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Physicochemical properties and chemical compositions of *Melaleuca leucadendron* leaf oils taken from the plantations in Java, Indonesia

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Abstract *Melaleuca leucadendron* Linn. leaf oils from Gunung Kidul, Gundih and Sukun, Java, Indonesia, at tree ages of 5, 10, and 15 years were analyzed to elucidate their qualities and chemical compositions. These oils gave yields from 0.61% to 1.59%. The samples from Gundih produced the highest yields (1.42–1.59%) compared to those from Gunung Kidul and Sukun. These oils were colorless with an odor typical of *Melaleuca* oils. The specific gravity of essential oils in this study ranged from 0.870 to 0.912. The samples from Gunung Kidul were the highest in specific gravity (0.905–0.912). The refractive index values of oil samples ranged from 1.468 to 1.470, optical rotation ranged from (–)2.47° to (–)0.98°, and ratio miscibility of oils in 70% ethanol ranged from 1:1 to 1:9.67. The organoleptic profiles and physicochemical properties of *M. leucadendron* Linn. leaf oils in this study were evaluated based on the Indonesian National Standard (SNI) 06-3954-2006 for standard quality of *Melaleuca* essential oils; only a few specific gravity values were below the standard. GC-MS spectrometry analysis indicated the presence of 26 compounds. Among them, 1,8-cineole (44.76–60.19%), α-terpineol (5.93–12.45%), D(+)-limonene (4.45–8.85%), and β-caryophyllene (3.78–7.64%) were the major components. Samples from each site tended to decrease in 1,8-cineole content and increase in β-caryophyllene content as plant age increased. α-Terpineol was highest at plant age 10 years, and D(+)-limonene varied according to plant site and age.

Key words *Melaleuca leucadendron* Linn. · Leaf essential oil · Physicochemical properties · Chemical compositions

Introduction

The Myrtaceae family is composed of at least 3,000 species in 130–150 genera, widely distributed in tropical and warm-temperate regions of the world.[1] *Melaleuca* species are included in the family Myrtaceae, which originated from Australia and spread to Southeast Asia including Indonesia. Some species found in Indonesia are *Melaleuca leucadendron* Linn., *Melaleuca cajeputi* Roxb., and *Melaleuca viridiflora* Corn. *Melaleuca leucadendron* Linn. is the species most often grown in Indonesia, known by the name "*Kayu Putih*." In Indonesia, *M. leucadendron* Linn. is grown mainly in natural forests and plantations. This plant is usually found in Java Island, Moluccas, Nusa Tenggara Timur, and Sulawesi Island. *Melaleuca leucadendron* Linn. was planted in Ponorogo of Java for the first time and distributed to Gunung Kidul in Yogyakarta and the other areas such as Gundih and Surakarta in Central Java, Mojokerto and Sukun in East Java, and Cikampek, Majalengka, and Indramayu in West Java. During the past four decades, forest landowners have exploited *M. leucadendron* Linn. leaves from the plantations in Java.[2]

Melaleuca leucadendron Linn. in Indonesia is mainly planted for production of essential oil from its leaves. This oil is used in herbal remedies, including antiseptics, antispasmodics, antineuralgics, and antirheumatics, and in cosmetics manufacture.[3,4] Several studies have also demonstrated the efficacy of *Melaleuca* species oil as antibacterial, antiviral, antitermite, and antifungal.[1,5–7]

Melaleuca leucadendron Linn. oil is a commercial non-timber forest product (NTFP) in Indonesia. Because it is a commercial product, it is necessary to know the quality of its oil. *Melaleuca leucadendron* Linn. oils have been assessed on the basis of individual requirements, the manner of assessment being dependent on its intended use. The physical characteristics of an oil, such as specific gravity, optical rotation, solubility in ethanol, odor, and color are not sufficient to characterize the quality of the essential oil. Therefore, chemical analysis of *M. leucadendron* Linn. leaf oil is also important.

In this study, *M. leucadendron* Linn. leaf oils of trees in plantations from several sites and of several plant ages in Java, Indonesia, were investigated. The aim of this study is to elucidate the physicochemical properties and chemical composition of these oils. The physicochemical properties

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were evaluated by using Indonesian National Standard (SNI) 06-3954-2006 for standard quality of *Melaleuca* essential oils. Chemical composition of oils was analyzed by gas chromatography-mass spectrometry (GC-MS).

