

Effect of *Melaleuca leucadendron*, *Cananga odorata* and *Pogostemon cablin* Oil Odors on Human Physiological Responses

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Abstract

The aims of this study were to evaluate the main compounds and the effect of *M. leucadendron*, *C. odorata* and *P. cablin* essential oil odors on human physiological responses. The chemical compounds of essential oils in this study were analyzed by gas chromatography-mass spectrometry (GC-MS) and the physiological effects of essential oils on human were analyzed via the sense of smell olfactory system. Physiological parameters of systolic blood pressure, diastolic blood pressure, pulse rate, stress index, and brain wave were recorded before and after sniffing essential oils. The result shows that the main compound of *M. leucadendron*, *C. odorata*, and *P. cablin* is 1,8-cineole, Caryophyllene and Patchouli Alcohol, respectively. Our results demonstrated that *M. leucadendron* and *C. odorata* oil odors possessed sedative effect and *P. cablin* oil odor had tendency to contain stimulating effect on human physiologies.

Keywords: *M. leucadendron*, *C. odorata*, *P. cablin*, chemical composition, physiological effect.

Introduction

Essential oils from natural sources are endowed with many physiological and pharmacological properties. They have many positive properties and effect such as being anti-inflammatory, antiseptic, appetite-stimulating, carminative, choleric, expectorant, circulation-stimulating, deodorizing, insecticidal, granulation-stimulating, hyperaemic, insect-repelling, and sedative (Steflitsch *et al.* 2008).

Essential oils can be absorbed into the body via the skin or the olfactory system. When essential oils are inhaled, they stimulate parts of the brain and influence physical, emotional, and mental health because of their structural complexity (Rho *et al.* 2006). Some of the odors of essential oils are used in the treatment of depression, anxiety and some types of cognitive disorders in aromatherapy (Kuroda *et al.* 2005).

The effect of odors on human are divided into physiological and psychological effects. Physiological measurement would be beneficial in order to elucidate actual physiological change in human. Physiological parameters which can be used as indicator for measure of the odor effects such as heart rate, blood pressure, electrodermal activity, electroencephalogram, slow potential brain wave, and eye blink rate or pupil functions (Hongratanaworakit 2004). A study found that olfactory stimulation produces immediate changes in physiological parameters such as blood pressure, muscle tension, pupil dilation, blink magnitude, skin temperature, skin blood flow, electrodermal activity, pulse rate, and brain activity (Rho *et al.* 2006).

The essential oils also have stimulating and sedative/relaxing properties and physiological test can be used to classify odors as stimulant or sedative. However, there is less scientific information about physiological effects

of essential oil from Indonesia. In this study, we observed the inhalation of three essential oils from Indonesian essential oil plants, namely *M. leucadendron*, *C. odorata* and *P. cablin*. *M. leucadendron* 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 *et al.* 1996). *C. odorata* is an evergreen tree indigenous to China, Burma, Thailand, Indonesia and the Philippines. Its oil is used in perfumery, aromatherapy, pharmaceutical, cosmetics and food industry (Kristiawan *et al.* 2008). Burdock and Carabin (2008) describes the use of *Cananga* flowers to add scent to coconut oil; the essential oil is used as a flavoring in candies, icings, baked goods, soft drinks, and chewing gum in Southeast Asia. This oil is also used topically as a sedative, antiseptic, hypotensive, and aphrodisiac. *Pogostemon cablin* from Southeast Asia is cultivated extensively in Indonesia, Philippines, and Malaysia. This essential oil is also called patchouli oil (Singh *et al.* 2002). Patchouli oil is one of the important natural essential oils used to give a base and lasting character to a fragrance in perfumery industry. This oil is very appreciated for its characteristic pleasant and long lasting woody, earthy, and camphor odor, as well as for its fixative properties, being suitable for use in soaps and cosmetic products. The patchouli essential oil also shows medicinal properties including anti-inflammatory, aphrodisiac, anti-depressive, astringent, carminative, febrifuge, diuretic, tonic, antiemetic, trypanocidal, antibacterial, and antifungal activity (Zhao *et al.* 2005).

