Utilization of *Melaleuca leucadendron* Essential Oil

Rini Pujiarti, Yoshito Ohtani, Titis Budi Widowati, and Kasmudjo

Abstract

The chemical composition, antioxidant, antifungal, and physiological effects of commercial *Melaleuca leucadendron* LINN. (*Kayu Putih*) oil from Sukun, East Java, Indonesia was investigated in this study. GC-MS analysis showed the majority compounds of this oil are mixtures of monoterpenes and sesquiterpenes, which 1,8-cineole (53.90%) was the most abundant compound in this oil. *In vitro* antioxidant assay showed this essential oil possessed antioxidant activity (IC_{50}: 4.24mg/ml). *In vitro* antifungal assay showed relatively strong antifungal activity of this oil against plant pathogenic fungi of *F. oxysporum* (IC_{50}: 0.44 mg/ml), *T. cucumeris* (IC_{50}: 0.97mg/ml) and *R. oryzae* (IC_{50}: 7.71mg/ml). The investigation on the effect of the odor of *M. leucadendron* oil in this study also showed functional fragrance of this oil for human physiological behavior controlling.

Keywords: *M. leucadendron* oil, chemical composition, antioxidant, antifungal, physiological effect.

Introduction

Melaleuca species are native of Australia and Southeast Asia. They consist of more than 100 species, some of which are known as essential oil rich species (Sakasegawa *et al*. 2003). *M. leucadendron* LINN. is one of *Melaleuca* species which found in northern Australia, Indonesia, Papua New Guinea, Thailand and Vietnam. The synonym name of this species are *M. cajuputi* Roxb., *M. cumingiana et lancifolia* Turccz., *M. minor* Sm., *M. saligna* Bl., *M. viridifolia* Gaertn., *Myrtus leucadendra* L. and *M. saligna* Gmel. (Dalimartha 2008).

M. leucadendron oil is essential oil obtained by distillation process from fresh leaves and terminal twigs of this tree (Yoshida 1996; Arnold and Perez 2001; Sakasegawa *et al*. 2003). In Indonesia this essential oil is famous with the name *Kayu Putih* oil or Cajaputi oil. This essential oil is one of the most important NTFP (Non-timber forest products) in Indonesia along with rattan, bamboo, resins, tengkawang seed, sandalwood oil, honey and shellac (Vantomme *et al.* 2002). Forest area of *M. leucadendron* in Indonesia is about 620.000 ha, more than 90% are natural forest and the other are forest plantations. The natural forest of *M. leucadendron* are mostly located in Maluku, Papua, East Nusa Tenggara, Southeast Sulawesi, South Sumatra and forest plantations are located in Java (Central Java, West Java, East Java and Yogyakarta) (Kasmudjo 2011).

This essential oil has been used as a perfume and a popular remedy (*Jamu* medicine) for the treatment of colic, cholera, headaches, toothache and various skin diseases in Indonesia (Perry 1980). They are used mainly in the manufacture of cosmetics, germicides and as antiseptic agents. They are also used as carminatives and in the treatment of several ailments (Yoshida 1996). In recent years, many studies have investigated about chemical composition and bioactivities of essential oil from *Melaleuca* species. However, there has been little research on chemical compositions and utilizations of *M. leucadendron* oil from Indonesia. For this reasons, the chemical composition and utilizations of essential oil of *M. leucadendron* was investigated. This study demonstrated chemical composition, antioxidant, antifungal effects and physiological effects on human behavior of *M. leucadendron* essential oil from Sukun, East Java, Indonesia in order to improve its commercial values and increase utilization.

Materials and Methods

Essential Oil Samples and GC-MS Analysis

M. leucadendron oil was provided by Perum Perhutani Unit II, East Java, Indonesia. This oil was obtained by steam distillation process from fresh leaves and terminal twigs of *M. leucadendron.*

GC-MS analyses were carried out on 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; 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. The chemical components were identified using gas chromatography by comparison of their Kovats retention indices (RI) (Van Den Dool 1963), and with National Institute of Standards and Technology (NIST) database library and authentic standards. The retention indices were determined using homologous series of *n*-alkanes (C_8~C_{22}, Wako Chemicals Co.) under the same operating conditions.