Materials and methods

Plant materials and extraction of essential oil

Leaves of *M. leucadendron* Linn. (*Kayu Putih*) were collected from three sites in Java, Indonesia. Sampling sites were selected from the area of *M. leucadendron* Linn. plantations in Gunung Kidul, Yogyakarta (L1) owned by the Department of Forestry in the Province of Yogyakarta, and in Gundih, Central Java (L2) and Sukun, East Java (L3) owned by Perum Perhutani (Fig. 1). Each sample was obtained from 5-year-old (A1), 10-year-old (A2), or 15-year-old (A3) plantations.

For each sample, 5–7 kg fresh leaves of *M. leucadendron* Linn. was extracted by the water–steam distillation method for 5 h. Oil yields were determined based on dry weight (wt) of leaves (w/w). The yields were calculated by the following equation:

$$\text{Yield (\%)} = (\text{wt oil total/wt dry leaves}) \times 100$$

where wt is the weight in the same unit. All distillations were done in triplicate; the combined oils were stored at approximately 0°C until analysis.

Determination of organoleptic profiles and physicochemical properties

The organoleptic profiles and physicochemical properties of oils were evaluated using Indonesian National Standard (SNI) 06-3954-2006[8] for standard quality of *Melaleuca* essential oils. Analyses determined color, odor, specific gravity at 20°C, refractive index at 20°C, miscibility in 70% ethanol, and optical rotation.

Oil color was analyzed based on visual observation, and odor was evaluated by direct smelling of paper strips containing the oil.

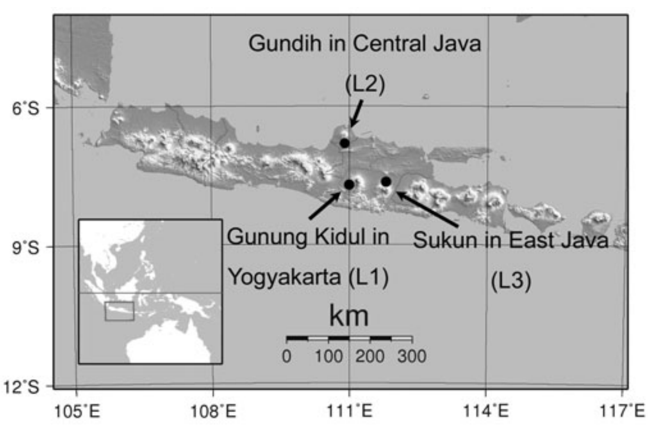


Fig. 1. Sites of *Melaleuca leucadendron* Linn. plantations in Gunung Kidul (*L1*), Gundih (*L2*), and Sukun (*L3*) in Java, Indonesia

Specific gravity was measured by pycnometer based on the weight ratio of oil and water in the same volume at the same temperature. This test used a 5-ml volume pycnometer. The empty pycnometer was weighed (m); it was then filled with distilled water (avoiding any air bubbles) and weighed again (m_1). The pycnometer was then washed with ethanol and subsequently with diethyl ether and dried. This pycnometer was filled with oil and weighed again (m_2). The specific gravity of oil was obtained using the following equation:

$$d^{20} = d^t + 0.0007 \times (t - 20)$$

where d^{20} is specific gravity at 20°C, t is ambient temperature (°C), and 0.0007 is a correction factor.

$$d^t = (m_2 - m)/(m_1 - m)$$

where d^t is specific gravity at ambient temperature (t°C), m (g) is weight of empty pycnometer, m_1 (g) is weight of pycnometer containing water at t°C, and m_2 (g) is weight of pycnometer containing oil at t°C.

Refractive index was determined by a Handy Refractometer (N-3000e; Atago, Tokyo, Japan). The refractive index was calculated by the following equation:

$$n_D = n^t{}_D + 0.0004\ (t - 20)$$

where n_D is index value at 20°C, n_D^t is index value at ambient temperature (t°C), and 0.0004 is a correction factor.

