Indonesian Journal of Forestry Research Vol. 10 No. 2, October 2023, 195-205 ISSN 2355-7079/E-ISSN 2406-8195

CHEMICAL CONSTITUENTS OF *Melaleuca leucadendron* Linn. LEAF ESSENTIAL OILS QUALITY UNDER DIFFERENT COLLECTING TIMES IN KPH YOGYAKARTA, GUNUNGKIDUL, INDONESIA

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Received: 30 September 2022, Revised: 21 October 20223, Accepted: 21 October 2023

CHEMICAL CONSTITUENTS OF *Melaleuca leucadendron* Linn. LEAF ESSENTIAL OILS QUALITY UNDER DIFFERENT COLLECTING TIMES IN KPH YOGYAKARTA, GUNUNGKIDUL, INDONESIA. *Melaleuca leucadendron* Linn is one of Indonesia's most important non-timber forest products as a commercial essential oil. The aim of this study was to investigate the variation of compounds in *M. leucadendron* essential oils when leaves were collected at different times from August 2017 to April 2018 at the Yogyakarta Forest Management Unit (FMU). The essential oils were isolated by steam distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 13 constituents were identified with major compounds, i.e., 1.8-cineole (57.8 to 76.0%), D (+)-limonene (4.40 to 12.1%), p-cymene (1.53 to 12.9%) and α-terpineol (4.98 to 9.98%). The results showed that the weather conditions in the higher rainfall and flowering were favorable for the production of essential oil (March) and increased the major compounds, especially from November to January (with the exception of α-terpineol, which was found to be higher in September). In contrast to these main compounds, α-pinene, β-pinene, and humulene are used as marker compounds to distinguish between the lowest and highest period. In addition, it is also noted that the flowering stage affects the monthly variation of the major compounds.

Keywords: *Melaleuca leucadendron*, Myrtaceae, Seasonal variation, Leaf essential oil, 1.8-cineole

KONSTITUEN KIMIA Melaleuca leucadendron *Linn. KUALITAS MINYAK ATSIRI DAUN PADA WAKTU PENGUMPULAN YANG BERBEDA DI KPH YOGYAKARTA, GUNUNGKIDUL, INDONESIA.* Melaleuca leucadendron *Linn merupakan salah satu hasil hutan non kayu terpenting di Indonesia sebagai minyak atsiri komersial. Tujuan dari studi ini adalah untuk menginvestigasi variasi senyawa dalam minyak atsiri* M. leucadendron *ketika daun dikumpulkan pada waktu yang berbeda dari Agustus 2017 hingga April 2018 di Kesatuan Pengelolaan Hutan (KPH) Yogyakarta. Minyak atsiri diisolasi dengan destilasi uap dan dianalisis dengan Gas chromatography-mass spectrometry (GC-MS). Sebanyak 13 konstituen diidentifikasi dengan senyawa utama, yaitu 1,8-cineole (57,8 -76,0%), D (+)-limonene (4,40–12,1%), p-cymene (1,53 - 12,9%) dan a-terpineol (4,98 - 9,98%). Hasil penelitian menunjukkan bahwa kondisi cuaca dengan curah hujan yang lebih tinggi dan berbunga, menguntungkan untuk produksi minyak atsiri (Maret) dan meningkatkan senyawa utama, terutama dari bulan November hingga Januari (kecuali a-terpineol, yang ditemukan lebih tinggi pada bulan September). Berbeda dengan senyawa utama tersebut, a-pinene, β-pinene, dan humulene digunakan sebagai senyawa penanda untuk membedakan periode curah hujan rendah dan tinggi. Selain itu, juga perlu dicatat bahwa tahap pembungaan mempengaruhi variasi bulanan senyawa utama.*

Kata kunci: Melaleuca leucadendron*, Myrtaceae, Variasi musim, Minyak atsiri daun, 1,8-cineole*

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doi:10.59465/ijfr.2023.10.2.195-205

I. INTRODUCTION

Melaleuca leucadendron, known as Cajuput oil-producing tree, is one of the most important commercial essential oils in Indonesia (Budiadi, Ishii, Sunarto, & Kanazawa, 2005; Pujiarti, Ohtani, & Ichiura, 2011). This plant belongs to the Myrtaceae family, which produces strong-scented essential oils with useful medicinal properties from leaves, twigs, and stems by water steam distillation (Budiadi et al., 2005; Farag et al., 2011). *Melaleuca* is commonly found in the islands of Java, Moluccas, East Nusa Tenggara, and Sulawesi Islands (Pujiarti et al., 2011). *M. leucadendron* trees planted on the island of Java were first established as a pioneer tree for reforestation in 1924. In addition, the commercial production of essential oil by the forest owner started in the 1960s (Budiadi et al., 2005).

