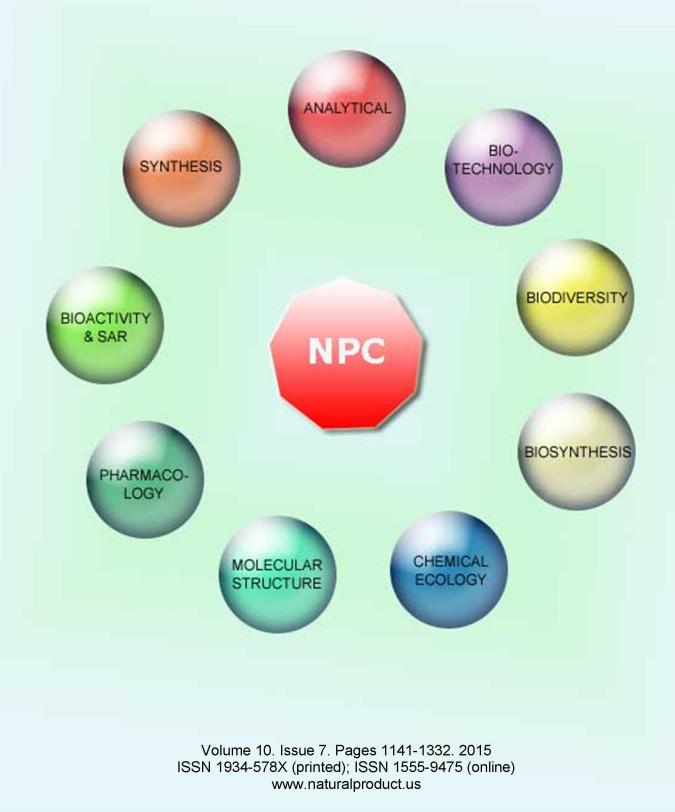
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Effect of Hinoki and Meniki Essential Oils on Human Autonomic Nervous System Activity and Mood States

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Meniki (*Chamecyparis formosensis*) and Hinoki (*C. obtusa*) are precious conifers with excellent wood properties and distinctive fragrances that make these species popular in Taiwan for construction, interiors and furniture. In the present study, the compositions of essential oils prepared from Meniki and Hinoki were analyzed by gas chromatography–mass spectrometry (GC/MS). Thirty-six compounds were identified from the wood essential oil of Meniki, including δ -cadinene, γ -cadinene, δ -cadinol, α -muurolene, calamenee, linalyl acetate and myrtenol; 29 compounds were identified from Hinoki, including α -terpineol, α -pinene, δ -cadinene, borneol, terpinolene, and limonene. Next, we examined the effect of Meniki and Hinoki essential oils on human autonomic nervous system activity. Sixteen healthy adults received Meniki or Hinoki by inhalation for 5 min, and the physiological and psychological effects were examined. After inhaling Meniki essential oil, participant's systolic blood pressure and heart rate (HR) were decreased, and diastolic blood pressure increased. In addition, sympathetic nervous activity (SNS) was significantly decreased, and parasympathetic activity (PSNS) was significantly increased. In addition, supprating lineares of the variability (HRV) in tested adults. Furthermore, in the Profile of Mood States (POMS) test, both Meniki and Hinoki wood essential oils stimulated a pleasant mood status. Our results strongly suggest that Meniki and Hinoki essential oils could be suitable agents for the development of regulators of sympathetic nervous system dysfunctions.

Keywords: Chamaecyparis formosensis, Chamaecyparis obtusa, Essential oil, Autonomic nervous system activity, Mood states.

False cypresses belong to the genus *Chamaecyparis* (Cupressaceae). They are known for their excellent wood properties and horticultural value. Meniki (*C. formosensis*) and Hinoki (*C. obtusa*) are regarded as the most precious conifers in Taiwan, and their excellent wood properties, beautiful texture and characteristic fragrances make them popular for construction, furniture and interior decoration materials. Extractives, including the essential oils, contribute to the properties of the wood, such as color, odor, and durability [1]. Previously, we obtained a non-biased overall profile of the fragrance compositions of the Meniki and Hinoki woods by using solid-phase microextraction (SPME) and GC/MS analysis. We also evaluated the antifungal activity of essential oil distilled from Meniki heartwood; the essential oil presented significant antifungal activity against *Laetiporus sulphureus* and *Trametes versicolor* [2].