Miscibility in 70% ethanol was estimated based on the volume ratio of oil to 70% ethanol: 1 ml oil was put in a 10-ml volumetric glass and 1 ml 70% ethanol was added; the solution becomes clear after mixing well. If the solution is not clear, another 1 ml 70% ethanol is added until a clear solution is obtained. The results are expressed as follows:

$$\text{Solubility in 70\% ethanol} = (\text{1 ml oil}):(\text{ml 70\% ethanol added})$$

Optical rotation of oil was measured by a disk polarimeter (WGX-4; Shanghai Benson Instrument, Shanghai, China). Optical rotation is expressed in degrees of circumference (°). Optical rotation *dextro* is a positive sign (+), and optical rotation *levo* is a negative sign (–).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical analysis was conducted by a GC-17A gas chromatograph (GC) coupled to a QP5050A mass spectrometer (MS) (Shimadzu, Kyoto, Japan) using a fused-silica capillary column (TC-1701; 0.25 mm i.d. × 15 m, 0.25-µm film thickness; GL Sciences). GC-MS was performed using the following conditions: carrier gas, He; flow rate, 20.6 ml/min; splitless injection; injection volume, 1.0 µl; injection temperature, 230°C; oven temperature programmed from 30°C (5-min hold) to 100°C at 10°C/min (5-min hold), and from 100°C to 230°C at 15°C/min (5-min hold); interface temperature, 230°C; and electron impact ionization at 70 eV.

Components of the oils were identified from the GC retention time and MS fragmentation patterns by comparison with those of the National Institute of Standards and Technology (NIST) database library and with those of authentic compounds. Relative area percentage of each compound was calculated from the GC peak area.

Statistical analysis

Each experiment was performed three times. Data were analyzed using Scheffe's test in the SPSS statistical program. P values <0.05 were considered as significant.

Results and discussion

Essential oil yield

The leaves of *M. leucadendron* Linn. were collected separately from Gunung Kidul, Gundih, and Sukun at plant age 5, 10, and 15 years. The yields of *M. leucadendron* Linn. leaf oils are shown in Table 1 and Fig. 2.

Water and steam distillation of *M. leucadendron* Linn. leaves gave yields of volatile oil in the range between 0.61% and 1.59%. Samples from Gundih had the highest oil yields (1.42–1.59%) compared to Gunung Kidul and Sukun. The lowest oil yield was obtained from the samples of Sukun (0.61–0.85%).

The leaves obtained from Gunung Kidul and Gundih showed oil yields decreased as plant age increased (5, 10, and 15 years, respectively). In contrast, the samples obtained from Sukun showed a tendency that as tree age increased the oil yields also increased (Table 1, Fig. 2). Leaf oil content is usually greater in younger trees. Leaves from Sukun showed relatively low oil yields, which may be the reason the oil yield tended to differ from those of other sites.

Table 1. Locations, plant age, and yields of essential oil of *Melaleuca leucadendron* Linn. leaves from Java, Indonesia

Location	Plant age	Yield* (%)
Gunung Kidul (L1)	A1 (5 years)	1.51 ± 0.10 bc
	A2 (10 years)	1.44 ± 0.16 b
	A3 (15 years)	0.91 ± 0.23 ab
Gundih (L2)	A1 (5 years)	1.59 ± 0.32 bc
	A2 (10 years)	1.49 ± 0.27 bc
	A3 (15 years)	1.42 ± 0.19 b
Sukun (L3)	A1 (5 years)	0.61 ± 0.11 a
	A2 (10 years)	0.85 ± 0.32 a
	A3 (15 years)	0.85 ± 0.10 a

*Values are the mean of three replicates ± SD; where the letters (a–c) are the same, there is no significant difference between the means in same column at $P < 0.05$

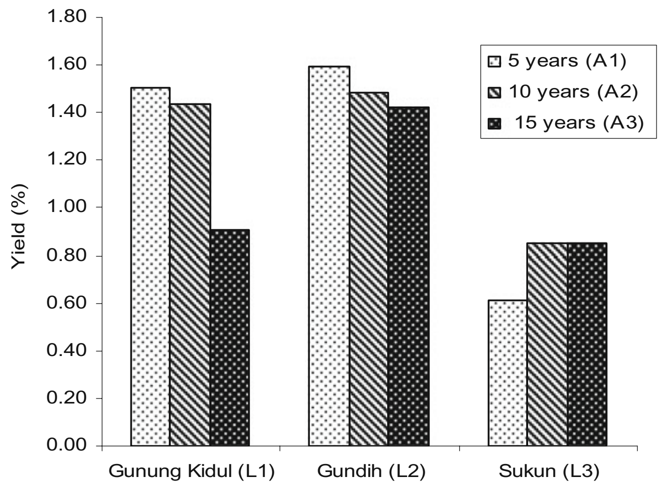


Fig. 2. Yield of *Melaleuca leucadendron* leaf oils from Gunung Kidul (L1), Gundih (L2), and Sukun (L3) by water and steam distillation method at tree age 5 years (A1), 10 years (A2), and 15 years (A3)

Physicochemical analysis

Organoleptic analysis of physicochemical properties is an important method to assess the quality of oil. The important physicochemical properties of essential oil include specific gravity, refractive index, miscibility in ethanol, and optical rotation. Study of the testing method of essential oils can help to judge whether the essential oils are genuine or fake. The results of physicochemical analysis of *M. leucadendron* Linn. leaf oil are shown in Table 2 and Fig. 3.

