

The effects of *d*-camphor inhalation on psychophysiological parameters among healthy participants

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ABSTRACT

This study aims to investigate the effects of *d*-camphor inhalation on psychophysiological parameters among healthy participants. Twenty-four healthy volunteers participated in the trials. *d*-Camphor was administered to determine the effects on autonomic nervous system (ANS), central nervous system (CNS) through electroencephalography (EEG) recordings as well as psychological parameters through emotional states. EEG recordings were conducted based on 10-20 system and EEG band power was calculated by Fast Fourier Transformation (FFT). For data analysis, *d*-camphor was inhaled and compared with sweet almond oil as base oil. Paired t-test was employed to measure the oil inhalation. The findings indicated that *d*-camphor inhalation caused significant changes in ANS parameters, psychological parameters through emotional states and central nervous system (CNS) through electroencephalography (EEG) recordings. After *d*-camphor inhalation, systolic blood pressure, heart rate, and the respiratory rate decreased significantly. Relaxed and calm feelings increased significantly while active and stressed feelings decreased significantly. The power of the alpha wave over all the brain areas including left anterior, right anterior, center, left posterior and right posterior brain areas increased significantly. *d*-Camphor caused significant changes in psychophysiological parameters indicating its sedative effects.

Key words:

d-Camphor; EEG; psychophysiological parameters; emotional states; sedative effects

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INTRODUCTION

d-Camphor is a white, crystalline substance with a strong odor and pungent taste obtained naturally from tropical plants called *Cinnamomum camphora* (L.) J. Presl which belongs to the Lauraceae family. *C. camphora* is the original source of camphor, which can be found in warm climates areas such as China, Japan, Taiwan, Vietnam, Australia, southern USA, southern Europe, southern and eastern Africa and Madagascar. Camphor

($C_{10}H_{16}O$) is an abundant monoterpenoid with a bicyclic framework structure known as bicyclic monoterpene ketone. Camphor is divided into two enantiomeric forms: (1S)-(-) or *l*-camphor and (1R)-(+)-camphor or *d*-camphor. *l*-Camphor is usually found in the synthetic production of camphor. It is also found in very small quantities in specific species of plants. *d*-Camphor is widely used as a fragrance in perfume and cosmetics, a food flavoring agent, and household cleaners¹⁻³.

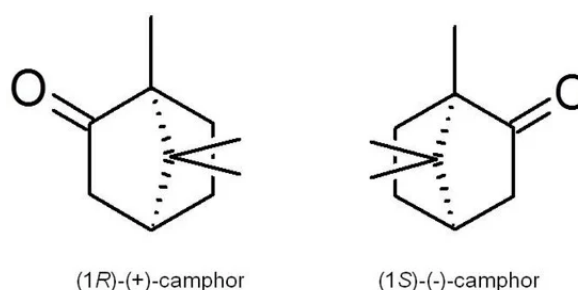


Figure1. The chemical structure of the (1R)-(+) and (1S)-(-) enantiomers of camphor³

It has been shown that *d*-camphor possesses numerous biological properties, i.e., antiviral⁴, antitussive⁵, antinoceptive⁶, insecticidal⁷, antimutagenic, and anticancer activities⁸. Because of its therapeutic properties, people have used *d*-camphor as an antiemetic, antidiarrheal, antiflatulent, and stimulant agent in Ayurvedic medicine⁹. Historically, the use of camphor could be traced back to the Pre-Sukhothai Period. The hospital inscription recorded camphor (karabun) as one of the plants with healing properties¹⁰.

Despite the effectiveness of aroma inhalation and its therapeutic properties, the effects on olfactory brain regions are still necessary to give more insight and new knowledge to promote specific olfaction uses of the odorant compounds. Current studies have been carried out to examine the effects and therapeutic properties of *d*-camphor. However, the current research studies on *d*-camphor inhalation in terms of

CNS, ANS and emotional states are still very limited. With respect to the literature review, this research study is one of the first clinical research studies in Thailand to investigate the effects of the inhalation of *d*-camphor on CNS or brainwave activities, ANS including respiratory rate, heart rate, blood pressure and skin temperature as well as emotional states.

MATERIALS AND METHODS

Study Design

The current research was a pre-test and post-test study design in which each participant served as his or her own control and the dependent variable was analyzed for each participant. The pretest-posttest design was used to evaluate the effectiveness of *d*-camphor as the intervention.

Participants

Based on the inclusion criteria, 24 healthy volunteers (both males and females) aged from 20 to 35 years were recruited for this study. They had normal cardiovascular health. They were healthy without these diseases namely neurological diseases, hypertension, otorhinolaryngologic diseases, upper respiratory infection, and cardiovascular disease. The volunteers had normal blood pressure (systolic up to 140 mmHg and diastolic 90 mmHg) and normal heart rate ranging from 60 to 90 bpm. A normal range of body mass index (BMI) from 18.5 to 22.9 kg/m² was applied according to the WHO and Asian criteria values¹¹. The volunteers did not take CNS medication or sedative drugs. Moreover, the Edinburgh Handedness Inventory scale was utilized to select right-handed volunteers only. Concerning the exclusion criteria, the female volunteers who were pregnant and the volunteers who were allergic to volatile compounds were excluded from this study.