The pleasant fragrances of Meniki and Hinoki wood are one of the reasons for their popularity. It has been proved that fragrance directly stimulates the limbic lobe and hypothalamus in humans, thus exerting a profound effect on the mind and body [3]. Kasuya *et al.* examined the effect of Hinoki oil on emotional behavior and stress-induced biomarkers. According to their results, Hinoki oil showed both anxiolytic-like and stress mitigation effects [4]. Recently, Park and his colleagues investigated the effects of Hinoki oil on early life stress, using maternal separation rats and found changes in gene expressions in the hippocampus of these animals caused by Hinoki oil by using a microarray approach. Their results indicated that Hinoki oil decreases maternal separation-induced anxiety-related behaviors, and modulates cytokines, particularly

CCL2 and IL6, in the hippocampus of MS rats [5]. However, the effect of Meniki and Hinoki essential oils on human autonomic system (ANS) activity and mood status were unexplored. ANS control 90% of body and mental activities, including heart rate, respiration rate, digestion, and sexual arousal. In our present study, the effects of essential oils on ANS activity and mood status were investigated. Besides, the compositions of essential oils were analyzed.

The physiological activities of the human body include extremely complex checks and balances and coordination processes that result in homeostasis. The main function of the ANS is to maintain a constant internal environment in the human body, so that the functions and activities among the various tissues and organs remain in the most coordinated state [6]. The autonomic nervous system is mainly distributed in smooth muscle, cardiac muscle, and various glands, and controls involuntary processes such as breathing, circulation, digestion, metabolism and other involuntary reflexes necessary for life [6]. The autonomic nervous system can be divided into the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). Most organs are under the dual control of both systems, with mutual antagonism between the two maintaining balance. Stimulation of the sympathetic nervous system results in changes such as quickening heartbeat, dilation of pupils, and increases in adrenaline, putting the body in a state of readiness to deal with emergencies; simulation of the parasympathetic nervous system, on the other hand, results in changes such as bradycardia, miosis, and bronchoconstriction, which produce a relaxed state [6,7].

Table 1: Composition of wood essential oil from C. formosensis and C. obtusa.

C	Concentration (%)		1/1	I.I	
Compounds	Cf	Co	— кі	Identification	
α-Pinene	3.3	11.8	935	MS, KI, ST	
Camphene	0.1	0.9	951	MS, KI, ST	
β-Myrcene	-	0.1	991	MS, KI, ST	
p-Cymene	0.1	0.4	1025	MS, KI, ST	
Limonene	0.2	3.8	1029	MS, KI, ST	
Sabinene hydrate	-	0.5	1032	MS, KI	
Terpinolene	-	4.7	1084	MS, KI, ST	
Fenchone	0.1	0.5	1087	MS, KI, ST	
Fenchol	0.2	3.9	1118	MS, KI, ST	
β-Pinene	0.4	0.1	1138	MS, KI	
Camphor	0.3	0.7	1146	MS, KI	
cis-Sabinene hydrate acetate	-	1.3	1151	MS, KI	
Borneol	0.4	6.8	1172	MS, KI, ST	
Terpinene-4-ol	0.1	1.3	1180	MS, KI	
Myrtenol	4.0	-	1188	MS, KI, ST	
Myrtenal	1.6		1193	MS, KI, ST MS, KI, ST	
α-Terpineol	-	35.2	1194	MS, KI, ST	
Linalyl acetate	5.2	-	1262	MS, KI, ST	
4-Terpinenyl acetate	0.3	2.2	1346	MS, KI	
α-Copaene	0.4	-	1372	MS, KI, ST	
β-Elemene	1.8	-	1386	MS, KI	
Isoledene	-	0.7	1387	MS, KI	
Isolongipholene	1.0	-	1398	MS, KI	
δ-Selinene	0.9	0.5	1470	MS, KI	
γ-Muurolene	2.4	0.9	1471	MS, KI	
Germacrene D	1.8	0.5	1472	MS, KI	
Valencene	1.6	0.5	1490	MS, KI	
α-Muurolene	7.0	2.2	1494	MS, KI, ST	
γ-Cadinene	9.9	3.0	1509	MS, KI	
δ-Cadinene	26.3	8.5	1514	MS, KI	
Calamenene	5.5	0.9	1518	MS, KI, ST	
β-Cadinene	1.8	-	1532	MS, KI	
α-Calacorene	1.8	_	1537	MS, KI	
Naphthalene	1.0	_	1545	MS, KI, ST	
Caryophyllene oxide	0.4	_	1583	MS, KI, ST	
10-epi-γ-Eudesmol	1.5	_	1625	MS, KI, ST	
1-epi-Cubenol	0.9	-	1629	MS, KI, ST	
α-Muurolol	3.5	-	1640	MS, KI, ST	
T-Muurolol	-	1.3	1641	MS, KI, ST MS, KI, ST	
T-Cadinol	3.3	1.5	1643	MS, KI, ST	
Cubenol	1.4	-	1645	MS, KI, ST MS, KI, ST	
δ-Cadinol	8.1	0.6	1646	MS, KI, ST MS, KI, ST	
α-Cadinol	-	2.1	1655	MS, KI, ST	
Cadalene	0.4	-	1671	MS, KI, ST	
Cauaiene	ohtung (Co)	Vovota roto	ntion index	, ,	