Essentials oils extracted from *M. leucadendron* Linn. leaves were colorless, with an odor of typical *Kayu Putih* oil or similar to *Melaleuca* oils. The specific gravity of oil samples ranged from 0.870 to 0.912. A few specific gravity values were below the SNI 06-3954-2006 for standard quality of *Melaleuca* oils, possibly because of the presence of D(+)-limonene and β-caryophyllene, which have specific gravity from 0.837 to 0.847 and from 0.856 to 0.865, respectively. Samples from Gunung Kidul had higher specific gravity (0.905–0.912) than those from Gundih and Sukun. Table 2 shows that the specific gravities of the oils from Gunung Kidul and Gundih are significantly different from one another, probably caused by differences in chemical content of oils, sampling sites, and plant age. Several previous studies have suggested that essential oil quality can vary depending on plant age, geographic location, and growing conditions.

The refractive index values of oil samples ranged from 1.468 to 1.470 and the optical rotation ranged from –2.47° to –0.98°. The refractive indexes and optical rotations of all samples were not significantly different, likely influenced by the highest 1,8-cineole content in oils (Table 3). 1,8-Cineole has a refractive index between 1.458 and 1.462 and optical rotation between –0.5° and +0.5° (see Table 2), also the result of essentials oils extracted from *M. leucadendron* Linn. Leaves in this study were colorless. Refractive index usually can be used to determine the purity level and quality of the oil. As refractive index is influenced by the color of the oil, increased oil clearness increases the refractive index. The greater refractive index, therefore, indicates oil of better quality.[9]

The values of miscibility of samples in 70% ethanol are not significantly different at $P < 0.05$, except the samples

Table 2. Organoleptic profiles and physicochemical properties of *Melaleuca leucadendron* Linn. leaf oils

Sample code	Color	Odor[a]	Specific gravity, 20°C[b]	Refractive index, 20°C[b]	Miscibility in 70% ethanol[b,c]	Optical Rotation** (°)
L1A1	Colorless	Typical *Kayu Putih* oil	0.905 ± 0.015 a	1.467 ± 0.002 a	1:1.33 ± 0.58 a	(–) 2.37 ± 1.30 a
L1A2	Colorless	Typical *Kayu Putih* oil	0.912 ± 0.007 a	1.469 ± 0.001 a	1:1.33 ± 0.58 a	(–) 1.10 ± 0.96 a
L1A3	Colorless	Typical *Kayu Putih* oil	0.912 ± 0.014 a	1.470 ± 0.003 a	1:9.67 ± 0.58 b	(–) 1.48 ± 0.70 a
L2A1	Colorless	Typical *Kayu Putih* oil	0.882 ± 0.015 b	1.469 ± 0.002 a	1:1.00 ± 0.00 a	(–) 1.68 ± 0.64 a
L2A2	Colorless	Typical *Kayu Putih* oil	0.879 ± 0.012 b	1.470 ± 0.000 a	1:1.00 ± 0.00 a	(–) 1.92 ± 0.44 a
L2A3	Colorless	Typical *Kayu Putih* oil	0.882 ± 0.052 b	1.470 ± 0.000 a	1:1.00 ± 0.00 a	(–) 1.30 ± 0.54 a
L3A1	Colorless	Typical *Kayu Putih* oil	0.870 ± 0.003ab	1.468 ± 0.001 a	1:1.33 ± 0.58 a	(–) 0.98 ± 0.86 a
L3A2	Colorless	Typical *Kayu Putih* oil	0.891 ± 0.003ab	1.470 ± 0.002 a	1:1.00 ± 0.00 a	(–) 2.47 ± 0.21 a
L3A3	Colorless	Typical *Kayu Putih* oil	0.901 ± 0.038 ab	1.470 ± 0.003 a	1:1.00 ± 0.00 a	(–) 1.58 ± 0.56 a
Standard[d]	Colorless to yellow-green	Typical *Kayu Putih* oil	0.900 to 0.930	1.450 to 1.470	1:1 to 1:10	(–) 4 to 0
1,8-Cineole	Colorless	Characteristic of camphor	0.905 to 0.925	1.458 to 1.462	nt	(–)0.5 to (+)0.5
D(+)-Limonene	Colorless to pale yellow	Mild-strong orange/ lime aroma	0.837 to 0.847	1.470 to 1.474	nt	(+)96 to (+)104
α-Terpineol	Colorless	Characteristic of turpentine	0.900 to 0.920	1.464 to 1.468	nt	(–)33 to (–)35
β-Caryophyllene	Colorless to pale yellow-green	Characteristic of camphor	0.856 to 0.865	1.498 to 1.504	nt	(–)5 to (–)10

