Chemical Compositions, Antioxidant and Antifungal Activities of *Melaleuca leucadendron* Linn. Leaf Oils from Indonesia

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Abstract

Melaleuca leucadendron Linn. Oil, also known as *Kayu Putih* oil, is one of the most important non-timber forest products in Indonesia. This oil has diverse bioactivity, such as insecticidal, antifungal, antibacterial, and antiviral. This study elucidated the chemical compositions and biological activities of *M. leucadendron* leaf oils from Java, Indonesia in order to evaluate their potency and improve their utilization. Leaf samples of *M. leucadendron* in this study were collected from plantation forest in Gunung Kidul-Yogyakarta (L1), Gundih-Central Java (L2) and Sukun-East Java (L3), Indonesia in different plant ages of 5 (A1), 10 (A2) and 15 (A3) years-old. Oil samples were distillated by water-steam distillation. Identification of chemical composition of these essential oils was conducted by GC-MS analysis. The *in vitro* antioxidant activity was examined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and antifungal activity was examined using a method by Wang *et al.* (2005) with slight modification against pathogenic fungi of *Fusarium oxysporum*, *Thanatephorus cucumeris* and *Rhizopus oryzae*. GC-MS investigations of *M. leucadendron* leaf oils showed 26 compounds have been identified. The result showed 1,8-cineole (44.76% to 60.19%) was the major compound in these oil, followed by α-terpineol (5.93% to 12.45%), D(+)-limonene (4.45% to 8.85%), and β-caryophyllene (3.78% to 7.64%), respectively. The *in vitro* DPPH assay showed anti-oxidative (IC_{50}: 7.21 to 9.46 mg/ml) properties of *M. leucadendron* leaf oils. This essential oils also revealed inhibitory effect against *F. oxysporum* (IC_{50}: 0.01 mg/ml to 0.11 mg/ml), *T. cucumeris* (IC_{50}: 0.52 mg/ml to 4.20 mg/ml) and *R. oryzae* (IC_{50}:1.35 mg/ml to 7.61 mg/ml). The antifungal activity showed *M. leucadendron* leaf oils were effective against fungi of *F. oxysporum* and *T. cucumeris*; but it showed less antifungal activity against *R. oryzae*. This study indicated that the *M. leucadendron* leaf oils can be used as antioxidant and sustainable eco-friendly bio fungicides.

Keywords: *Melaleuca leucadendron*, essential oil, chemical compositions, antioxidant, antifungal.

Introduction

The family Myrtaceae is one of the most important essential oil-producing species. This family has at least 3000 species distributed in 130~150 genera, and wide distribution in tropical and warm-temperate regions of the world, including Indonesia (Lee *et al*. 2008). In Indonesia *Melaleuca* is planted mainly in natural forests and plantations. This plant is usually found in Java Island, Moluccas, East Nusa Tenggara and Sulawesi Island. One species which can be found in Indonesia and produces commercial oil is *Melaleuca leucadendron* Linn. that is known as *Kayu Putih*.

The leaves and stems of several Myrtaceae families produce strong scented essential oils, some of which have useful medicinal properties (Farag *et al*. 2004). Several studies showed essential oils of Myrtaceae family have diverse bioactivity, such as antioxidant, insecticidal, antifungal (Lee *et al*. 2008), antibacterial, and antiviral (Carson *et al*. 2006; Cheng *et al*. 2007; Cleber *et al*. 2007). *M leucadendron* Linn. included in Myrtaceae family, has chemical and biological properties due to its components. This essential oil may provide potential alternatives to antioxidant and antifungal agents because it constitutes a rich source of bioactive chemicals.

This study analyzed chemical compositions of essential oils obtained from *M. leucadendron* leaves. The *in vitro* antioxidant and antifungal activities of *M. leucadendron* leaf oils and their major compounds were also analyzed. The aims of this study were to elucidate chemical compositions, inhibitory concentration, efficiency and potency of *M. leucadendron* leaf oils from Java, Indonesia and examine the potential of their major components as antioxidant and antifungal agents.

