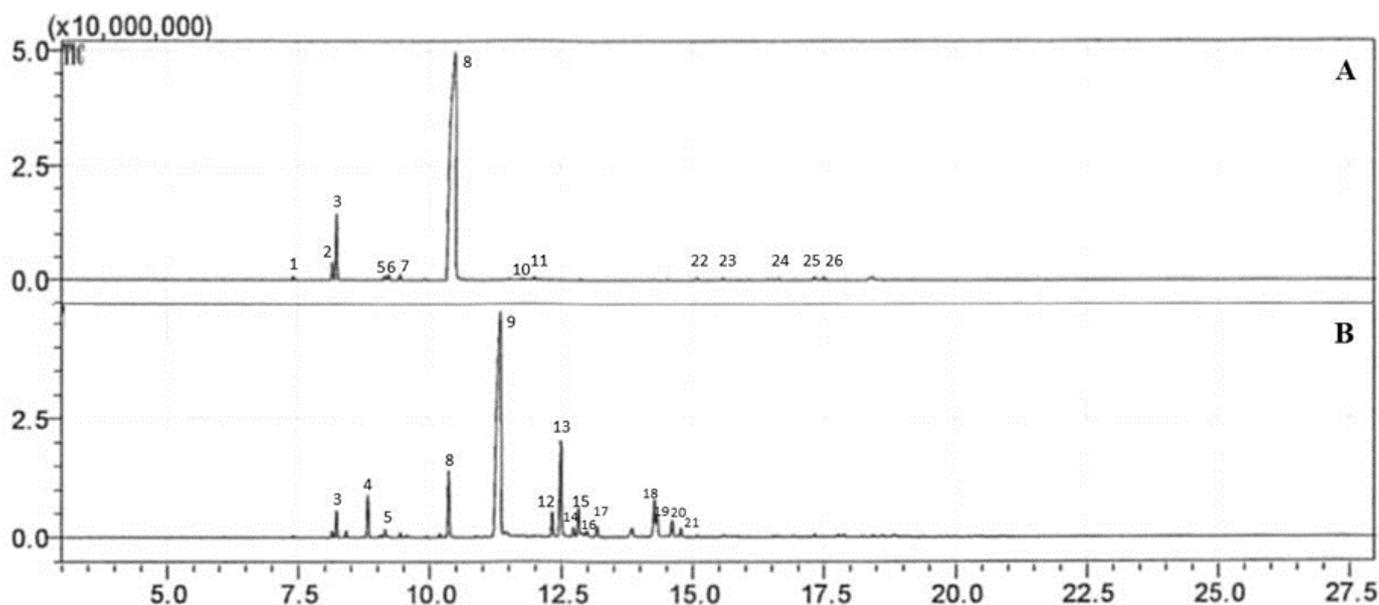


Figure 2. Gas chromatography chromatogram of *C. hystrix* leaf essential oils. (A) East Sumba, (B) Central Java.

Table 1. Composition of *C. hystrix* leaf essential oils from East Sumba and Central Java.