C. formosensis (Cf), C. obtusa (Co), Kovats retention index (KI), Mass spectrometry (MS), Spot test (ST).

People in East Asian countries, especially in Japan, Taiwan, China, and Korea believe that forest bathing and walking (Japanese: shinrin-yoku; green shower) have potential benefits to human health [8,9]. It is believed that walking in the forest and breathing the phytoncides emitted from the trees is not only pleasant and refreshing but is also beneficial for stress management and relaxation [10]. Phytoncides are defined as the antimicrobial volatile organic compounds emitted from plants. Chemically, the composition of phytoncides is closely related to essential oils produced by plants. Hydrodistillation of wood vielded 16.2 mL/kg Meniki and 18.0 mL/kg Hinoki wood essential oils. Table 1 shows the results of GC/MS analyses of the essential oils from Meniki and Hinoki. In total, 36 compounds were identified from the wood essential oil of Meniki and 29 from the essential oil of Hinoki. These findings indicated that the compounds emitted from the wood are different from the composition of the wood essential oils. The fragrance of Meniki and wood essential oil are sweet and Hinoki and its wood oils have a pungent smell.

Previous studies have revealed that wood essential oils promote human nervous system activity. Therefore, we next examined whether Meniki essential oil could improve human nervous system, blood pressure, HR, SNS, and PSNS activities. As shown in Table 2, after inhalation of Meniki essential oil for 5 minutes, the average HR of 16 adults tested was reduced from 75 beats/min to 70 beats/min. In addition, systolic blood pressure significantly (p =0.048) fell from 123 to 116 mmHg, and diastolic blood pressure was significantly (p = 0.038) raised to 78 mmHg from 74 mmHg.

Table 2: Effect of Meniki wood oil on human autonomic nervous system activity.

Parameters	Before inhalation	After inhalation	<i>t</i> -value	<i>p</i> -value
SBP (mmHg)	122.7 ± 16.4	116.1 ± 13.7	2.21	0.048*
DBP (mmHg)	73.8 ± 5.2	77.8 ± 7.6	-2.36	0.038*
HR (beats/min)	74.4 ± 8.2	70.2 ± 6.8	2.13	0.056
HRV (ms)	60.3 ± 28.6	66.0 ± 26.8	-1.21	0.253*
SNS (%)	60.8 ± 9.6	51.25 ± 12.69	3.06	0.011*
PSNS (%)	39.0 ± 9.5	48.7 ± 13.14	-2.86	0.016
vstolic blood pressur	e (SBP). Diastolic blo	ood pressure (DBP	. Heart rate	(HR). Hea

System blodd pressure (SDF), Diastine blodd pressure (DF), Heart rate (TR), Heart rate variability (HRV), Sympathetic nervous system activity (SNS), Parasympathetic nervous system activity (PSNS), * p < 0.05.