Materials and Methods

Plant Materials and Extracts

M. leucadendron leaves were collected from forest plantations in Gunung Kidul-Yogyakarta (L1), Gundih-Central Java (L2) and Sukun-East Java (L3), Indonesia. Each leaf was collected in different ages of 5 (A1), 10 (A2) and 15 (A3) years-old. Each sample used 5~7 kg of fresh leaves of *M. leucadendron* extracted by water-steam distillation for 5 h. All distillations were run in triplicate and the oils produced were stored at approximately 0°C prior to analysis.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The chemical analysis was conducted using a GC-17A gas chromatograph (GC) coupled to a QP5050A mass spectrometer (Shimadzu Co. Ltd, Kyoto, Japan) using a fused-silica capillary column TC-1701 (0.25 mm i.d. x 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; 1.0 µl injection volume; 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. The identification of chemical components was confirmed by comparison of retention times and calculated Kovats retention indices with National Institute of Standards and Technology (NIST) database library, authentic hydrocarbon standards and comparison with literature values.

Antioxidant Activity

The antioxidant activity of *M. leucadendron* leaf oils were elucidated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. An ethanol solution (1 ml) of sample was added to a solution (10 ml) of 0.25 mM DPPH in ethanol. The solution was rapidly mixed and was kept in water bath at 30°C for 30 min and the absorbance was measured at 515 nm using U-2810 spectrophotometer (Hitachi, Japan). Only ethanol (1 ml) was added to a solution (10 ml) of 0.25 mM DPPH in ethanol and this solution was subjected to the same method mentioned above. Absorbance of this solution was used as the control. The percentage of inhibition was calculated by the following equation:

$$\% \text{ Inhibition} = [(Ac - As)/Ac] \times 100$$

where Ac is absorbance of the control and As is the absorbance of sample tested. Various amounts of oil and authentic compound (collected using the same procedure mentioned above) were used in the assays to determine the ability of the inhibitors needed to encompass the IC_{50} values, which represented the concentrations of the essential oils that caused 50% inhibition. The assays were repeated within this range of inhibitor concentrations. If any inhibitors caused a significant change in the shape of the concentrations versus percent inhibition, the rates were calculated from steady state rates fitted with linear curves. The concentration of inhibitor needed to inhibit 50% was obtained by extrapolation of % antioxidant index versus concentration curves.

Antifungal Activity

The fungi used in this study were *Fusarium oxysporum* (NBRC 31213), *Thanatephorus cucumeris* (NBRC 30937) and *Rhizopus oryzae* (NBRC 31005). The fungi were obtained from the Department of Biotechnology, National Institute of Technology and Evaluation, Chiba, Japan.

Antifungal activity was examined using method of Wang *et al.* (2005) with slight modification. PDA (Potato Dextrose Agar, Difco) plates were prepared using Petri dishes (9 cm diameter). The known weight of oil samples and authentic compounds (1,8-cineole, α-terpineol, D(+)-limonene, and β-caryophyllene) were dissolved in methanol. The different concentration of the oil samples and authentic compounds were serially diluted with methanol and were added into 20 ml of PDA. The Petri dishes with the prepared media were kept in clean bench over night in order to remove volatile methanol. Each agar mycelium plug (5 mm diameter) taken from stock culture of the fungi was inoculated at the center of the Petri dish. After inoculation, the plates were incubated in dark at 25ºC. Colony growth diameter was measured every day for 14 days or while the fungal growth in the control treatment had completely covered the Petri dishes. PDA plates used as a control contained only methanol without essential oil or authentic compound solutions. All treatments were replicated three times and the data were averaged. The antifungal index was calculated by the following equation:

$$\% \text{ Antifungal Index} = (1-Sa/Sb) \times 100$$

where Sa is surface area of mycelium growth of treatment (cm^2) and Sb is surface area of mycelium growth of control (cm^2). IC_{50} values which represented the concentrations of the essential oils that caused 50% inhibition were determined by linear or logarithmic regression analysis.

Statistical Analysis

The data on antioxidant and antifungal indices were compared using Scheffe's test from SPSS statistical program and expressed as mean ± SD. *P*-values of < 0.05 were considered significant. IC_{50} of antioxidant and antifungal activity were analyzed using Excel program.

Results and Discussion

Chemical Compositions of the Essential Oils

The results on GC-MS analyses of nine oil samples are summarized in Table 1. Twenty-six compounds have been identified in *M. leucadendron* leaf oil samples. This study showed that the components of essential oils from Gunung Kidul, Gundih and Sukun in different plant age resembled each other, with a few differences in percentages of the contents. The compounds were mostly monoterpene hydrocarbons, oxygenated monoterpes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes.