nt, not tested
[a] Odor typical of *Kayu Putih* oil indicates that oils have the same odor as *Melaleuca* oils
[b] Values are the mean of three replicates ± SD; where the letters (a, b) following an entry are the same, there is no significant difference between the means in same column at $P < 0.05$
[c] Ratio between 1 ml oil and volume (ml) of 70% ethanol
[d] Standard based on Indonesian National Standard (SNI) number 06-3954-2006 for quality standard of *Melaleuca* oils

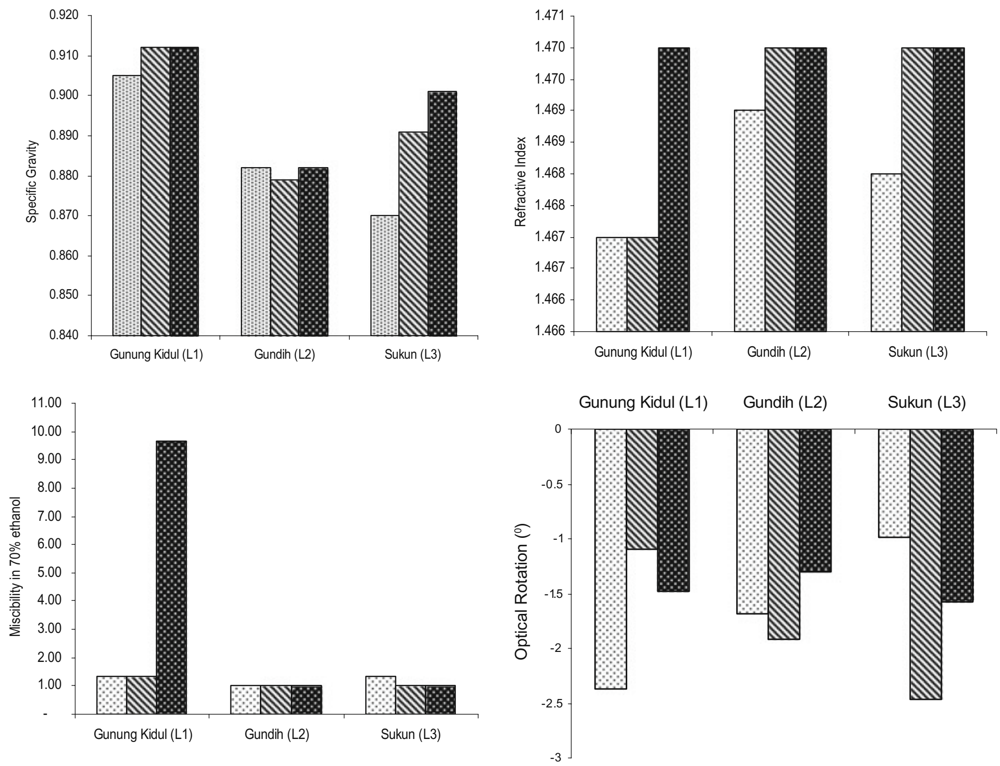


Fig. 3. Specific gravity, refractive index, miscibility in 70% ethanol, and optical rotation of *Melaleuca leucadendron* Linn. leaf oil from Gunung Kidul (L1), Gundih (L2), and Sukun (L3) at plant age 5 years (*light stippling*), 10 years (*diagonal hatching*), and 15 years (*dark stippling*)

from Gunung Kidul at tree age 15 years. These samples have a ratio of 1 : 9.67. GC-MS analyses showed (see Table 3) that samples from Gunung Kidul of 15-year-old plants had the highest sesquiterpene content; the sesquiterpenes are not as volatile as the monoterpene.[9,10] The low miscibility of oil in 70% ethanol in this study may be caused by the higher content of unsaturated sesquiterpenes in the oil, which makes the oil difficult to dissolve in ethanol; in consequence, a higher ethanol concentration would be required to dissolve this oil in the same volume of solution.