The aims of this study were to examine major compounds and effects of *M. leucadendron*, *C. odorata* and

P. cablin essential oils from Indonesia on human physiological responses by observing the changes of systolic and diastolic blood pressures, pulse rate, stress index, and brain wave after sniffing the essential oil samples.

Materials and Methods

Essential Oil Samples

Samples were obtained from Indonesian essential oil plants of *M. leucadendron*, *C. odorata*, and *P. cablin*. Essential oils of each sample was obtained by hydrodistillation method extraction from fresh leaves of *M. leucadendron*, fresh flowers of *C. odorata* and dry leaves of *P. cablin*. Extract processes were conducted in Non Wood Forest Product Laboratory, Forest Product Technology Department, Faculty of Forestry, Gadjah Mada University, Indonesia. The oils produced were kept in labeled bottles at approximately 0°C until used.

GC-MS Analysis

The chemical analysis of the essential oils were conducted by 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.

Identification of chemical compounds was carried out by comparing the retention time with National Institute of Standards and Technology (NIST) database library and literatures.

Physiological Analysis

The participants in this study were 10 volunteers with normal olfaction. Data were collected from five males and five females volunteers, with aged 22 to 35 years (mean: 26 six years). The physiological parameters of systolic (maximum), diastolic (minimum) blood pressures, pulse rate, stress index, and intensities of brain wave were evaluated in this study. The parameters were measured before and after sniffing (3 min) *M. leucadendron*, *C. odorata* and *P. cablin* oils in paper strip. Two control tests were conducted by using normal condition without sniffing essential oils (C_a) and by only sniffing the distilled water in paper strip test (C_b). Each parameter was determined by averaging the three replicates data.

The systolic (maximum), diastolic (minimum) blood pressures and pulse rate were measured by digital sphygmometer. In this study, human blood pressure rate

value were divided into: low (min./max.: 40 ~ 60 / 70 ~ 90 mmHg), ideal (min./max.: 60 ~ 80 / 90 ~ 120 mmHg), pre-high blood (min./max.: 80 ~ 90 / 120 ~ 140 mmHg), and high (min./max.: 90 ~ 100 / 140 ~ 190 mmHg).

The stress index was measured by Cocorometer (CM-1.1, NIPRO Co.) based on the amylase activity of saliva (Yamaguchi and Yoshida 2005). Higher stress index values mean to be highly stressed. Stress levels were evaluated in this study. Stress level determined by Cocorometer was classified into: no stress (0 ~ 30 KU/L), low stress (30 ~ 45 KU/L), medium stress (46 ~ 60 KU/L), and high stress (> 61 KU/L).

Essential oil odors produce cortical brain wave activity responses involving alpha, beta, delta and theta waves. The highest ratio of the alpha wave indicates decrease of stress. The intensities of wave brain (α (slow: 7 ~ 8Hz (α_s), mid: 9 ~ 11Hz (α_m), fast: 12 ~ 14Hz (α_f)), β (15 ~ 23Hz), θ (4 ~ 6Hz) – waves) were measured by Mind Sensor II (Naryoku Kaihatsu Kenkyusho Co.). The mental condition was evaluated as the relaxation value = $(3\alpha_m + \alpha_s + \theta) \times 100\%$.

Statistical Analysis

The results were analyzed using *t*-test to evaluate the differences in effectiveness of essential oils on human physiological responses. Results with $P < 0.05$ were considered to be statistically significant.

Results and Discussion

Major Compounds of Essential Oils

The major compounds of GC-MS analysis and percentages of three essential oils are shown in Table 1. Major compounds are presented in Table 1 based on the percent content which has percentage more than 5%.