The result showed 1,8-cineole (44.76 to 60.19%) was the major compound in these oils, followed by α-terpineol (5.93 to 12.45%), D(+)-limonene (4.45 to 8.85%), and β-caryophyllene (3.78 to 7.64%). The increasing of tree age resulted in decrease of the cineole content of the sample at each location, while on the contrary increased β-caryophyllene content. The highest quantity of cineole was obtained at tree age of 5 (A1) years, followed by 10 (A2) and 15 (A3) years-old, whereas the highest quantity of β-caryophyllene was obtained at tree age of 15 (A3) years, followed by 10 (A2) and 5 (A1) years-old. The highest quantity of α-terpineol at each location was obtained at tree age of 10 (A2) years. The amount of D(+)-limonene and

other compounds of oils at each place and age were varied. The amounts of compounds in this study vary slightly according to the tree locations and ages. Several previous studies reported that essential oil contents can vary based on tree age, geographical location and growing condition (Lee *et al*. 2002; Coppen 2002; Marcio *et al*. 2005; Cleber *et al*. 2007; Siramon and Ohtani 2007).

Antioxidant Activity

The *M. leucadendron* leaf oils were analyzed for their antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging. The DPPH assay measures the ability of the extract to donate electron to the DPPH radical, resulting in decolorization of the DPPH solution. The greater the decolorization, the higher antioxidant activity, which is reflected in a lower IC_{50}. The *in vitro* antioxidant activities of the major components and positive control of BHA (butylated hydroxyanisole) were also compared (Table 2).

The IC_{50} of L1A1 (IC_{50}: 9.12 mg/ml), L1A2 (IC_{50}: 8.82 mg/ml), L1A3 (IC_{50}: 8.66), L2A1 (IC_{50}: 9.23 mg/ml), L2A2 (IC_{50}: 7.21 mg/ml), L2A3 (IC_{50}: 7.71 mg/ml), L3A1 ((IC_{50}: 9.46 mg/ml), L3A2 (IC_{50}: 8.79 mg/ml), L3A3 (IC_{50}: 8.99 mg/ml) and four major compounds in essential oils, 1,8 cineole (IC_{50}: 4.92 mg/ml), D(+)-limonene (IC_{50}: 4.58mg/ml), β-caryophyllene (IC_{50}: 3.68 mg/ml), and α-terpineol (IC_{50}: 4.59 mg/ml) revealed antioxidant activities, but these essential oils have relatively low scavenging activities compare with BHA (IC_{50}: 25.68×10^{-3} mg/ml).

Table 1. Chemical composition of the *M. leucadendron* leaf oils

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

Table 2. Antioxidant activities and IC_{50} of *M. leucadendron* leaf oils and others authentic compounds by DPPH method.

Sample	Concentration (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)
L1A1	5	17.88 ± 0.42a	9.12
	7.5	39.84 ± 0.11d	
	10	55.58 ± 0.16h	
L1A2	5	18.15 ± 0.08a	8.82
	7.5	41.21 ± 0.19de	
	10	58.66 ± 0.82i	
L1A3	5	18.48 ± 0.10a	8.66
	7.5	43.54 ± 0.21e	
	10	59.52 ± 0.48i	
L2A1	5	23.77 ± 0.23b	9.23
	7.5	39.58 ± 0.09d	
	10	54.59 ± 0.45h	
L2A2	5	29.41 ± 0.49c	7.21
	7.5	50.53 ± 0.34g	
	10	78.96 ± 1.09k	
L2A3	5	27.10 ± 0.15c	7.71
	7.5	44.33 ± 0.34ef	
	10	72.79 ± 0.90j	
L3A1	5	24.64 ± 0.31b	9.46
	7.5	38.65 ± 0.23d	
	10	53.13 ± 0.51h	
L3A2	5	28.38 ± 0.20c	8.79
	7.5	41.00 ± 0.05d	
	10	57.82 ± 0.58hi	
L3A3	5	25.54 ± 0.18b	8.99
	7.5	39.64 ± 1.81d	
	10	56.83 ± 0.20h	
1,8- cineole	2.5	23.29 ± 0.18b	4.92
	5	52.64 ± 0.15lgh	
	7.5	76.60 ± 0.08k	
D(+)-limonene	2.5	28.49 ± 0.25c	4.58
	4	44.31 ± 0.02ef	
	5	54.17 ± 0.09h	
β-caryophyllene	1	21.73 ± 0.28ab	3.68
	3	44.67 ± 0.04ef	
	5	60.76 ± 0.13i	
α-terpineol	2.5	29.06 ± 0.23c	4.59
	4	44. 41± 0.04ef	
	5	53.88 ± 0.06h	
BHA (butylated hydroxyanisole)	20×10^{-3}	43.47 ± 0.41e	25.68×10^{-3}
	50×10^{-3}	71.41 ± 0.14ij	
	80×10^{-3}	82.09 ± 0.23l	
Eugenol	3×10^{-4}	8.29 ± 0.91m	3.25×10^{-3}
	6×10^{-4}	25.79 ± 0.07b	
	6×10^{-3}	82.08 ± 0.22l	