Sample Size Calculation

The sample size in this study was calculated according to the sample size calculation of the previous study^{1 2} conducted on rosemary oil inhalation, measuring the mean and SD values of EEG changes in the eye closed stage. More than 10% of the total population was recruited to compensate for the expected drop-outs during the experiment and guarantee the research confidence. A significance level lower than 0.05 ($Z_{\alpha} = 1.96$) and a test power at 80% were used.

This study was an experimental one in which the researchers conducted a pre-test and post-test design. This study was part of the research project investigating the inhalation effects of four different volatile compounds diluted in sweet almond oil on ANS parameters, emotional states, and brain wave activities through EEG recordings. Four different volatile compounds required four experimental

groups each of which recruited 24 healthy participants. Therefore, all four experimental groups contained 96 healthy participants in total. The 24 healthy participants were randomly assigned for this study.

Materials

d-Camphor (CAS number 464-49-3) was purchased from Sigma-Aldrich, USA. Six percent (w/v) of *d*-camphor was diluted in sweet almond oil from Chemipan Corporation Co., Ltd., Thailand and delivered from an oxygen pump system via a plastic tube through a face mask at the constant rate of 2 L/min.

Outcome measurements

ANS parameters

The Biolight M7000 Multi-Parameter Patient Monitor (BIOM7000) was utilized to record the physiological parameters including skin temperature, heart rate, respiratory rate, systolic and diastolic blood pressure.

Psychological parameters of emotional states

The Geneva Emotion and Odor Scale¹³ Thai version (Cronbach's alpha 0.752)¹⁴ was used to measure the emotional states of the participants. This scale was invented to assess subjective personal feelings by using a 100-mm visual analog scale on five types of emotional states, including pleasant feeling (good), unpleasant feeling (bad, disgusted, frustrated, uncomfortable and/or stressed), sensual feeling (romantic), relaxation (relaxed, drowsy and serene), and refreshing (refreshed, energetic).

EEG parameters

EEG recordings were conducted based on the international 10-20 system by applying a set of 31 electrodes with one additional ground placed at FP1, FP2, FZ, F3, F4, F7, F8, FT7, FC3, FCZ, FC4, FT8, T3, T4, T5, T6, TP7, TP8, C3, CP3, C4, CZ,

CP4, P3, P4, PZ O1, O2 and OZ¹⁵. Both mastoids were used as the recording references, with an average of both mastoids equal to $A1+A2/2$. The electrooculogram (EOG) was monitored by placing four electrodes in both external canthi (HEOL and HEOR), left supraorbital (VEOU), and infraorbital (VEOL) regions. Each participant was asked to put on an electro cap of elastic spandex-type fabric with recessed silver or silver chloride (Ag or AgCl) electrodes attached to the fabric. Electrode impedances were set below five k Ohms. The recording system known as Acquire Neuroscan version 4.3 (Compumedics Neuroscan, Australia) was utilized. The online filter was set to a band-pass with the low pass equal to 60 Hz and the high pass equal to 0.1. and the A/D rate was 500 Hz. The gain was set at 19. The notch filter was open at 50 Hz¹⁶. During EEG analysis, the continuous EEG data were cut into 2,00 milliseconds-length EEG epochs. The post-recording filter was set as band-pass at 0.3-30 Hz, and artifact rejection was assigned at ± 80 Hz for all EEG channels¹⁷. The power spectrum of the respective frequency bands was analyzed based on Fast Fourier Transformation (FFT), ranging from delta (0.5-4 Hz), theta (4.5-8 Hz), alpha (8.5-13 Hz), and beta (13.5-30 Hz)¹⁸.

Data Collection

The general characteristics of each participant including age, weight, height, BMI as well as the data of ANS physiological parameters including skin temperature, heart rate, respiratory rate, and systolic and diastolic blood pressure were collected. A set of scales on 12 types of emotional states was completed by all the participants to measure their psychological parameters. EEG recordings collected and interpreted brain wave activities which were translated into frequency bands made up of five brain regions e.g. left anterior (Fp1, F3, F7), right anterior (Fp2, F4, F8),

center (Fcz, Cz, Cpz), left posterior (P3, T5, O1) and right posterior (P4, T6, O2) brain regions.

Data Analysis

Descriptive statistics, percentage, mean values and standard deviation (SD) were applied to report the general characteristics of all the participants. The SPSS statistical package version 22 was used for data analyses of the effects of the volatile compound on physiological and emotional changes before and after the *d*-camphor inhalation and ANS parameters, psychological parameters of emotional states and EEG parameters were analyzed by paired *t*-tests. A *p*-value less than 0.05 is considered as statistically significant. Shapiro-Wilk Test was used for normality test.