No.	Compound		Area component (%)		
			East Sumba	Central Java	Common*
1	α -Pinene	Monoterpene hydrocarbon	0.38	-	-
2	α -Sabinene	Monoterpene hydrocarbon	1.79	-	2,79
3	β -Pinene	Monoterpene hydrocarbon	7.02	2.44	0,33
4	3-Carene	Monoterpene hydrocarbon	-	3.98	-
5	D-limonene	Monoterpene hydrocarbon	0.58	0.79	0,13
6	Eucalyptol	Oxygenated monoterpene	0.72	-	-
7	β -Ocimene	Monoterpene hydrocarbon	0.65	-	0.44
8	Linalool	Oxygenated monoterpene	86.06	6.85	3.46
9	Citronellal	Oxygenated monoterpene	-	56.99	85.07
10	L-4-Terpineol	Oxygenated monoterpene	0.16	-	-
11	L- α -Terpineol	Oxygenated monoterpene	0.35	-	-
12	Cyclohexane, 2,4-diethyl-1-methyl	Non-terpene hydrocarbon	-	2.68	-
13	Citronellol	Oxygenated monoterpene	-	11.66	-
14	β -Citral	Oxygenated monoterpene	-	0.86	-
15	(R)-Citronellol	Oxygenated monoterpene	-	2.75	-
16	Octane,1-ethoxy	Oxygenated non-terpene	-	0.60	-
17	α -Citral	Oxygenated monoterpene	-	1.09	-
18	Cyclohexanol, 2-(2-hydroxy-2-propyl)-5-methyl-	Oxygenated non-terpene	-	6.75	-
19	Citronellol acetate	Oxygenated monoterpene	-	1.74	2.77
20	Geranyl acetate	Oxygenated monoterpene	-	0.82	0.61
21	β -Elemene	Sesquiterpene hydrocarbon	0.31	-	-
22	Caryophyllene	Sesquiterpene hydrocarbon	0.26	-	1.77
23	γ -Gurjunene	Sesquiterpene hydrocarbon	0.23	-	-
24	E-Nerolidol	Oxygenated sesquiterpene	0.39	-	-
25	β -Germacrene	Sesquiterpene hydrocarbon	0.34	-	-
26	β -Myrcene	Monoterpene hydrocarbon	-	-	1.04
27	Linalool epoxide	Oxygenated monoterpene	-	-	0.70
28	Linalool oxide	Oxygenated monoterpene	-	-	0.33
29	Cyclo-Germacrene	Monoterpene hydrocarbon	-	-	0.30
30	Cadinene	Sesquiterpene hydrocarbon	-	-	0.22

from the three different leaf EOs. Some minor compounds were detected in the leaf EO from East Sumba but not in the leaf EO from Central Java as well in the common leaf EO from the reference and the other way around. These differences were displayed as different small peaks appear in Figure 1 and also from the Venn diagram (Figure 3).

The results of fruit EOs from East Sumba and Central Java displayed a more similar composition profile than the leaf EOs (Figure 4 and Table 2). Three major compounds were detected in each of *C. hystrix* fruit EO. Two compounds, L- β -Pinene dan D-Limonene, were present as the same major