Values are the mean of three replicates ± SD. The value in the column followed by the same letter is not significantly different at $p < 0.05$.

In this study, the leaf oils of L1A3, L2A2, and L3A2 had higher antioxidant activity than the others with different ages from the same site. This is probably due to the presence of phenolic compound in these oils such as eugenol. DPPH radical-scavenging assay of eugenol (IC_{50}: 3.25 x 10^{-3}) showed that eugenol had high antioxidant activity. Several studies also reported eugenol exhibited highly antioxidant activity (Ogata *et al*. 2000; Hidalgo and De la Rosa 2009). The result obtained could be explained partially by the presence of higher amounts of eugenol in the oils of L2A2 and L2A3, which were responsible for these activities.

Antifungal Activity

The antifungal indices of *M. leucadendron* leaf oils are shown in Table 3 and the antifungal indices of authentic compounds are shown in Table 4. Various concentrations of oils and authentic compounds were used to determine a range of the inhibitory effects needed to encompass the IC_{50} value.

Antifungal indices of *M. leucadendron* leaf oils against *F. oxysporum* at concentrations of 0.1 mg/ml (49.85 to 62.15%), 5 mg/ml (68.23 to 81.96%), and 10 mg/ml (78.16 to 90.74%) were not significantly different. The antifungal indices of α-terpineol against *F. oxysporum* showed highest inhibitory effect compared to 1,8-cineole, β-caryophyllene and D(+)-limonene, with the growth of *F. oxysporum* on PDA medium with α-terpineol was completely inhibited at concentration 5 mg/ml, but D(+)-limonene did not affect at all.

Table 3 Antifungal indices of *M. leucadendron* leaf oils against three plant pathogenic fungi.

Fungi	Conc. (mg/ml)	Antifungal Index (%)								
		L1A1	L1A2	L1A3	L2A1	L2A2	L2A3	L3A1	L3A2	L3A3
*F. oxysporum**	0.1	49.85 ±10.39 d	62.15 ± 3.45 abcd	52.51 ± 3.06 d	54.53 ± 3.14 cd	60.79 ± 2.87 abcd	55.02 ± 3.08 cd	52.53 ± 1.53 d	58.46 ± 1.43 bcd	55.04 ± 2.28 cd
	5	79.15 ± 3.54 abcd	79.70 ± 6.64 abcd	72.60 ± 4.64 abcd	81.94 ± 8.02 abcd	74.79 ± 5.56 abcd	81.96 ± 2.45 abcd	78.71 ± 5.20 abcd	68.23 ±16.49 abcd	76.51 ± 6.68 abcd
	10	88.78 ± 7.65 abc	90.74 ± 5.27 a	85.91 ± 2.90 ab	88.11 ± 1.35 ab	89.85 ± 2.69 a	89.41 ± 3.58 a	86.13 ± 1.10 ab	78.16 ± 2.79 abcd	88.53 ± 5.99 ab
*T. cucumeris**	1	-	-	-	-	57.44 ± 7.20 defgh	-	-	59.52 ± 2.99 defgh	-
	2.5	54.08 ± 6.60 efgh	58.85 ± 8.82 defgh	33.78 ± 7.12 h	36.68 ±13.86 h	78.80 ± 3.46 abcdef	64.29 ± 3.92 cdefgh	62.47 ± 6.21 cdefgh	71.34 ± 6.10 abcdef	57.15 ± 4.17 defgh
	5	89.30 ± 4.68 abc	65.28 ±49.10 cdefgh	41.18±20.33 gh	47.84 ± 0.00 fgh	79.22 ±19.03 abcdef	70.64 ±21.33 abcdef	81.95 ± 6.95 abcde	87.50 ±17.68 abcde	57.68 ±22.57 defgh
	10	99.55 ± 0.79 a	100.00 ± 0.00 a	100.00 ± 0.00 a	94.65 ± 5.57 abc	94.44 ± 2.20 abc	80.35 ± 7.47 abcdef	98.01 ± 3.45 ab	99.80 ± 0.35 a	100.00 ± 0.00 a
R. oryzae$^{\Delta}$	5	44.58 ± 0.00 abc	34.66 ± 0.64 bc	30.50 ± 4.63 c	70.27 ±21.86 abc	48.18 ±11.54 abc	24.86 ±14.31 c	33.06 ±14.31 bc	63.49 ±20.95 abc	54.10 ± 0.59 abc
	10	72.37 ± 9.10 abc	79.11 ±20.50 abc	75.08 ±11.73 abc	81.70 ± 0.67 abc	55.34 ±11.51 abc	91.24 ± 8.90 ab	57.53 ±11.21 abc	88.89 ± 0.00 abc	98.26 ± 0.72 ab
	20	97.64 ± 0.00 ab	98.70 ± 0.18 ab	98.56 ± 0.16 ab	98.30 ± 0.24 ab	98.44 ± 0.11 ab	97.71 ± 0.83 ab	98.22 ± 0.16 ab	98.40 ± 0.06 ab	98.29 ± 0.13 ab
	30	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a	100.00 ± 0.00 a