It is known that the quality of M. leucadendron essential oil depends on the yield of 1,8-cineole, a pharmacologically active component of the aromatic essential oil (Milthorpe, Brooker, Slee, & Nicol, 1998). In Indonesia, the quality requirement of excellent *M. leucadendron* essential oil is expected to have 1,8-cineole levels ranging from 50 to 65%, as per the Indonesian National Standard (Badan Standardisasi Nasional, 2006). Certain major constituents from these plants have some biological activity and also flavor and fragrance additives.

Some factors, such as the quality of the site and the season of harvesting, can affect the quality of the essential oil (Castelo, Del Menezzi, & Resck, 2012; Lee, Brooks, Rossetto, Henry, & Baverstock, 2002). In addition, rainfall, temperature, and humidity are also reported to affect the quality and quantity of essential oil (Usano-Alemany, Palá-Paúl, & Herráiz-Peñalver, 2014, 2016; Pellegrini et al., 2018; García-Caparrós et al., 2019; Sadono, Soeprijadi, & Wirabuana, 2019; Karalija, Dahija, Tarkowski, & Zeljković, 2022). Therefore, the quality of *M. leucadendron* essential oil is assumed to depend on the collecting time. In previous studies, some authors reported variations in the chemical composition of essential oils depending on the month of collection and season within the same or different families in certain species., such as *Blepharocalyx salicifolius*, *Psidium myrsinites*, *Vitex trifolia* var. *Purpurea* and *Mentha* longifolia (Castelo et al., 2012; Dehsheikh et al., 2019; Zouari-Bouassida, Trigui, Makni, Jlaiel, & Tounsi, 2018). However, to our knowledge, research related to the influence of harvesting time in different months on the chemical composition of *M. leucadendron* essential oil is still limited. The influence of different ages and regions of the tree on the chemical composition and biological activity of essential oils has been relatively little studied in this species (Pujiarti et al., 2011, 2012).

The objective of this study was to investigate the variation of the major constituents from *M. leucadendron* essential oils with varying collection times in months. In addition, variations in the chemical composition of *M. leucadendron* essential oil and its major compounds have also been studied. Knowledge of the 1,8-cineole level and variation in its chemical composition may help predict the best time to collect the fresh leaves of *M. leucadendron*. Investigation of the potential of the major compound from *M. leucadendron* will support the pharmaceutical industry, including the dairy, vitamin, and cosmetic industries.

II. MATERIAL AND METHOD

A. Materials

The leaves of *M. leucadendron* leaves were collected 15 kg from *M. leucadendron* plantations with tree ages ranging from 21 to 43 years old, which were planted in plot 31 at Forest Management Unit (FMU) Yogyakarta or KPH Yogyakarta (Indonesia) in Karangmojo district (7.570 'S, 110.400 'E), Gunungkidul, Indonesia. The plant was identified by Mr. Andi Nugroho based on compared to digital herbarium plants from the Royal Botanic Garden Kew. The voucher number of the plant is K000793405. Plot 31 contained a homogenous category based on remote sensing mapping. The plots were selected randomly, and 9 plot experiments were obtained as representatives. The leaves of *M. leucadendron* were taken in each experimental plot with a size of 20 x 20 m with nine replication measurements on the same trees every month. In addition, to analyze the effect of seasonality on the essential oils of this species, the collection period was nine months, from August 2017 to April 2018. Comprehensive data on field conditions and plot experimental selection were defined in parallel work (Mulyana, Rohman, & Wardhana, 2018; Mulyana, Siallagan, Yuwono, & Purwanto, 2019). During the collection period, higher rainfall was recorded in the months of November, December, January, and March (Table 1). On the other hand, lower precipitation was observed during the months of August and September (Badan Pusat Statistik, 2020). Furthermore, Meteorology, Climatology, and Geophysical Agency (BMKG, 2023) also categorized the rainfall rate, namely 0.5 - 20 mm day-1 (green): light rain; 20-50 mm day-1 (yellow): moderate rain; 50 - 100 mm day-1 (orange): heavy rain. 100 - 150 mm day^{-1} (red): very heavy rain.