Antioxidant Assay

The antioxidant activity of *M. leucadendron* oil was determined by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Ethanol solutions (1 ml) of

samples were added to a solution (10 ml) of 0.25 mM DPPH in ethanol. The control sample was prepared using only ethanol without essential oil and butylated hydroxyanisole (BHA) was used as positive control. The solution was kept in water bath at 30ºC for 30 min. The absorbance of reaction mixture was spectrophotometrically measured at 515 nm using U-2810 spectrophotometer (Hitachi, Japan). Percent 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 tested sample. The concentration of 50% inhibition (IC_{50}) was obtained by extrapolation of the curves, percent antioxidant indices versus concentration.

Fungal Strains and Antifungal Assay

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

Antifungal activity was determined by method of Wang *et al.* (2005) with slight modification. PDA (Potato Dextrose Agar, Difco) plates were prepared using Petri dishes (9 cm diameter). The different concentration of oil samples were serially diluted with methanol and added to 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 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 contain methanol without essential oil. Percent antifungal was calculated by the following equation:

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

where Sa is surface area of mycelium growth of treatment (cm^2) and Sb is surface area of mycelium growth of control (cm^2). The concentration of 50% inhibition (IC_{50}) was obtained by extrapolation of the curves, percent antifungal indices versus concentration.

Physiological Effect Analysis

The examinees were volunteers with normal olfaction. Data were collected from 10 students, five males and five females, aged 22 to 35 years (mean: 26 years). Tests were conducted in laboratory with room temperature 26ºC. Influence of the essential oil on human physiological behaviors includes systolic (maximum) and diastolic (minimum) blood pressures, pulse rate and stress index before and after sniffing (3 minutes) *M. leucadendron* oil in paper strip. Two control tests were conducted by using normal condition without sniffing essential oil and sniffing distilled water in paper strip. The systolic (maximum), diastolic (minimum) blood pressures and pulse rate were measured by digital sphygmometer. The stress index was measured by Cocorometer (CM-1.1, NIPRO Co.) based on the amylase activity of saliva (Yamaguchi and Yoshida 2005).

Statistical Analysis

All experiments were replicated three times, and the data were averaged. The results were analyzed using SPSS statistical program and expressed as mean ± SD. Significant differences of antioxidant and antifungal activities were determined by Scheffe`s test and significant differences of physiological effects were determined by *t*-test.

Results and Discussion

Chemical Composition

GC-MS investigations of the compounds of *M. leucadendron* oil are presented in Table 1. Eighteen compounds have been identified in this essential oil. Compared with previous study (Pujiarti *et al.* 2011) of *M. leucadendron* leaf oils extracted by water-steam distillation, this oil extracted by steam distillation contains less chemical compounds.

The compounds of this oil are mostly monoterpene hydrocarbons (α-thujene, α–pinene, β-pinene, β-myrcene, carene, D(+) limonene, γ-terpinene and terpinolene), oxygenated monoterpenes (1.8-cineole, linalool, terpinene-4-ol, ocimeol and α-terpineol), sesquiterpene hydrocarbons (β-caryophylene and β-eudesmene) and oxygenated sesquiterpene (viridiflorol and cubenol). The result showed, 1,8-cineole (53.90%) was the major compound in these oils, followed by α-terpineol (9.53%), D(+)-limonene (6.52%) and β-caryophyllene (4.11%). Several studies previously also reported that 1,8-cineole is the major compound in *M. leucadendron* oils. (Sakasegawa *et al.* 2003; Farag *et al.* 2004; Pujiarti *et al.* 2011).

Antioxidant Activity

DPPH radical scavenging ability of *M. leucadendron* oil is shown in Table 2. The result shows concentration of the oils affect the radical scavenging activities. Antioxidant activity of *M. leucadendron* is expressed as IC_{50} value, which is defined as the concentration of antioxidant capable of decreasing the activity by 50%. The IC_{50} of *M. leucadendron* oil in this study is 4.24mg/ml. If compared with positive control of BHA (IC_{50}: 25.68 x 10^{-3}mg/ml) this oil has weak antioxidant activities. The low antioxidant activity of this oil compared with positive control is due to the absence of phenolic compounds, which phenolic compounds have high antioxidant activities.

The results of this study show *M. leucadendron* oil has

antioxidant activity. The antioxidant ability of *M. leucadendron* oil (IC_{50}: 4.24mg/ml) is probably due to presence of 1,8-cineole, D(+)-limonene, and β-caryophyllene. This finding correlated with our previous study on the antioxidant activities of 1.8-Cineole (IC_{50}: 4.92mg/ml), D(+)-limonene (IC_{50}: 4.58mg/ml), and β-caryophyllene (IC_{50}: 3.68mg/ml). It seems to be a general trend that the essential oils which contain monoterpene hydrocarbons, oxygenated monoterpenes and/or sesquiterpenes have greater antioxidant properties (Tepe *et al.* 2004). Another research shows that some oils rich in non-phenolic compounds also have antioxidant potentials (El-Massry *et al.* 2002).