Moreover, the SNS activity (low frequency) was significantly (p = 0.011) decreased to 51% from 61%, whereas PSNS activity (high frequency) was significantly (p = 0.016) increased to 48% from 39%, which suggest that Meniki essential oil promotes ANS (restand-digest) activities. Furthermore, with response to the inhalation of Meniki essential oil, the HRV was increased to 66 ms from 60 ms. Previous studies also reported that δ -cadinene and α -muurolol, components of the essential oil of *Cananga odorata*, regulate ANS through stimulation of PSNS [11]. Interestingly, the Meniki essential oil contains large amount of δ -cadinene (26.3%) and α -muurolol (3.5%), which may be the reason for the increase of PSNS activity after inhalation of the essential oil. Moreover, the data strongly suggest that the increased HRV by Meniki essential oil could improve ANS activity.

Next, we examined whether Hinoki essential oil could improve ANS parameters, including blood pressure, HR, HRV, SNS, and PSNS. As shown in Table 3, after inhalation of Hinoki essential oil for 5 minutes, the HR was decreased to 70 beats/min from 72 beats/min. However, in contrast with the results of Meniki essential oil, inhalation of Hinoki essential oil reduced systolic blood pressure from 120 mmHg to 117 mmHg, whereas the diastolic blood pressure was unaffected. In addition, inhalation of Hinoki essential oil increased SNS activity from 48% to 55%, whereas the PSNS activity was decreased from 52% to 45%. Interestingly, inhalation of Hinoki essential oil significantly increased the HRV from 56 ms to 71 ms. These data also support the idea that Hinoki essential oil at least partially stimulates ANS activity. Miyazaki and his coworkers also evaluated the effects of the fragrance of Taiwan Hinoki (C. obtusa; syn. C. taiwanensis) essential oil on adults. They also found that the maximal blood pressure was decreased after inhalation of Hinoki essential oil [12]. It has also been reported that octopamine, an endogenous biogenic amine widely distributed in the nervous system of vertebrates, acts as a neurotransmitter, neurohormone, and neuromodulator [13]. Wood essential oils such as eugenol and α -terpineol were reported to induce octopaminergic system in insects [14]. Moreover, the structure-activity relationship analysis (SRA) revealed that the high content of α -terpineol (35.1%) in Hinoki essential oil might be the reason for the increase of sympathetic nervous system activity of Hinoki essential oil.

Table 3: Effect of Hinoki wood oil on human autonomic nervous system activity.

Parameters	Before inhalation	After inhalation	<i>t</i> -value	<i>p</i> -value
SBP (mmHg)	120.1 ± 8.6	115.9 ± 8.2	2.089	0.057
DBP (mmHg)	73.1 ± 1.7	72.4 ± 1.6	1.979	0.067
HR (beats/min)	72.3 ± 10.3	70.7 ± 9.3	1.933	0.072
HRV (ms)	56.8 ± 24.1	71.5 ± 31.8	-2.41	0.031*
SNS (%)	48.1 ± 12.8	54.7 ± 10.1	1.914	0.078
PSNS (%)	51.9 ± 12.8	45.2 ± 9.9	1.934	0.075

Systolic blood pressure (SBP), Diastolic blood pressure (DBP, Heart rate (HR), Heart rate variability (HRV), Sympathetic nervous system activity (SNS), Parasympathetic nervous system activity (PSNS), * p < 0.05.