Values are the mean of three replicates ± SD. The value in the column followed by the same letter is not significantly different at $p < 0.05$, $^{\Delta}IC_{50}$ measured after 2 days incubation, $^{*}IC_{50}$ measured after 10 days incubation, and $^{*}IC_{50}$ measured after 14 days incubation, Dashes means sample were not analyzed

Table 4. Antifungal indices of authentic compounds against three plant pathogenic fungi.

Fungi	Conc. (mg/ml)	Antifungal Index (%)			
		1,8- cineole	D(+)-limonene	β-caryophyllene	α-terpineol
*F. oxysporum**	0.1	34.45 ± 8.70 e	ne	47.38 ± 1.10 de	64.68 ± 0.90 cd
	1	64.00 ± 6.07 cd	ne	65.52 ± 6.26 cd	70.97 ± 4.76 c
	5	79.73 ± 1.35 bc	ne	76.85 ± 2.66 bc	100.00 ± 0.00 a
	10	96.25 ± 1.08 a	ne	93.34 ± 3.04 ab	-
*T. cucumeris**	0.1	49.91 ± 3.39 c	22.69 ± 7.97 d	-	67.41 ± 5.80 bc
	0.5	-	47.87 ± 8.59 cd	-	100.00 ± 0.00 a
	1	66.96 ± 4.61 bc	70.54 ± 5.42 bc	50.53 ±15.73 c	-
	2.5	70.65 ± 1.50 bc	71.55 ± 4.18 bc	81.81 ± 7.19 ab	-
	5	77.67 ± 2.24 ab	82.17 ± 2.97 ab	87.59 ± 1.51 ab	-
	10	92.87 ± 2.74 ab	86.40 ± 4.38 ab	93.91 ± 1.51 ab	-
R. oryzae$^{\Delta}$	0.1	-	-	-	0.00 ± 0.00 j
	0.25	-	-	-	48.77 ± 6.37 hi
	0.5	-	-	-	84.58 ± 0.51 bc
	1	-	-	-	99.30 ± 0.35 a
	2.5	14.20 ± 5.88 i	-	-	-
	5	82.66 ± 1.57 bcd	41.73 ± 2.37 hi	54.94 ± 3.43 ghi	100.00 ± 0.00 a
	10	92.79 ± 2.87 ab	60.73 ± 6.44 fgh	62.23 ± 2.48 fgh	-
	30	100.00 ± 0.00 a	82.35 ± 1.02 bcd	67.33 ± 1.57 efg	-
	40	-	86.58 ± 1.00 abc	69.48 ± 2.02 def	-
	50	-	-	76.54 ± 0.49 cde	-

Values are the mean of three replicates ± SD. The value in the column followed by the same letter is not significantly different at $p < 0.05$, $^{\Delta}IC_{50}$ measured after 2 days incubation, $^{*}IC_{50}$ measured after 10 days incubation, and $^{*}IC_{50}$ measured after 14 days incubation, ne means no effect of authentic compounds as antifungal, Dashes means sample were not analyzed.