Antifungal Activity

The antifungal indices and IC_{50} of *M. leucadendron* oil against three pathogenic fungi of *R. oryzae*, *T. cucumeris* and *F.oxysporum* are shown in Table 3. Any concentrations of the solution tested inhibited the fungal growth with varying degree of effectiveness. The result showed that the antifungal activity of *M. leucadendron* oil higher against *T. cucumeris* and *F.oxysporum* than against *R. oryzae*. The IC_{50} value of *M. leucadendron* oil against *F. oxysporum* was 0.44mg/ml, *T. cucumeris* 0.97mg/ml and *R. oryzae* 7.71mg/ml.

The effectiveness of this essential oil against pathogenic fungi is probably due to the complex compounds in this oil. The major compounds might influence the antifungal activity, but possible synergic and antagonistic effects of compounds also play important role on fungal inhibition (Deba *et al.* 2008). Previous study showed α-terpineol has the highest inhibitory effect against some pathogenic fungus, also β-caryophyllene was very toxic against *Fusarium* species on the antifungal activities of essential oils against some agricultural pathogenic fungal species (Cakir *et al*. 2004). Another monoterpene alcohol, linalool, reported its had antifungal activity (Pattnaik *et al.* 1997) and 1,8-cineole also have antifungal activity (Vilela *et al.* 2009). The present study indicates the effectiveness of *M. leucadendron* as antifungal agent. Antifungal activity of *M. leucadendron* oil in this study is probably due to the presence of α-terpineol, β-caryophyllene, 1,8-cineole and mixed numerous compounds.

Physiological Effect

Influence of *M. leucadendron* oil on human physiological behaviors was analyzed via the sense of smell or olfactory system, which in turn may cause physiological effects. Paired sample *t*-tests were used to analyze the physiological data before and after stimulation by distilled water (control group) or *M. leucadendron* oil. Physiological parameters include blood pressure, pulse rate and stress index, and the results before and after sniffing *M. leucadendron* oil are shown in Table 4 and Table 5.

Table 1. Chemical composition of *M. leucadendron* oil.

No.	RI[a]	Compounds	Percent Compound (%)
1	934.67	α-Thujene[b]	0.26
2	946.79	α -Pinene[c]	2.79
3	988.32	2-Pentanone[b]	3.83
4	998.57	β-Pinene[b]	1.96
5	1015.64	β-Myrcene[c]	0.58
6	1035.14	Carene [c]	0.36
7	1042.53	D(+) Limonene[c]	6.52
8	1060.48	1.8-Cineole[c]	53.90
9	1071.23	γ-Terpinene[b]	2.74
10	1099.06	Terpinolene[c]	1.20
11	1196.79	Linalool[c]	0.21
12	1296.80	Terpinene-4-ol[c]	0.60
13	1328.79	α-Terpineol[c]	9.53
14	1440.69	Ocimeol [b]	1.08
15	1503.31	β-Caryophylene[c]	4.11
16	1555.87	β-Eudesmene[b]	2.19
17	1802.88	Viridiflorol[b]	0.28
18	1810.94	Cubenol[b]	0.26

[a] Retention indices on the column relative to C_8-C_{22} *n*-alkanes

[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

Table 2. Antioxidant activities and IC_{50} of *M. leucadendron* oil by DPPH method assay.

Sample	Concentration (mg/ml)	Inhibition (%)[a]	IC_{50} (mg/ml)
M. leucadendron oil	1	13.82 ± 0.40 a	4.24
	2.5	31.69 ± 0.44 b	
	5	61.44 ± 0.07 c	
	7.5	85.89 ± 0.25 d	
BHA (butylated hydroxyanisole)	20×10^{-3}	43.47 ± 0.41 a	25.68×10^{-3}
	50×10^{-3}	71.41 ± 0.14 b	
	80×10^{-3}	82.08 ± 0.23 c	

[a] Value followed by different letter are significantly different by the Scheffe test ($P<0.05$)

Table 3. Antifungal indices and IC_{50} of *M. leucadendron* oil against three plant pathogenic fungi.