Table 3. Chemical composition of *Melaleuca leucadendron* Linn. leaf oils

Components	Composition[a] (%)								
	L1A1	L1A2	L1A3	L2A1	L2A2	L2A3	L3A1	L3A2	L3A3
Monoterpene hydrocarbons									
α-Thujene[b]	0.22	0.29	0.68	0.28	0.31	0.29	0.28	0.29	0.30
α-Pinene[c]	3.70	2.32	1.47	2.12	1.82	2.18	1.50	1.29	4.16
β-Pinene[b]	2.90	1.78	0.79	1.72	1.37	1.64	1.42	1.08	2.83
β-Myrcene[c]	0.78	0.63	0.31	0.68	0.74	0.67	0.78	0.95	0.74
Carene[c]	0.34	0.49	1.18	0.36	0.63	0.71	0.29	0.45	0.47
D(+)-Limonene[c]	8.76	6.38	4.45	6.39	5.58	5.92	5.93	5.39	8.85
γ-Terpinene[b]	2.02	2.81	6.72	2.39	2.76	3.15	1.82	2.88	3.16
Terpinolene[c]	0.93	1.42	3.62	1.16	1.56	1.68	0.67	1.32	1.71
Oxygenated monoterpenes									
1,8-Cineole[c]	54.24	52.34	44.76	55.04	51.32	49.22	60.19	56.22	46.44
Linalool[c]	0.39	0.10	0.04	0.16	0.08	0.17	0.00	0.00	0.42
Terpinene-4-ol[c]	0.64	0.77	0.97	0.63	0.67	0.71	0.78	0.81	0.78
Ocimenol[b]	0.11	0.20	0.09	0.10	0.11	0.11	0.12	0.14	0.17
α-Terpineol[c]	7.14	10.67	7.72	8.79	10.70	10.42	10.63	12.45	5.93
γ-Terpineol[b]	2.06	0.74	0.90	1.57	1.13	1.16	1.46	0.49	0.36
Sesquiterpene hydrocarbons									
Cedrene[c]	0.00	0.18	0.61	0.14	0.19	0.00	0.00	0.00	0.00
β-Caryophyllene[c]	4.46	6.33	6.83	5.03	7.33	7.64	3.78	5.48	6.14
Humulen[b]	0.56	0.66	0.88	0.53	0.75	0.84	0.53	0.68	0.71
β-Eudesmene[b]	1.29	1.74	3.51	2.30	2.18	2.23	0.98	1.72	2.44
Patchoulene[b]	0.82	1.63	4.37	2.80	2.09	2.91	0.77	2.19	2.88
Germacrene D[b]	0.60	0.41	0.29	0.17	0.24	0.33	0.54	0.26	0.29
Aromadendren[b]	0.00	0.27	0.15	0.16	0.12	0.00	0.00	0.00	0.00
Oxygenated sesquiterpenes									
Globulol[c]	2.70	1.80	1.54	1.28	1.37	1.61	3.60	1.49	2.05
Viridiflorol[b]	0.00	0.00	0.36	0.16	0.29	0.25	0.00	0.00	0.00
Cubenol[b]	0.18	0.16	1.15	1.00	0.17	0.18	0.23	0.00	0.89
Others									
Eugenol[c]	2.91	3.98	4.85	3.27	4.55	4.35	2.68	3.34	3.30
2-Pentanone[b]	1.91	1.82	1.65	1.75	1.76	1.65	0.99	0.94	0.88
Amount of identified compounds (%)	99.64	99.94	99.90	99.97	99.82	100.00	99.98	99.85	95.88

[a] Percentage of chemical compounds
[b] Compounds were identified by comparison with National Institute of Standards and Technology (NIST) database library
[c] Compounds were identified by comparison with authentic compounds