Four major compounds identified in *M. leucadendron* oil are 1,8-cineole (55.04%), α -terpineol (8.79%), *d*-limonene (6.39%) and β -caryophyllene (5.03%), five major compounds in *C. odorata* are Caryophyllene (36.44%), Germacrene D (17.23%), α -caryophyllene (9.61%), Benzyl benzoate (7.18%), and Linalool (5.97%), and five major compounds in *P. cablin* oil are Patchouli Alcohol (34.40%), Azulene (15.01%), β -humulen (13.15%), α -panasinsen (9.63%) and 7-menthanoazulene (6.91%).

The GC-MS analysis showed that the main compound in *M. leucadendron* oil was monoterpene compound of 1,8-cineole (55.04%), in *C. odorata* oil was sesquiterpene compound of Caryophyllene (36.44%), and in *P. cablin* was sesquiterpene compound of Patchouli Alcohol (34.40%). Several studies previously also reported that 1,8-cineole is the main compound in *M. leucadendron* oils (Sakasegawa *et al.* 2003; Farag *et al.* 2004; Pujiarti *et al.* 2011), main compound in *C. odorata* is Caryophyllene (Kristiawan *et al.* 2008) and the main compound in *P. cablin* is Patchouli Alcohol (Hu *et al.* 2006).

Table 1. Major compound of essential oils.

Plant	Major compound	Percent major compound (%)
<i>M. leucadendron</i>	1,8-cineole	55.04
	α -terpineol	8.79
	<i>d</i> -limonene	6.39
	β -caryophyllene	5.03
<i>C. odorata</i>	Caryophyllene	36.44
	Germacrene D	17.23
	α -caryophyllene	9.61
	Benzyl Benzoate	7.18
	Linalool	5.97
<i>P. cablin</i>	Patchouli Alcohol	34.40
	Azulene	15.01
	β -humulen	13.15
	α -panasinsen	9.63
	7-menthanoazulene	6.91

Table 2. Effects of sniffing essential oil on human physiological responses.

Parameter	Before sniffing essential oil (Control)		After sniffing essential oil		
	C _a *	C _b **	<i>M. leucadendron</i>	<i>C. odorata</i>	<i>P. cablin</i>
Systolic blood pressure (mmHg)	106.43 ± 11.51 a	103.87 ± 14.60 a	100.20 ± 10.26 a	105.20 ± 10.72 a	107.37 ± 13.84 a
Diastolic blood pressure (mmHg)	70.60 ± 10.53 a	67.83 ± 11.61 a	66.93 ± 9.44 a	69.20 ± 11.71 a	69.37 ± 11.83 a
Pulse rate (bpm)	75.33 ± 7.55 a	75.40 ± 6.59 a	72.97 ± 5.61 a	73.40 ± 7.38 a	73.23 ± 7.60 a
Stress index (KU/L)***	73.33 ± 35.53 a	54.40 ± 22.43ab	43.13 ± 15.81 b	49.50 ± 18.34ab	57.53 ± 27.38ab
Brain wave	222.90 ± 16.89 a	224.83 ± 11.27 a	231.17 ± 16.13 a	225.67 ± 19.48 a	222.70 ± 17.85 a

*: normal condition, not sniffing anything, **: sniffing distilled water, ***: KU/L of alpha-amylase

Means followed by the same alphabetical letters are not significantly different by *t*-test at 5% probability level among the samples.

Physiological Analysis

Table 2 shows human physiological responses before (C_a-C_b) and after sniffing *M. leucadendron*, *C. odorata* and *P. cablin* oils. Some parameters were not significant different but the result showed tendency that essential oils of *M. leucadendron*, *C. odorata*, and *P. cablin* can influence human physiologies.