Fungi	Concentration (mg/ml)	Antifungal Indices (%)	IC_{50} (mg/ml)
R. oryzae	1	10.18 ± 2.28 a	7.71
	5	39.21 ± 1.79 b	
	10	66.22 ± 5.90 c	
	15	85.19 ± 12.22 c	
T.cucumeris	0.5	11.38 ± 4.43 a	0.97
	1	76.66 ± 13.53 b	
	5	92.32 ± 4.21 b	
	10	94.42 ± 1.11 b	
F. oxysporum	0.01	37.04 ± 18.79a	0.44
	0.05	42.39 ± 11.04a	
	0.1	43.66 ± 31.62a	
	1	62.56 ± 15.41b	

The result shows that in the control group and after sniffing *M. leucadendron* oil were not significant differences in the systolic (max.), diastolic (min.) blood pressure, pulse rate and stress index. But, this study elucidated that systolic and diastolic blood pressure decreased after sniffing *M. luecadendron* oil (Figure 1 and Figure 2). Several studies explained that blood pressure is considered to be associated with cardiovascular health (Zi-lin *et al.* 2009) and inhalation of essential oil could lead to a decrease in blood pressure and an increase in attentiveness (Hongratanaworakit *et al.* 2002, 2004).

It was also found pulse rate decreased after sniffing *M. leucadendron* oil (Figure 3). These variations suggest that parasympathetic nervous activity increases and physiological arousal decreases when exposed to this fragrance, leading to relaxation and emotional alleviation (Ouyang *et al.* 2002 in Zi-lin *et al.* 2009). This finding likely indicates that *M. leucadendron* oil fragrance possesses a sedative effect. A review article explained that the common physiological measurements of heart activity are pulse rate, change of pulse rate as indices for sedative effect (Hongratanaworakit 2004). Faster heartbeat is often caused by stress. Previous studies also used changes of heartbeat rate (pulse rate) and blood pressure for indicator to measure the sedative effect of fragrances (Yamaguchi 1990; Brauchli *et al.* 1995; Ohtani *et al.* 2009).

The stress index in this study was evaluated by amylase activity of saliva. Higher values mean to be highly stressed. After sniffing *M. leucadendron* oil, stress index is decreased (Figure 4). It means that *M. leucadendron* fragrance makes human relaxed. This study observed that *M. leucadendron* oil has positive effect on human physiological behavior and can be used as a beneficial mental relaxation therapy (aromatherapy).

Table 4. Paired-samples *t*-test of control group.

Parameters	Control Group		*t*	*df*	*P**
	Normal condition	Control (sniffing distilled water)			
Systolic Blood Pressure (mmHg)	106.44 ± 11.51	103.87±14.60	0.437	9	0.67
Diastolic Blood Pressure (mmHg)	70.60 ± 10.53	67.83±11.61	0.558	9	0.58
Pulse Rate (bpm)	75.33 ± 7.55	75.40± 6.59	-0.020	9	0.98
Stress Index (KU/L)**	73.33 ± 35.53	54.40 ±22.43	1.424	9	0.17

* $p<0.05$ means correlation is significant at the 0.05 level (2-tailed test), **KU/L of alpha-amylase

Table 5. Paired-samples *t*-test of the effects of *M. leucadendron* oil.

Parameters	Before sniffing *M.leucadendron* oil (Control / sniffing distilled water)	After sniffing *M. leucadendron* oil	*t*	*df*	*P**
Systolic Blood Pressure (mmHg)	103.87 ± 14.60	103.80 ± 11.61	0.011	9	0.99
Diastolic Blood Pressure (mmHg)	67.83 ± 11.61	66.93 ± 9.23	0.192	9	0.85
Pulse Rate (bpm)	75.40 ± 6.59	72.77 ± 6.25	0.916	9	0.37
Stress Index (KU/L)**	54.40 ± 22.43	49.93 ± 22.66	0.443	9	0.66

* $p<0.05$ means correlation is significant at the 0.05 level (2-tailed test), **KU/L of alpha-amylase

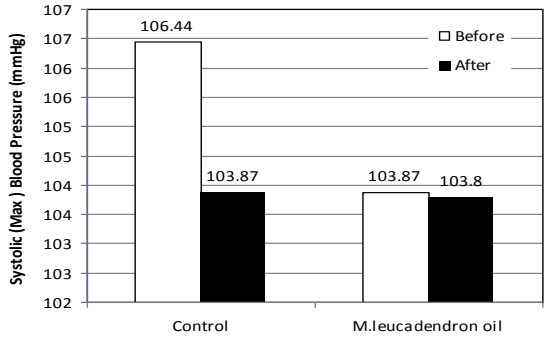

Figure 1. Systolic (max.) blood pressure of control group and stimulant before and after sniffing *M. leucadendron* oil.