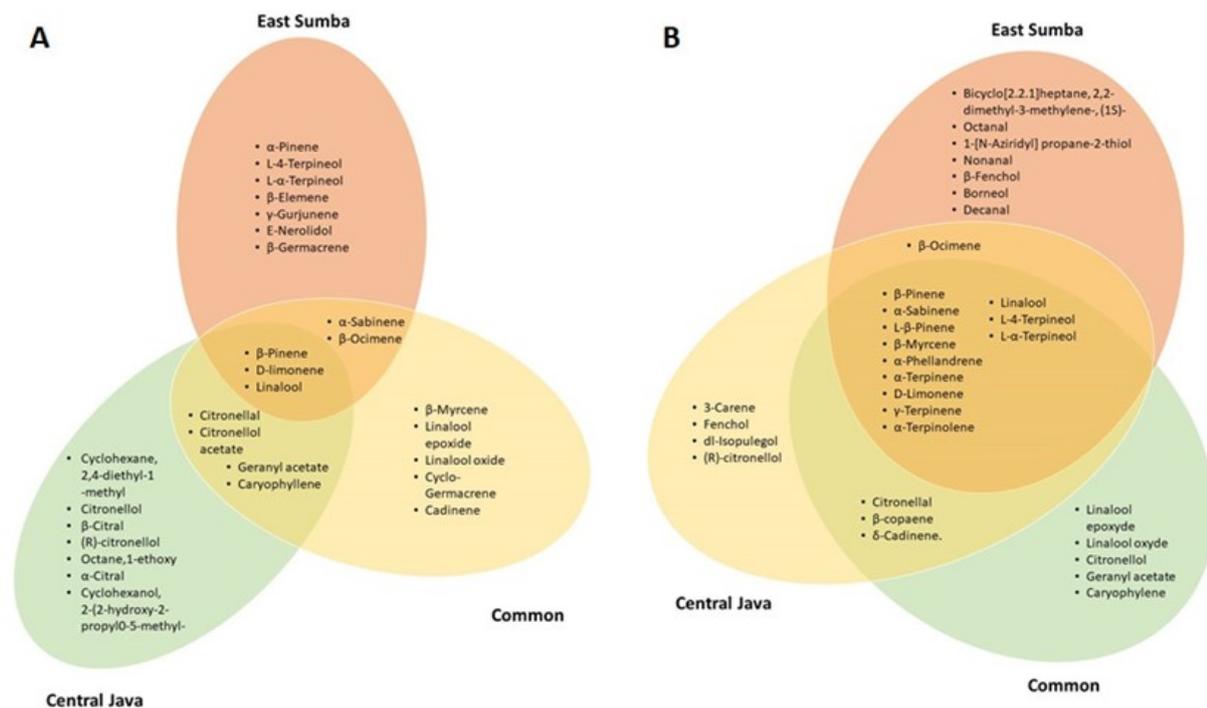


Figure 3. The Venn diagram of essential oils' chemical constituents from the (A) leaf and (B) fruit of *C. hystrix* originated from East Sumba, Central Java, as well as from common *C. hystrix*.

components in the three different fruit EOs. In the fruit EO from East Sumba and common *C. hystrix*, L-β-Pinene was more dominant, while in the fruit EO from Central Java, the result indicated D-Limonene as the more dominant compound. β-pinene belongs to the monoterpenes group and is found in many plants EOs. A wide range of biological properties have been reported, such as antibiotic, anticoagulant, antitumor, antimicrobial, antimalarial, antioxidant, anti-inflammatory, and analgesic effects (Salehi et al. 2019). Limonene is one of the major compositions of citrus peel, which contributes to the smell of citrus peel. It has many biological activities,