Systolic and Diastolic Blood Pressure

The Systolic and diastolic blood pressures in this study tended to decrease after sniffing *M. leucadendron* oil. Systolic and diastolic blood pressures after sniffing *C. odorata* oil were lower than C_a but higher than C_b and systolic blood pressure after sniffing *P. cablin* oil increased and diastolic blood pressure was lower than C_a but higher than C_b (Figure 1 and 2). In this study, systolic and diastolic blood pressures after sniffing *M. leucadendron* and *C. odorata* oils were reduced. 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 (Hongratanaworakit *et al.* 2002; 2004). On the other hand *P. cablin* oil odor caused an increase of systolic and diastolic blood pressure. This finding likely indicates that *M. leucadendron* and *C. odorata* oils odors possesses a sedative effect (decrease of blood pressure), in contrast *P.*

cablin oil odor possess a stimulant effect (increase of blood pressure). This result shows that *M. leucadendron* and *C. odorata* oils can reduce blood pressure and keep blood pressure in ideal condition.

Pulse Rate

Pulse rate volunteers in this study tended to decrease after sniffing each oil sample (Figure 3). The results showed that *M. leucadendron* oil was the best in reducing the pulse rate, followed by *P. cablin* and *C. odorata* oils, respectively. Romine *et al.* (1999) explained that pulse rate can change under olfactory stimulation. Specifically, it was found that sedative odors decrease pulse rate (Jellinek 1994). A review article explained that the common physiological measurement of heart activity is pulse rate, change of pulse rate as indices for sedative effect and faster heartbeat is often caused by stress (Hongratanaworakit 2004). Several studies previously also used changes of pulse rate and blood pressure as indicates for the measurement of sedative effect of odors (Yamaguchi 1990; Brauchli *et al.* 1995; Ohtani *et al.* 2009). Each essential oil in this study can keep pulse rate (beats/min) in normal blood pressure or in relaxed condition.

Stress Index

This study found that each essential oil seems to have influence on reducing stress index (Figure 4). Among the oil samples, *M. leucadendron* oil was the most effective in reducing stress. Stress index before (C_a : 73.33 KU/L) and after sniffing *M. leucadendron* oil (43.13 KU/L) was significantly different. *M. leucadendron* can reduce stress level from high level to low level. This essential oil showed sedative effect which it can make human feel more relax after sniffing this essential oil. Stress index of *C. odorata* (49.50 KU/L) and *P. cablin* (57.53 KU/L) oils also showed the effect in reducing stress level from high level to medium level.

Brain Wave

Alpha brain wave is increased when the person is relaxed, the highest ratio of the alpha wave indicates decreased of stress. In this study, *M. leucadendron* and *C. odorata* oils can increase alpha brain wave or increase relaxation value. In contrast, relaxation value decreased after sniffing essential oil of *P. cablin* (Figure 5). The result showed that *M. leucadendron* oil is most effective in reducing stress followed by *C. odorata*. However *P. cablin* has tendency to increasing alpha brain wave or have stimulating effect to improving the activity of brain.

In general, *M. leucadendron* and *C. odorata* oils can be used alone as aromatherapy because its have sedative

effect. This effect is probably due to the major compound and sedative odors of these essential oils. It is convinced by a study which reported that 1,8-cineole compound has sedative effect (Ilberger *et al.* 2001). *C. odorata* oil consists of several sesquiterpene and a monoterpene group, which make this essential oil has not strong odor. Previous study reported that *C. odorata* oil consists of sesquiterpene hydrocarbons, alcohols, esters, ethers, phenols, and aldehydes which make this oil has medium aroma (Burdock and Carabin 2008). On the other hands, *P. cablin* oil has tendency to content stimulating effect. It is possible because this oil has strong smell, and usually strong smell possesses stimulating effect. This is probably because main compound of this oil is Patchouli Alcohol which is included in the sesquiterpene group. A previous study reported that *P. cablin* has a very strong odor because the composition of *P. cablin* is unique and complex. It consists of more sesquiterpenes compounds rather than a blend of different mono-, sesqui- and di-terpene compounds. The sesquiterpenes are responsible for the typical *P. cablin* odor (Deguerry *et al.* 2006). This study also shows that control test with sniffing the distilled water (C_b) may indicate an influence of placebo effect. By only sniffing the distilled water, blood pressures, pulse rate, stress index and brain wave has changed, compared to the control in normal condition (C_a).