The antifungal activity of essential oils against *T. cucumeris* at concentrations of 1, 2.5, 5, and 10 mg/ml ranged from 57.44 to 59.52%, 33.78 to 78.80%, 41.18 to 89.30%, and 80.35 to 100%, respectively. Antifungal indices of α-terpineol against *T. cucumeris* also showed higher inhibitory effects than those of 1,8-cineole, β-caryophyllene and D(+)-limonene, with growth of *T. cucumeris* on PDA medium with α-terpineol was also completely inhibited at concentration 0.5 mg/ml.

Antifungal activities of essential oils against fast-growing species *R. oryzae* at concentrations of 5, 10, 20, and 30 mg/ml showed antifungal indices ranged from 24.86 to 70.27%, 55.34 to 98.26%, 97.64 to 98.70%, and 100%, respectively. The 100% inhibition was obtained at concentration of 30 mg/ml. Antifungal indices of 1,8-cineole at concentrations 2.5, 5, 10, and 30 mg/ml, D(+)-limonene at concentrations of 5, 10, 30, and 40 mg/ml, β-caryophyllene at concentrations of 5, 10, 30, 40, and 50 mg/ml and α-terpineol at concentrations of 0.1, 0.25, 0.5, 1, and 5mg/ml were used. This species, compared to *F. oxysporum* and *T. cucumeris,* required higher concentration in order to obtain higher inhibitory effects.

Table 5. IC_{50} of antifungal indices of *M. leucadendron* leaf oils and authentic compounds against three plant pathogenic fungi.

Sample	IC_{50} (mg/ml)		
	F. oxysporum*	*T. cucumeris**	*R. orizae*$^{\Delta}$
L1A1	0.11	1.94	5.42
L1A2	0.01	2.17	6.11
L1A3	0.08	4.20	6.80
L2A1	0.05	3.96	1.35
L2A2	0.02	0.52	6.09
L2A3	0.05	0.76	6.43
L3A1	0.07	1.50	7.61
L3A2	0.01	0.64	2.04
L3A3	0.05	2.49	2.68
1,8-cineole	0.35	0.13	3.78
D(+)-limonene	n.e	0.51	6.79
β-caryophyllene	0.16	0.71	2.55
α-terpineol	0.03	0.04	0.30

$^{\Delta}IC_{50}$ measured after 2 days incubation, $^{*}IC_{50}$ measured after 10 days incubation, $^{*}IC_{50}$ measured after 14 days incubation and ne means no effect.

Table 5 shows the IC_{50} of *M. leucadendron* Linn. leaf oils and its authentic compounds (1,8-cineole, β-caryophyllene, D(+)-limonene, and α-terpineol). IC_{50} of *M. leucadendron* leaf oils against *F. oxysporum* ranged from

0.01 to 0.11 mg/ml and those against *T. cucumeris* ranged from 0.52 to 4.20 mg/ml, with the lowest IC_{50} was obtainedfrom the oil at plant age of 10 years-old (A2). IC_{50} of the oils against *R. oyzae* ranged from 1.35 to 7.61 mg/ml. The IC_{50} of the oils against *R. oryzae* showed the lowest activity. The other studies also showed the low antifungal activity of essential oils on *R. oryzae* (Yazdani *et al*. 2009). The IC_{50} of authentic compounds showed the highest inhibitory effect of α-terpineol on *F. oxysporum, T. cucumeris* and *R. oryzae*. Therefore, higher activity of *M. leucadendron* leaf oils from plant age of 10 (A2) years was probably due to the highest content of α-terpineol in this plant. These essential oils were effective against plant pathogenic fungi of *F. oxysporum* and *T. cucumeris*, but they showed the lowest antifungal activity against *R. oryzae*. The higher inhibitory effect of *M. leucadendron* leaf oils was probably due to the existence of α-terpineol.

Conclusions

In conclusion, this study indicated the potential of antioxidant and antifungal activities of *M. leucadendron* leaf oils. *M leucadendron* oil can be used as a potential source of sustainable antioxidant and eco-friendly bio-fungicides. However, further study on bioactivity of *M. leucadendron* oils is needed.

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