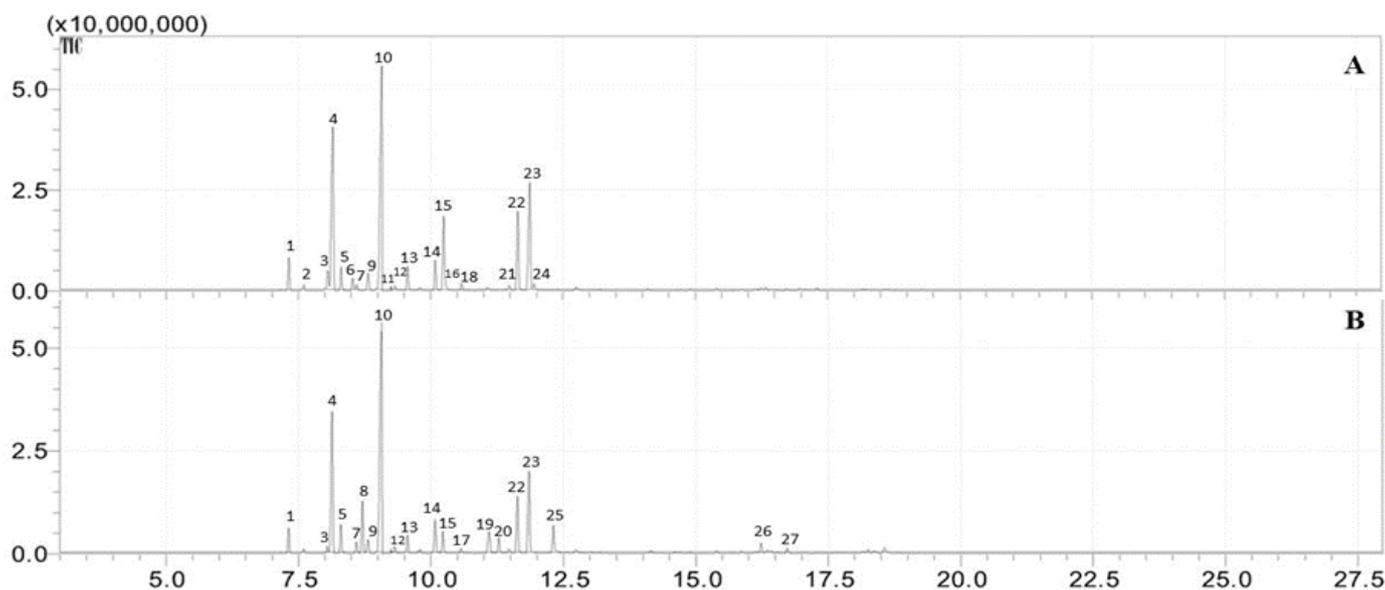


Figure 4. Gas chromatography chromatogram of *C. hystrix* fruit essential oils. (A) East Sumba, (B) Central Java.

Table 2. Composition of *C. hystrix* fruit essential oils from East Sumba and Central Java.

No.	Compound		Area component (%)		
			East Sumba	Central Java	Common*
1	α -Pinene	Monoterpene hydrocarbon	3.5	2.5	1.26
2	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	Monoterpene hydrocarbon	0.4	-	-
3	α -Sabinene	Monoterpene hydrocarbon	1.91	0.75	9.21
4	L- β -Pinene	Monoterpene hydrocarbon	21.55	17.6	21.44
5	β -Myrcene	Monoterpene hydrocarbon	2.2	2.78	1.98
6	Octanal	Oxygenated non-terpene	1.31	-	-
7	α -Phellandrene	Monoterpene hydrocarbon	0.64	1.22	0.10
8	3-Carene	Monoterpene hydrocarbon	-	5.58	-
9	α -Terpinene	Monoterpene hydrocarbon	1.89	1.63	1.23
10	D-Limonene	Monoterpene hydrocarbon	28.14	32.4	12.59
11	1-[N-Aziridyl] propane-2-thiol	Non-terpene hydrocarbon	0.15	-	-
12	β -Ocimene	Monoterpene hydrocarbon	0.51	0.74	-
13	γ -Terpinene	Monoterpene hydrocarbon	2.28	1.73	2.29
14	α -Terpinolene	Oxygenated monoterpene	3.03	3.65	0.62
15	Linalool	Oxygenated monoterpene	8.09	2.2	4.23
16	Nonanal	Oxygenated non-terpene	0.26	-	-
17	Fenchol	Oxygenated monoterpene	-	0.53	-
18	β -Fenchol	Oxygenated monoterpene	0.68	-	-
19	Citronellal	Oxygenated monoterpene	-	3.11	20.91
20	dl-Isopulegol	Oxygenated monoterpene	-	1.62	-
21	Borneol	Oxygenated monoterpene	0.52	-	-
22	L-4-Terpineol	Oxygenated monoterpene	9.04	6.61	11.93
23	L- α -Terpineol	Oxygenated monoterpene	13.77	10.5	5.16
24	Decanal	Oxygenated non-terpene	0.58	-	-
25	(R)-Citronellol	Oxygenated monoterpene	-	3.23	-
26	β -copaene	Sesquiterpene hydrocarbon	-	1.1	0.18
27	δ -Cadinene.	Sesquiterpene hydrocarbon	-	0.57	0.23
28	Linalool epoxide	Oxygenated monoterpene	-	-	3.29
29	Linalool oxide	Oxygenated monoterpene	-	-	1.57
30	Citronellol	Oxygenated monoterpene	-	-	0.46
31	Geranyl acetate	Oxygenated monoterpene	-	-	0.43
32	Caryophyllene	Sesquiterpene hydrocarbon	-	-	0.24

including antioxidant, anti-inflammatory, anticancer, analgesic, antidiabetic activity, and some effects on the gastrointestinal and respiratory tract (Soulimani et al. 2019). The third major component in the fruit EOs from East Sumba and Central Java was L- α -Terpineol, while in the common *C. hystrix*, Citronellal was one of the major compositions (20.19%). These results have some degree of similarities with a previous study. Research conducted by Ngan et al. (2019) reported that kaffir lime essential oil from Vietnam was rich in β -Pinene (35.54%), sabinene (23.64%), and D-Limonene (19.08), while the kaffir lime essential oil from Thailand rich in Citronellal

(23.85), and the kaffir lime essential oil from Malaysia rich in Sabinene (35.2%), β -Pinene (16.8%), and D-Limonene (19.8%).