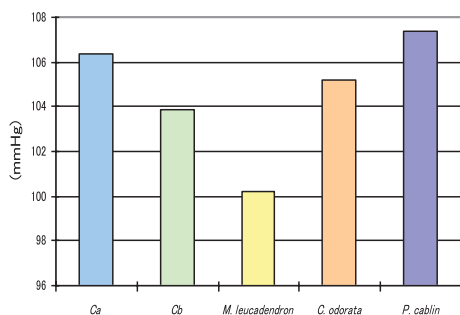


Figure 1. Effect of essential oils on systolic (max.) blood pressure (C_a : normal condition, not sniffing anything, C_b : sniffing distilled water).

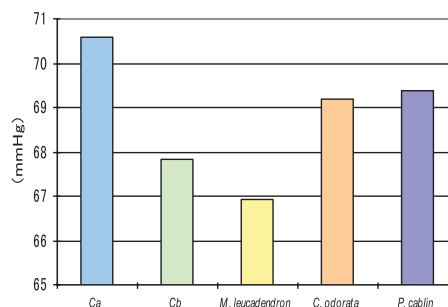


Figure 2. Effect of essential oils on diastolic (min.) blood pressure (C_a : normal condition, not sniffing anything, C_b : sniffing distilled water).

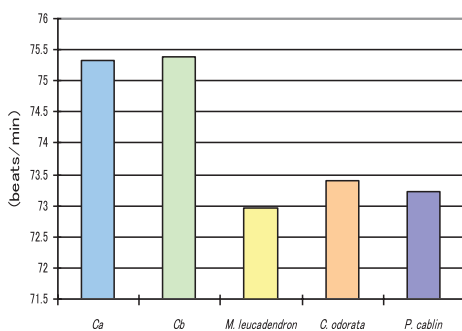


Figure 3. Effect of essential oils on pulse rate (C_a : normal condition, not sniffing anything, C_b : sniffing distilled water).

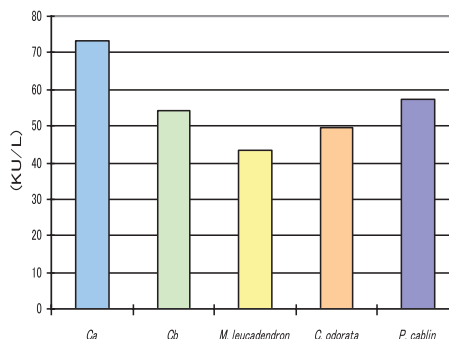


Figure 4. Effect of essential oils on stress index (C_a : normal condition, not sniffing anything, C_b : sniffing distilled water).

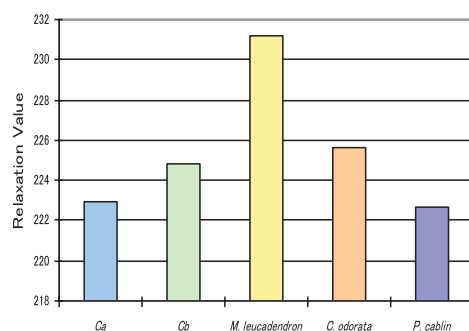


Figure 5. Effect of essential oils on brain wave (Ca: normal condition, not sniffing anything, Cb: sniffing distilled water).

Conclusions

The *M. leucadendron* and *C. odorata* oils possessed sedative effect and *P. cablin* oil odor had tendency to contain stimulating effect on human physiological responses. This result was influenced by the odors of its main compounds.

There is an interaction of odor and mood. Essential oils have certain therapeutic effects on the user or human organism when its fragrance is inhaled.

This study can be considered as a preliminary study for physiological effects of essential oils on human. Further experiments are needed to obtain deep information.

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