B. Isolation of essential oils

15 kg of fresh leaves sample was isolated by water steam distillation in 3 of the plant chambers of a stainless-steel apparatus (3 kg per plant chamber) for 6 hours. Each month, all distillations were carried out in triplicate, and the essential oils produced were purified from the remaining water using magnesium sulfate monohydrate ($MgSO_4.H_2O$). Then, the essential oil was stored in the vial at approximately 0°C prior to the GC-MS analysis.

C. Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical constituent was identified by Gas chromatography-mass spectrometry (GC-MS-QP 2010, Shimadzu, Japan) under the following conditions: DB-1 capillary column (30 m x 0.25 mm I.D. and 0.25 µm; GL Sciences, Japan); carrier gas He; injector temperature 290°C; oven initial temperature 70° C held for 5 min; temperature 6°C per min; final temperature 285° C held for 20 min; 1.0 µl of *M. leucadendron* essential oil injection. The transfer-line temperature was 200°C, and the split ratio used was 1:50. Compounds were investigated by comparing experimental GC-MS data with the NIST MS library (NIST 2011), and their retention indices based on literature research (Davies, 1990; Fall, Ngom, Sall, Sembene, & Samb, 2017; Pujiarti et al., 2011, 2012; Monzote et al., 2020). The relative area percentage of the essential oil constituents was expressed as a percentage obtained by a computer integrator based on

Table 1. Rainfall information on the collection period of *M. leucadendron*

Year	Month	Rainfall number of days	Rainfall trend category	Total rainfall	Temperature (°C)			Average of humidity (%)
					Max	Min	Average	
2017	August	1.28	Light rain	4.28	34.8	19.8	25.1	81
	September	3.72	Light rain	44.28	33.6	18.6	25.8	81
	October	10.89	Light rain	121.7	33.8	22.8	26.9	84
	November	16.22	Light rain	577.2	33.6	22.0	25.8	90
	December	14.44	Light rain	277.2	32.6	21.2	26.3	86
2018	January	19.11	Light rain	479.4	30.4	22.9	25.9	85
	February	14.06	Light rain	200.6	30.9	22.8	26.0	84
	March	13.22	Light rain	241.5	31.3	23.1	26.4	83
	April	7.83	Light rain	90.72	32.1	23.4	26.4	82

Source: Badan Pusat Statistik (2020)

the total ion chromatography (TIC) peak area, with corresponding response coefficients are considered one (Bardakci, Servi, & Polatoglu, 2019; Bugayong, Cruz, & Padilla, 2019; Jin, Ai, Qu, Cui, & Wang, 2019; Mohamed, Mohafez, Khalil, & Alhaider, 2019; Niczad, Sharafzadeh, Alizadeh, Amiri, & Bazrafshan; Qu et al., 2019).

III. RESULT AND DISCUSSION

A. Physical properties and yield of essential oils

The physical traits, i.e., specific gravity, refractive index, and solubility in alcohol as well as essential oil yield, ranged from 0.874 to 0.955, 1.461 to 1.468, 1:1 to 1:7, and 0.17 to 0.67%, respectively (Mulyana et al., 2019). In general, all parameters were qualified according to SNI (2006) (Badan Standardisasi Nasional, 2006) and ISO 4730 (the International Organization for Standardization 4730, 2017). In our previous studies, the highest content of essential oil was investigated in March, while the lowest essential oil yield was obtained in August. Although March and August are in the same category (light rain) based on the BMKG category, they have different numbers of rainfall per day and total rainfall (Table 1). From these rainfall data, April to September is the lowest rainfall period, while October to March is the highest rainfall period. *M. leucadendron* trees produced higher essential oil content during the month of March, indicating higher rainfall rates than in August, where rainfall was lowest. Other species with the same family (Myrtaceae), such as *Leptospermum petersonii*, had higher yields of essential oils in the rainy season compared to the dry season (Demuner et al., 2011). Pirzad, Alyari, Shakiba, Zehtab-Salmasi, and Mohammadi (2006) reported that the content of essential oil from *Matricaria chamomilla* had a higher essential oil content under irrigation at 85% of field capacity compared with irrigation at 55%. In addition, Castelo et al. (2012) mentioned that the essential oil of *Psidium myrsinites* grown in Brazil had the highest essential oil content in the dry season (July). Rathore et al. (2022) also stated that in *Rosmarinus officinalis*, the levels of essential oil were greater in the autumn and summer seasons compared to the rainy season. Similar findings with higher essential oil content were reported in *Nectandra* spp. (Ferraz et al., 2018) and *Achillea fragrantissima* (Forssk.) Sch. Bip. (Elsharkawy & Nahed, 2018) during the autumn and summer season, respectively. In addition, the highest essential oil content of this result was also found at high humidity and low temperature. Kaul, Rajesware, Bhattacharya, and Singh (1999) reported that the essential oil of Rose-Scented Geraniums was positively correlated with rainfall and humidity but negatively correlated with temperature. The same pattern was also found in the species of *Mentha arvensis* L. *var. piperascens* Holmes and *Melaleuca alternifolia* under the influence of day temperature (Duriyaprapan, Britten, & Basford, 1986; Lemos, Rocha, Melo, Visser, & Pinheiro, 2008).