In order to understand the effects of Meniki essential oil on human mood states, the Profile of Mood States (POMS) test was used to assess mood changes after inhalation of Meniki essential oil. As shown in Figure 1, inhalation of Meniki essential oil significantly

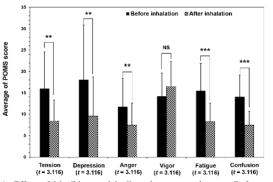


Figure 1: Effect of Meniki essential oil on human mood states. Before and after inhalation of Meniki essential oil, the mood states were examined by POMS test and the test scores shown are the average of 16 individuals. Each value is expressed as the mean \pm SD of 16 individuals (n =16). ** p<0.01 and *** p<0.001 are statistically significant compared with before treatment. NS is not statistically significant.

decreased the average scores of tension from 16 to 8 (p = 0.009), depression from 18 to 9 (p = 0.002), anger 12 to 7 (p = 0.009), fatigue 15 to 8 (p = 0.0001), and confusion 14 to 7 (p = 0.0001). In contrast, the average score for vigor was slightly increased to 14 from 15, but the value was not statistically significant (p = 0.112). Moreover, the mood state scores after inhalation of Meniki essential oil are highly comparable with baseline scores. These data strongly support the results obtained from ANS analysis. Inhalation of Meniki essential oil decreased systolic blood pressure, increased diastolic blood pressure and decreased heart rate; in addition, sympathetic activity was decreased and parasympathetic activity was increased. After inhaling Hinoki wood essential oil, systolic blood pressure, diastolic blood pressure and heart rate were all decreased, whereas sympathetic activity was increased and parasympathetic activity decreased.

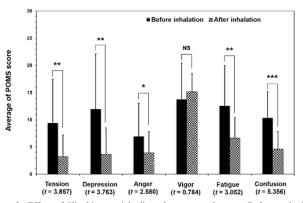


Figure 2: Effect of Hinoki essential oil on human mood states. Before and after inhalation of Hinoki essential oil, the mood states were examined by POMS test and the test scores shown are the average of 16 individuals. Each value is expressed as the mean \pm SD (n =16). ** p<0.01 and *** p<0.001 are statistically significant compared with before treatment. NS is not statistically significant.

As shown in Figure 2, inhalation of Hinoki essential oil significantly decreased the average scores of tension from 9 to 3 (p = 0.002), depression from 12 to 4 (p = 0.003), anger 7 to 4 (p = 0.023), fatigue 13 to 7 (p = 0.009), and confusion 10 to 5 (p = 0.0001). In contrast, the average score for vigor was slightly increased to 16 from 14, but the value was not statistically significant (p = 0.447). Moreover, the mood state scores after inhalation of Hinoki essential oil are highly comparable with baseline scores. These data partially support the results obtained from ANS analysis. In addition, there was no significant difference in vitality after treatment with either Meniki or Hinoki essential oils; however, the trends in vitality were improved.

Results obtained from this study support the notion that the fragrances of these two wood essential oils may enhance mood. We recognize that both physiological and psychological mechanisms are involved in the effects of fragrance inhalation on sympathetic activity. We conclude that inhalation of Hinoki and Meniki essential oils may regulate the autonomic nervous system and stimulate positive mood states. Our results suggest the possible application of Hinoki and Meniki oils as regulators of dysfunctions of the sympathetic nervous system.

Experimental

Plant materials and essential oil preparation: The 80 years aged Meniki and 60 years aged Hinoki logs used in this study were collected from Huisun Experimental Forest, Nantou, Taiwan in June 2011. Prof. Yen-Hsueh Tseng (Department of Forestry, National Chung-Hsing University) confirmed taxonomic identification, and voucher specimens were deposited at the Herbarium of the Department of Forestry, National Chung-Hsing University, National Chung-Hsing University, Taiwan. Heartwood chips were prepared from a green cut tree. The air-dried wood chips were subjected to hydrodistillation for 8 h using a Clevenger type apparatus, giving 16.2 mL/kg for Meniki and 18.0 mL/kg for Hinoki oil. The moisture-free oil was obtained by treating with anhydrous Na₂SO₄.

Gas chromatography-mass spectrometry analyses of essential oils: The compositions of the essential oils were analyzed by an ITQ Series GC mass system, equipped with a DB-5 capillary column (30 m \times 0.25 mm i.d., 0.25 μ m film thickness; J & W Scientific). The temperature program was as follows: 40°C for 1 min, then increased by 4°C/min to 260°C and held for 4 min. The other parameters were as follows: injection temperature, 270°C; ion source temperature, 280°C; EI, 70 eV; carrier gas, He at 1 mL/min; injection volume, 1 µL; spilt ratio, 1:50; and mass range, m/z 45-425. Quantification was obtained from percentage peak areas from the gas chromatogram. A Wiley/NBS Registry of Mass Spectral Data search and authentic reference compounds were used for substance identification. The Kovats retention index (KI), which is a parameter calculated in reference to *n*-alkanes that converts retention times into system-independent constants, was also confirmed [15]. Chromatography results expressed as area percentages were calculated with a response factor of 1.0.