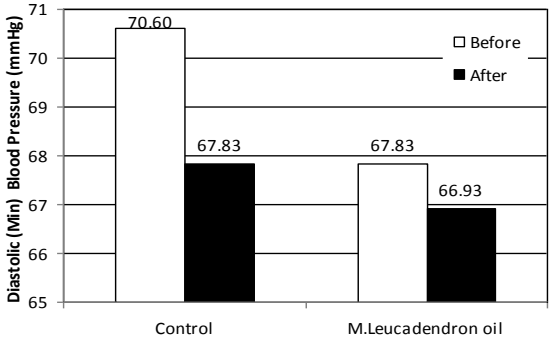

Figure 2. Diastolic (min.) blood pressure of control group and stimulant before and after sniffing *M. leucadendron* oil.

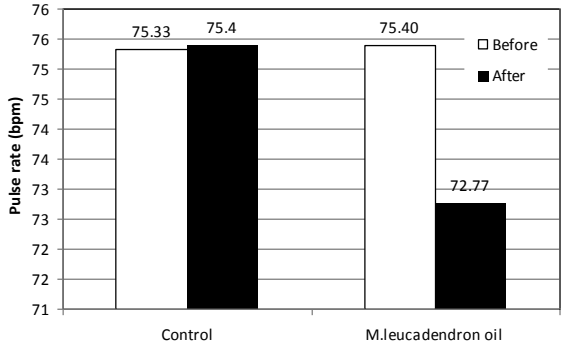

Figure 3. Pulse rate of control group and stimulant before and after sniffing *M. leucadendron* oil.

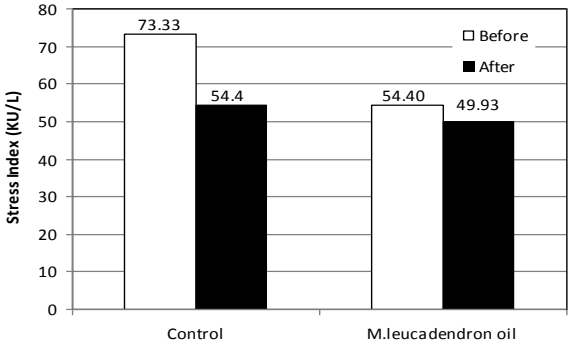

Figure 4. Stress Index of control group and stimulant before and after sniffing *M. leucadendron* oil.

Conclusions

This present study shows that *M. leucadendron* essential oil possesses compounds with antioxidant and antifungal properties that can be used as sources of natural antioxidant and antifungal agents (especially antifungal agents in plant diseases). This essential oil also shows physiological effects on human behavior such as sedative effect on autonomic nerve activity and mood state.

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References

Arnold, J.E.M.; M.R. Perez. 2001. Can Non-Timber Forest Products Match Tropical Forest Conservation and Development Objectives?. Ecol. Econ. 39: 437–447.

Brauchli, P.; P.B. Ruegg; F. Etzweiler; H. Zeier. 1995. Electrocortical and Autonomic Alteration by Administration of A Pleasant and An Unpleasant Odor. Chem. Senses 20: 505-515.

Dalimartha, S. 2008. Atlas of Herbs Medicines in Indonesia, Chapter 5. Pustaka Bunda. Jakarta.

Cakir, A.; S. Kordali; H. Zengin; S. Izumi; T. Hirata. 2004. Composition and Antifungal Activity of Essential Oils Isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. J. Flavor and Fragrance 19: 62–68.

Deba, F.; T.D. Xuan; M. Yasuda; S. Tawata. 2008.

Chemical Composition and Antioxidant, Antibacterial and Antifungal Activities of the Essential oils from *Bidens pilosa* Linn. var. *Radiata*. Food Control 19: 346–352.

El-Massry, K.F.; A.H. El-Ghorab; A. Farouk. 2002. Antioxidant Activity and Volatile Components of Egyptian *Artemisia judaica* L. Food Chemistry 79: 331–336.

Farag, R.S.; A.S. Shalaby; G.A. El-Baroty; N.A. Ibrahim; A. Ali; E.M. Hassan. 2004. Chemical and Biological Evaluation of the Essential Oils of Different *Melaleuca* Spesies. Phytotherapy Research 18:30-35.