In view of this condition, water supply is, therefore, a critical factor affecting the production of essential oil. The desired rainfall is a maximum of <1500 mm year-1 (Sadono et al., 2019). In general, although irrigation may still be necessary where rainfall is less than 1000 mm year-1, this species is relatively well adapted to dry climates (Kartikawati, Nirsatmanto, Rimbawanto, Sumardi, & Prastyono, 2021). In addition, there appears to be a flowering stage in *M. leucadendron* trees, which can also affect the yield of essential oils. Corryanti and Sugito (2015) reported the flowering stage in *M. leucadendron* trees starts from January to April. Serralutzu et al. (2020) reported that essential oil content was greater in the longer flowering period than in the short period in *Rosmarinus officinalis*. The same result was also stated by Rathore et al. (2022) in the same species. Previous studies in different species have shown that the content of essential oils is higher in the flowering stages of *Mentha longifolia*. As stated by Golparvar, Hadipanah, and Mehrabi (2015) compared to the vegetative stage, the flowering stage is known to be the most effective time for essential oil extraction. In addition, Kofdis, Bosabalidis, and Kokkini (2004) also indicated that the maximum yield of essential oil was found at the flowering stage. It is known that many factors, such as season, temperature, humidity, reproductive stage, and species, can lead to qualitative and quantitative differences in the essential oils production. On the other hand, the distinction between yields of essential oils may also be related to whether younger or older leaves have been collected. Yuan et al. (2016) demonstrated that young leaves of *Blumea balsamifera* had the highest essential oil yield, followed by mature leaves and senescent leaves.

B. Chemical composition of essential oils

The GC-MS analysis detected nine essential oil samples with different collection times from August 2017 to April 2018 are shown (Table 2). Thirteen compounds were found in *M. leucadendron* leaves essential oil samples. These studies clarified and explained the variations

Table 2. Chemical constituent of the *M. leucadendron* leaves essential oils

Sr. No	Compound	RT	RI*	RI Lit.**	Concentration (%)								
					2017					2018			
					Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
1	α-Thujene	8.36	928	926	-	0.46	-	-	1.75	-	-	-	-
2	α-Pinene	8.62	948	948	1.5	-	0.44	-	2.91	2.36	3.77	4.44	2.82
3	β-Pinene	9.99	976	978	-	-	-	-	1.26	-	1.96	2.58	2.18
4	β-Myrcene	10.25	989	991	-	-	-	-	0.36	-	-	-	-
5	*p*-Cymene	11.44	1010	1016	2.23	1.80	4.94	2.51	12.9	1.53	5.16	4.65	-
6	D-Limonene	11.57	1042	1044	8.14	8.42	9.67	12.1	4.20	5.18	4.40	6.13	-
7	1,8-Cineole	11.70	1059	1062	63.6	67.6	67.7	57.8	68.2	76.0	69.8	63.6	74.0
8	α-Terpineol	16.67	1333	1330	9.41	9.98	6.7	9.09	5.44	4.98	2.55	6.68	7.09
9	β-Caryophyllene	22.97	1431	1426	5.03		4.09	-	-	-	1.69	1.16	-
10	Aromadendrene	24.64	1433	1446	-	-	-	-	0.51	-	-	-	-
11	Humulene	24.82	1456	1451	2.36	2.06	1.7	2.47	-	-	-	-	-
12	α-Bisabolol	27.00	1593	1595	1.20	1.05	-	-	1.76	-	-	-	-
13	β-Eudesmol	27.21	1647	1648	-	-	1.09	0.29	0.29	-	-	-	-
	Monoterpene Hydrocarbons				11.9	10.7	15.05	14.6	23.38	9.07	15.3	17.8	5.00
	Oxygenated Monoterpenes				73	77.6	74.4	66.9	73.64	81	72.4	70.28	81.1
	Sesquiterpene Hydrocarbons				7.39	2.06	5.79	2.47	0.51	0	1.69	1.16	0
	Oxygenated Sesquiterpenes				1.20	1.05	1.09	0.29	2.05	0.00	0.00	0.00	0.00