Based on Palazzolo et al. (2013), the major chemical component of most of the types of fruit citrus oils is Limonene. The Limonene content ranges are about 32 to 98%. In particular, the Limonene content ranges from 68 to 98% in sweet orange, from 45 to 76% in lemon, and from 32 to 45% in bergamot. These values are more significant than the Limonene content of *C. hystrix* from East Sumba (28.14%) and common *C. hystrix* (12.59%), while *C. hystrix* from Central Java had a value that fell in the range (32.4%). In addition, some minor constituents were detected in the fruit EO from East Sumba but not in the fruit EO from Central Java as well in the common leaf EO from the reference, and the other way around (Figure 3).

Oxygenated monoterpene is the dominant group of composition in the leaf EOs from East Sumba, Central Java, and in the common leaf EO from reference (Table 3). However, in the leaf EO from East Sumba, the majority of oxygenated monoterpene consist of Linalool, while in the leaf EO from Central Java and in the common leaf EO, the major oxygenated monoterpene was Citronellal. On the other hand, in the three different fruit EOs, two dominant groups of compounds were monoterpene hydrocarbon and oxygenated monoterpene (Table 3). In the fruit EOs from East Sumba and Central Java, monoterpene hydrocarbon was more dominant (approximately two times higher), while in the common fruit EO, monoterpene hydrocarbon and oxygenated hydrocarbon had relatively the same percentage.

The varied compositions of essential oils in this study can be caused by differences in plant origin, age, morphology, and aroma. Moreover, environmental factors can affect the production of essential oils. Both *C. hystrix* from the BBG collection were planted in the same area and closed to each other. However, *C. hystrix* from Central Java was located in a part that was shaded by other plants, while *C. hystrix* from East Sumba was in an area that was exposed to direct sunlight. According to Yang et al. (2018), plant secondary metabolites accumulation depends on various environmental factors such as light, temperature, and soil water. For most plants, a change in an individual also factor may alter the content of secondary metabolites even if other factors remain constant.

In conclusion, the results showed that leaf and fruit EOs composition analysis of *C. hystrix* from East Sumba and Central Java using GC-MS indicated a variation of detected volatile compounds between *C. hystrix* from the two regions compared to common *C. Hystrix* from reference. The main composition of *C. hystrix* leaf EOs from East Sumba was Linalool, while in *C. hystrix* from Central Java and common *C. hystrix* was Citronellal. Meanwhile, the *C. hystrix* fruit EOs from East Sumbawa and Central Java has almost the same profile with the three main components, namely L- β -Pinene, D-Limonene, and L- α -Terpineol, while in common *C. hystrix* were L- β -Pinene, D-Limonene, and Citronellal.

Table 3. Class of compounds in *C. hystrix* fruit essential oils.

No.	Group of compounds	Total peak (%)					
		East Sumba		Central Java		Common	
		Leaf	Fruit	Leaf	Fruit	Leaf	Fruit
1	Monoterpene hydrocarbons	10.4	62.5	7.2	66.9	5.0	50.1
2	Oxygenated monoterpenes	87,3	35.1	82.7	31.5	92.9	48.6
3	Sesquiterpene hydrocarbons	1.1	-	-	1.7	2.0	0.65
4	Oxygenated sesquiterpenes	0,4	-	-	-	-	-
5	Non-terpene hydrocarbons	-	0.2	2.7	-	-	-
6	Oxygenated non-terpenes	-	2.2	7.4	-	-	-

AUTHORS CONTRIBUTION

All authors have an equal contribution to the research and publication. IPA was designed for the study, observed, and collected the research samples from the garden. KDP and FD analyzed the data. IPA, KDP, and FD wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest from this manuscript.

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