Hongratanaworakit, T.; E. Heuberger; G. Buchbauer. 2002. Influence of Ylang-ylang Oil on Mental, Emotional and Human Physiological Parameters. Proc. 33rd International Symposium on Essential Oils (ISEO 2002), Lisbon, Portugal. p 37.

Hongratanaworakit, T. 2004. Physiological Effects in Aromatherapy. Songklanakarin J. Sci Technol. 26 (1): 117–125.

Kasmudjo. 2011. Non-timber Forest Products. Cakrawala. Yogyakarta (in Indonesian).

Ohtani, Y.; P. Siramon; R. Pujiarti; H. Ichiura. 2009. New Utilization Methods of Esssential Oils from Woody Biomass. Transactions of the Materials Research Soc. of Japan 34(4): 715-718.

Pattnaik, S.; V.R. Subramanyam; M. Bapaji; C.R. Kole. 1997. Antibacterial and Antifungal Activity of Aromatic Constituents of Essential Oils. Microbios. 89: 39–46.

Perry, L.M. 1980. Medicinal Plants of East and Southeast Asia, the MIT Press, Cambrige, Massachusettes 9.284.

Pujiarti, R.; Y. Ohtani; H. Ichihura. 2011. Physicochemical Properties and Chemical Compositions of *Melaleuca leucadendron* Leaf Oils Taken from the Plantations in Java, Indonesia. J. Wood Sci. 57: 446-451.

Sakasegawa, M.; H.Keko; M. Yatagai. 2003. Composition and Antitermite Activities of Essential oils from *Melaleuca* spesies. J. Wood Sci. 49: 181-187.

Tepe, B.; E. Donmez; M. Unlu; F. Candan; D. Daferera; G. Vardar-Unlu. 2004. Antibacterial and Antioxidative Activities of the Essential Oils and Methanol Extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chemistry 84:519–525.

Van Den Dool, H.; P. Dec Kratz. 1963. A Generalization of the Retention Indices System Including Linear Temperature Programmed Gas-Liquid Partition Chromatography. J. of Chromatography 11:463-471.

Vantomme, P.; P.A. Markkula; R.N. Leslie. 2002. Non-Wood Forest Products in 15 Countries of Tropical Asia: An Overview. EC-FAO Partnership Programme 2000–2002. Bangkok, Thailand. p 188.

Vilela, G. R.; G.S., Almeida; M.A.B.R. D'Arce; M.H. Moraes; J.O. Brito; F.G.F. Maria; S. Cruz; S.M.S. Piedade; M.A.C. Domingues; E.M. Gloria. 2009. Activity of Essential Oil and Its Major Compound, 1,8-Cineole, from *Eucalyptus globulus* Labill., Against the Storage Fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. J. of Stored Products Research 45: 108–111.

Wang, SY.; PF. Chen; S.T. Chang. 2005. Antifungal Activities of Essential Oils and their Constituents from Indigenous Cinnamon (*Cinamomum osmophloeum*) Leaves Against Wood Decay Fungi. Bioresource Technol. 96: 813-818.

Yamaguchi, H. 1990. Effects of Odor on Heart Rate. In: The Psychophysiological Effects of Odor, Aromachology, Koryo. p 168.

Yamaguchi, M.; H. Yoshida. 2005. Chemical Sensors. 21(3): 92-98.

Yoshida, T.; T. Maruyama; A. Nitta; T. Okuda. 1996. An Hydrolysable Tannin and Accompaning Polyphenols from *Melaleuca leucadendron*. Phytochemistry 42: 1171-1173.

Zi-lin, J.; L. Xia; Z. Qi-xiang; P. Hui-tang; A.N. Xue. 2009. Human Responses to Flower Fragrance of *Lilium* 'Siberia' and *Rosa* 'Escimo'. For. Stud. China 11(3): 185–189.

Rini Pujiarti, Titis Budi Widowati and Kasmudjo
Department of Forest Product Technology
Faculty of Forestry, Gadjah Mada University
Jl. Agro No. 01, Bulaksumur, Yogyakarta, 55281, Indonesia.
Tel. : 0274-550541
Fax. : 0274-550543
E-mail : rpujiarti@gmail.com
titis_budiw@yahoo.co.id
kasmudjo@yahoo.com

Yoshito Ohtani
Department of Forest Science
Faculty of Agriculture, Kochi University.
B-200 Monobe, Nankoku, Kochi 783-8502, Japan.
Tel. : +81-88-864-5143.
Fax : +81-88-864-5143.
E-mail : ohtani@kochi-u.ac.jp.