Remarks:
- not detected
* Calculated retention indices on a DB-1 column
** Reference retention indices based on the literature
Sr. No. 1-6 (Monoterpene Hydrocarbons)
Sr. No. 7-8 (Oxygenated Monoterpenes)
Sr. No. 9-11 (Sesquiterpene Hydrocarbons)
Sr. No. 12-13 (Oxygenated Sesquiterpenes)

in the quality of bioactive compounds and the bioactivity of essential oils of *M. leucadendron* leaves collected from different collecting months in KPH Yogyakarta. These classes were monoterpene and sesquiterpene hydrocarbons and oxygenated forms (monoterpene and sesquiterpene). Constituents of monoterpene hydrocarbons from these essential oils, such as α-thujene, α-pinene, β-pinene, β-myrcene, and D (+)-limonene, have been identified in previous studies in *M. Leucadendron* (Fall et al., 2017; Patramurti, Amin, Nastiti, & Hariono, 2020; Monzote et al., 2020; Pujiarti et al., 2011, 2012). In addition, 1,8-cineole is the most abundant in these oils, with a value ranging from 57.8% to 76.0%. Previous studies have also stated that 1,8-cineole was the major compound in *M. leucadendron* leaves (Budiadi et al., 2005; Fall et al., 2017; Patramurti et al., 2020; Pujiarti et al., 2011, 2012). In addition, other primary components have also been detected in each sample, i.e., D (+)-limonene (4.40 to 12.1 %), p-cymene (1.53 to 12.9%), and α-terpineol (4.98 to 9.98%) (Table 2).

These main components, besides 1,8-cineole, were also observed in previous studies in *M. leucadendron* (Fall et al., 2017; Monzote et al., 2020; Patramurti et al., 2020; Pujiarti et al., 2011, 2012). Moreover, certain components of sesquiterpene hydrocarbon groups, i.e., β-caryophyllene, humulene, aromadendrene, have been found in previous studies in *M. leucadendron* (Pujiarti et al., 2011, 2012). Furthermore, β-eudesmol was also detected in *M. Leucadendron* (Fall et al., 2017; Monzote et al., 2020). Some of the components detected in this result have been also mentioned in ISO 4730 (2017). However, the main constituent of terpinen-4-ol (tea tree oil) was not found. This may be ISO 4730 (2017) based on the species of *M. alternifolia* or *M. linariifolia*. However, the α-terpineol compound in this study was still detected in which terpinen-4-ol is an isomer of the terpineol compound.

C. Effect of different collecting time

The chemical composition of *M. leucadendron* shows that the major compound groups in these samples were oxygenated monoterpenes (ca. 66.9-81.1%) (Figure 1). The second major group was found in monoterpene hydrocarbons (5-23.4%), followed by sesquiterpene hydrocarbons (0-7.39%) and oxygenated sesquiterpenes (0-2.05%). Monoterpene hydrocarbons increased from August to December after a slight decrease in September, with the highest constituents found in both D (+)-limonene and p-cymene during the highest rainfall condition, low temperature, and high humidity period (November and December) (Figure 2). In addition, this class showed a fluctuating pattern, with the largest and smallest amounts reported in December (23.4%) and April (5.0%), respectively. Oxygenated monoterpenes differed significantly in each month, with the highest levels in January and