Chemical compositions of *M. leucadendron* Linn. leaf oils

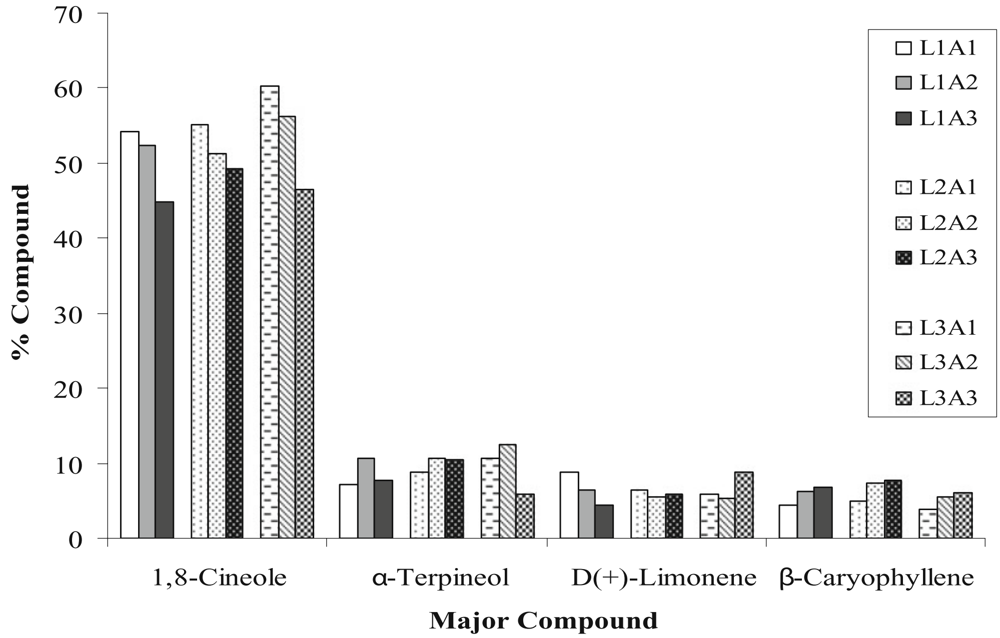
Twenty-six compounds were identified in *M. leucadendron* Linn. leaf oil samples: 8 monoterpene hydrocarbons, 6 oxygenated monoterpenes, 7 sesquiterpene hydrocarbons, 3 oxygenated sesquiterpenes, and 2 other compounds (see Table 3).

Basically, nine samples in this study have mostly the same chemical components, because the seeds for all *M. leucadendron* Linn. plantations in this study originated from Ponorogo. *Melaleuca leucadendron* Linn. was planted in Java for the first time in Ponorogo and spread to Gunung Kidul, Gundih, and Sukun.

The results showed that 1,8-cineole (44.76–60.19%) was the major compound in these oils, followed by α-terpineol (5.93–12.45%), D(+)-limonene (4.45–8.85%), and β-caryophyllene (3.78–7.64%). Several studies also indicate that the major compound of *M. leucadendron* Linn. oil is 1,8-cineole.[5–7] The 1,8-cineole content of the sample at each location indicates that increase of tree age results in a decrease of 1,8-cineole and conversely an increase of β-caryophyllene content. The highest quantity of 1,8-cineole was obtained at tree age of 5 years, followed by 10 and 15 years, whereas the highest quantity of β-caryophyllene was obtained at tree age 15 years, followed by 10 and 5 years. The highest quantity of α-terpineol at each location was obtained at tree age 10 years. The amounts of D(+)-limonene and the other compounds in the oils varied slightly according to tree location and age (Fig. 4). Several studies previously reported that essential oil content varied by tree age, geographic location, and growing conditions.[1,5,11–14]

In conclusion, in this study the organoleptic profiles and physicochemical properties of *M. leucadendron* Linn. from different sites and at different plant ages are very similar, satisfying the quality prescribed by the Indonesian National Standard (SNI) number 06-3954-2006 for standard quality of *Melaleuca* oils; only a few values of specific gravity are below the standard. The major compounds of oils in this study show a tendency for 1,8-cineole content to decrease with increasing plant age, and conversely that β-caryophyllene content increases with increasing plant age. The highest α-terpineol content was obtained at tree age of 10 years, and D(+)-limonene content varied slightly according to plant site and age. GC-MS spectrometry analysis suggests that *M. leucadendron* Linn. leaf oil has bioactive compounds and medicinal properties, which will be investigated in future studies.

Fig. 4. Contents (%) of major compounds of *Melaleuca leucadendron* Linn. leaf oils from Gunung Kidul (L1), Gundih (L2), and Sukun (L3) at plant age 5 years (A1), 10 years (A2), and 15 years (A3)



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