Human subjects: In order to avoid age and gender influences, we randomly selected 8 male and 8 female college students within the age group of 20-21 years. All subjects were healthy and not undergoing any medical treatment during the experimental period. The subjects were instructed to have sufficient sleep in the previous night and not to drink, eat, or be involved in sports for at least 1 h prior to each test. Verbal and written informed consent were obtained from all volunteers after informing them of the study design, intervention, data collection, and the rights of the participants. Interventions and data collection were performed by the researcher and trained research assistants. The non-invasive human study was approved by the University Ethics Committee and performed in accordance with the ethical standards of the responsible committee on human experimentation outlined in the 1942 Helsinki declaration.

Experimental procedure: To understand the effects of the autonomic nervous system activity after inhalation of essential oils, all 16 volunteers were subjected to the experiments. The test room was controlled by temperature at $22 \pm 2^{\circ}$ C, 55% humidity, and 200 lux illuminance. Prior to the experiment, each volunteer's blood pressure, heart rate (HR), sympathetic nervous system activity (SNS), parasympathetic nervous system activity (PSNS), and heart

rate variability (HRV) were recorded. To measure the effect of Hinoki essential oil, Hinoki was dissolved at a concentration of 20%, w/w, in olive oil. The Hinoki essential oil solution (100 μ L) was applied to a piece of absorbent cotton (0.8 cm × 0.8 cm), and the cotton piece was fitted under the subject's nose (*i.e.*, the philtrum) and the odor of Hinoki essential oil was inhaled during ordinary breathing for 5 min. Then, each subject's blood pressure, HR, HRV, SNS, and PSNS were measured using an ANSWatch wrist monitor (Taiwan Scientific Corporation, Taipei, Taiwan; Taiwan Department of Health, medical device product registration number 001525), as described previously [16]. A similar protocol was applied to measure the effect of Meniki essential oil on the second day.

Instrument and data collection: To monitor HRV, most researchers have used electrocardiography (ECG) data due to their availability in research laboratories. However, very few studies have reported HRV measurements obtained from finger blood pressure waveform using an optical sensor [17]. Compared with ECG, correlation coefficients of HRV parameters from optical sensors were in the range of 0.75 to 0.99. In this study, we used an advanced ANSWatch wrist monitor, which subjects multiple piezo-electrical sensors enclosed in the wrist to measure directly the blood pressure waveform in the radial artery [18]. According to the product information, the device accuracy on HRV parameters in terms of correlation coefficient is in the range of 0.90 to 1.0, using ECG as the control [18]. This portable device requires neither electrodes nor

other disposables, and can conduct tests in sitting or lying (supine) postures. Each ANSWatch® test takes about 7 min and outputs 8 parameters, including systolic blood pressure, diastolic blood pressure, HR, SNS activity (low-frequency, LF), PSNS (high-frequency, HF), sympatho-parasympathetic balance index LF/HF, HRV, and number of irregular heartbeats. The data obtained from ANSWatch wrist monitor were analyzed by ANSWatch® Manager Pro software.

POMS analysis: In this study, the Profile of Mood States (POMS), a globally standardized, self-administered, 65-item questionnaire (including 7 dummy items), was used to assess moods before and after inhalation of essential oils. Each item was rated on a 5-point Likert scale of 0 to 4, ranging from "not at all" to "extremely". These raw scores were added to generate 6 subscales of emotional state: tension–anxiety, depression–dejection, anger–hostility, vigor, fatigue, and confusion, as described previously [19].

Statistical analysis: Data are expressed mean \pm SD. Statistical comparisons of the results were made using the Paired-Samples *t*-Test. Significant differences (*p < 0.05 and **p < 0.01).

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