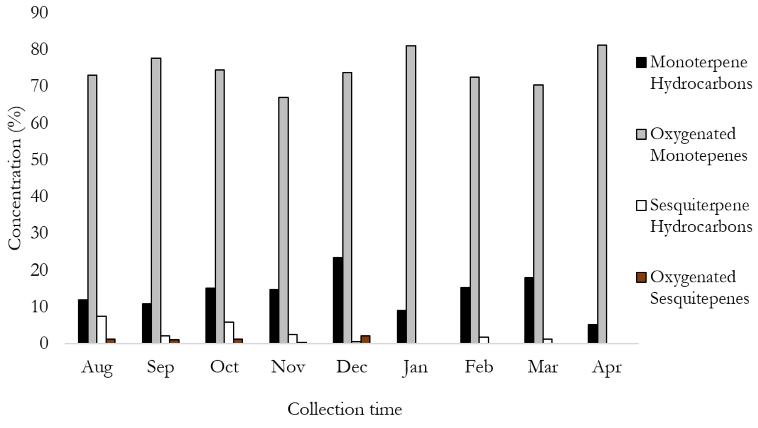

Figure 1. Chemical composition based on peak area percentage from *M. leucadendron*

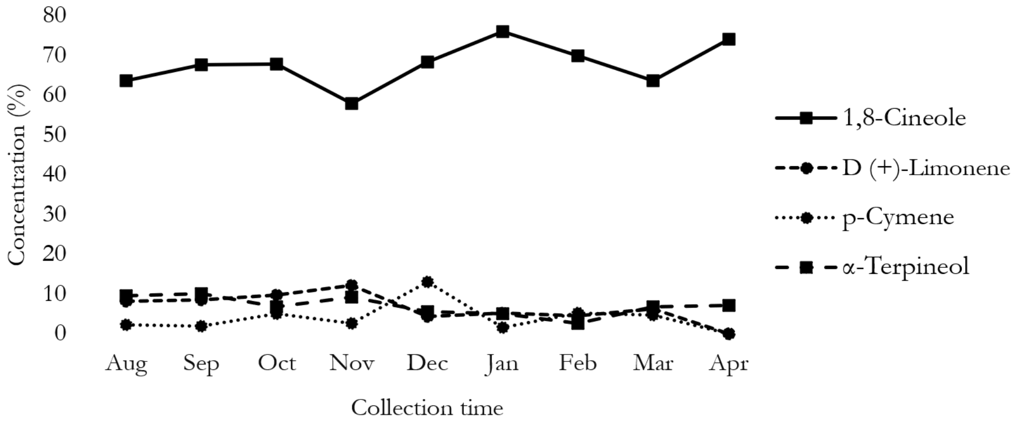

Figure 2. Major constituents' variation of essential oil of *M. leucadendron* leaves collected at different time

April (81.1%) relative to the lowest levels in November (66.9%).

1,8-cineole and α-terpineol were the main constituents of oxygenated monoterpenes. The 1,8-cineole content increased steadily from the lowest amount in November (57.8 %) to the highest amount (76.0 %) in January (highest rainfall). On the other hand, when comparing the lowest with the highest rainfall period, α-terpineol was found to be higher in the dry season compared to the rainy season, especially in September (9.98 %), with the smallest concentration in February (2.55 %). In addition, other major compounds have become significant components between the lowest and highest rainfall conditions, such as D (+)-limonene and p-cymene during the higher rainfall (November and December) and α-terpineol during the lower rainfall period (September).

Of the periods analyzed, the November and December periods with higher rainfall and humidity were the richest in volatile compounds such as p-cymene, D (+)-limonene, and other monoterpene hydrocarbons (Table 2, Figure 1). These major compounds may have marked the flowering period of this species in those months. It starts in January, with the highest amount of 1.8-cineole. The presence of the volatile compound in the essential oil can be related to the protection of the plant from predators or as a defense mechanism against external conditions such as pathogens. Castelo et al. (2012) showed that p-cymene, α-pinene, α-terpineol, and myrcene were higher in the fruiting period of *B. salicifolius* and *P. myrsinites* which serves as a major signal to protect against predators. The highest concentration of 1,8-cineole was found during flowering (January), which seems to attract its natural pollinators, bees or small insects. In previous studies, Holopainen (2007) and Demuner et al. (2011) reported that major compounds such as citronella and β-citronellol (oxygenated monoterpenes) of *L. Petersonii* flowers attract pollinators and serve as a protecting mechanism to protect the reproductive organs and their germination cells against pathogens or damages caused by ozone.

From this study, it is believed that the secondary metabolite production by plants may be influenced by environmental factors such as seasonality rainfall, and these factors may affect the yield and chemical composition of essential oils Gobbo-Neto and Lopes (2007) and Ferraz et al. (2018). On the other hand, climatic conditions can influence the enzymatic activity of certain plant species and then affect the certain secondary metabolites biosynthesis, including terpenic compounds (Barros, Zambarda, Heinzmann, & Mallmann, 2009). In addition, Zouari-Bouassida et al. (2018) noted that the yield of most compounds in different species, such as Mentha longifolia leaves, was higher in the flowering stage (winter) of oxygenated monoterpenes such as pulegone, 1,8-cineole, L-menthone, α-pinene, β-pinene, cis iso-pulegone, and piperitenone.

On the other hand, these results also revealed that the highest daily rainfall, especially in January, was the time with the highest concentration of 1,8-cineole, which is the most commercial compound of *M. leucadendron*. A variety of biological and pharmacological properties were assigned to 1,8-cineole. Usually, 1,8-cineole was used to treat both upper and lower airway diseases, such as bronchial asthma (Juergens et al., 2003). In addition, with respect to D (+)-limonene, p-cymene, and α-terpineol, these compounds had great potential in the food, pharmaceutical, and cosmetic industries.

Jidong (2007) has shown that D (+)-limonene has been used as a food flavoring agent. For many years, D (+)-limonene has been used as a flavor and fragrance additive in food, beverage, and consumer products. In addition, Joglekar, Panaskar, and Arvindekar (2014) mentioned that p-cymene is used to prevent cough and eliminate phlegm. Additionally, this component is used as a flavoring agent and also in the production of fungicides and pesticides (Bonjardim et al., 2012). The last compound, α-terpineol, has been investigated as a common fragrance ingredient used in perfumes, cosmetics, flavoring foods, and beverages (Khaleel, Tabanca, & Buchbauer, 2018).

In addition to the main compounds, significant amounts of α-pinene, β-pinene, and humulene were found (Table 2). In general, α-pinene, and β-pinene were present in all rainy periods and high humidity, whereas in the lowest rainfall conditions, these compounds were absent. On the other hand, the reverse pattern was found for the concentration of humulene. The results observed here differed from those reported by Demuner et al. (2011). They found that in the dry season, especially in the case of α-pinene and β-pinene, they were significantly higher than in the rainy season in both *L. madidum* and *L. flavescens* species. In addition, this study also showed that monoterpene hydrocarbons predominated in the highest rainfall period, while sesquiterpene hydrocarbons were found to be greater in the lowest rainfall period (Figure 2). In contrast, Demuner et al. (2011) showed that the concentration of monoterpene hydrocarbons of L. madidum spp and L. flavescens (Myrtaceae family) was the largest in the dry season, while sesquiterpenes hydrocarbons were greater in the rainy season. Kaul et al. (1999) reported α-pinene and β-pinene were positively correlated with relative humidity, whereas those compounds were negatively correlated with atmospheric temperature. Conversely, citronellol, citronellyl formate, citronellyl tiglate and 2-phenylethyl tiglate correlated positively with atmosphere temperature and negatively with relative humidity.

It should be noted that although studies are conducted using the same species, the quantity and quality of essential oil production can vary depending on the genetic, soil, extraction and climatic characteristics of the region (Castelo et al., 2012; Yuan et al., 2016). However, this study only showed variations in chemical constituents over a period of nine months. The patterns and comparisons between the highest and lowest rainfall periods are incomplete, especially during the lowest rainfall periods. Further research in 12 months (one year) is therefore needed to observe a complete trend of variation and comparison, both in rainy and dry seasons, in order to obtain the best time for the harvesting of essential oils from *M. leucadendron* leaves.

IV. CONCLUSION

In conclusion, the results showed that weather conditions in March with higher rainfall, humidity, and flowering stage were favorable for the essential oil production and increased by the amounts of 1,8-cineole (January), D (+)-limonene (November) and p-cymene (December). Although the number of essential oils decreased during the lowest rainfall condition, the plant collected during this season contained a higher concentration of α-terpineol (September). In addition, α-pinene, β-pinene, and humulene were markers of weather condition differences. In general, α-pinene and β-pinene were found in the high precipitation period (during the rainy season), while humulene was only found in the low precipitation period (during the dry season). Therefore, the knowledge of the impact of environmental factors, such as seasonal variation and rainfall, would significantly help producers to know and choose the best time for plant harvesting and production to obtain a high amount of the desired compounds for the pharmaceutical and food industries.

ACKNOWLEDGEMENT

The authors would like to thank KPH Yogyakarta for providing the sample of *M. leucadendron* trees.

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