Ethical Considerations

This study was approved by the Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University, Permissions No. COA No. 074/2020 and conducted in an EEG laboratory, Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University on Salaya campus in Nakhon Pathom province, Thailand from 1st April, 2020 to 1st December, 2020.

Procedures

Each participant was appointed to take part in the experiments in two sessions: one for ANS physiological parameters, emotional states and the other for EEG recordings. The experiments in each session were conducted in an air-conditioned, quiet room at 24 ± 1 °C with relative humidity between 50-65%. The experiments were carried out in the morning between 8.00-12.00 a.m. to reduce the impact of the circadian rhythm.

In the first session as the first appointment, the electrodes were attached to suitable positions to measure the ANS physiological parameters including heart rate, respiratory rate and skin temperature recorded every minute while both systolic and diastolic blood pressure were recorded every 2.5 minutes. The experiments were divided into three periods (10 minutes each): the first period (baseline or resting period), the second period (sweet almond oil inhalation) and the third period (*d*-camphor in sweet almond oil inhalation). In addition, the researchers asked each participant to rate his/her emotional states

at baseline, after the sweet almond oil inhalation, and after the *d*-camphor inhalation.

The second session was scheduled at least seven days after the first session as the washout period. The second session for EEG recordings was divided into four periods (eight minutes each). First, the EEG recordings were conducted as a baseline while each participant was opening and closing his/her eyes (five minutes each). Then, he/she was asked to inhale sweet almond oil for eight minutes. Finally, each participant was asked to inhale *d*-camphor for eight minutes.

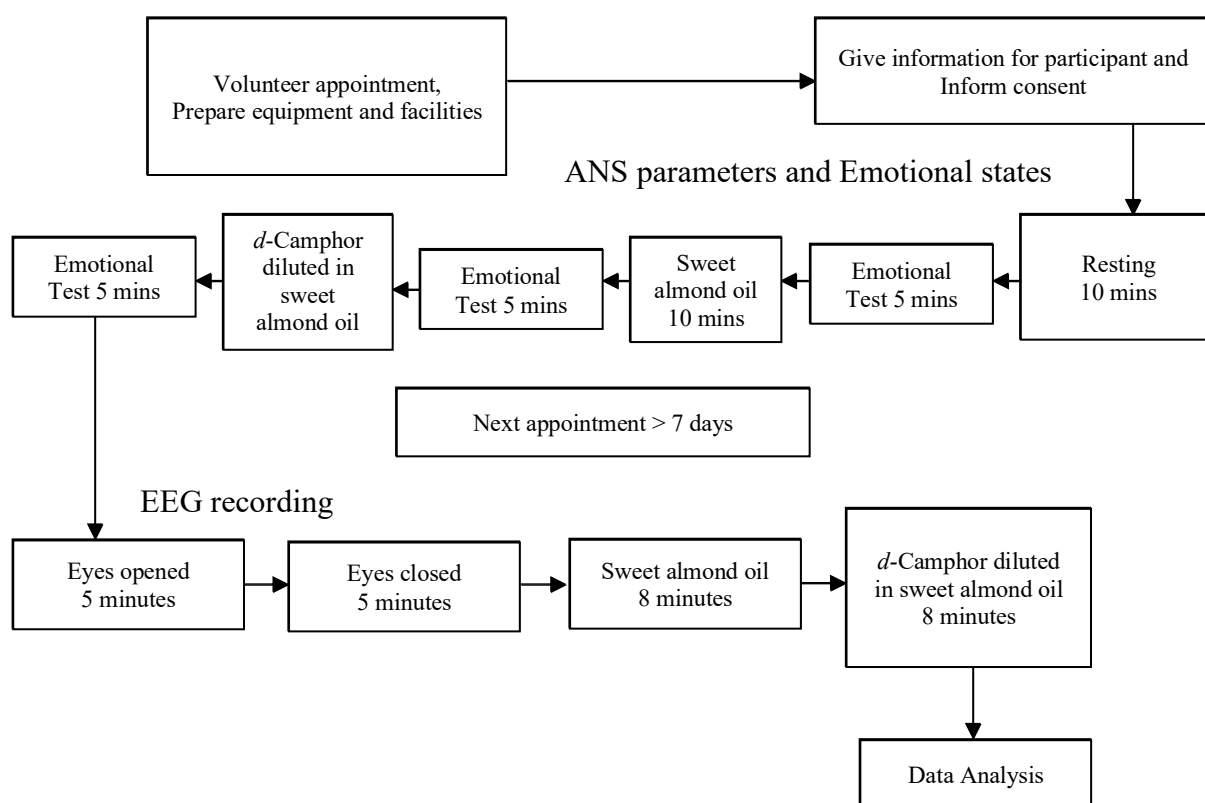


Figure 2. The procedures are sequenced as in a following map.

RESULTS

Twenty-four participants (12 males and 12 females) aged between 20 and 35 years with normal body mass index were asked to inhale sweet almond oil and *d*-camphor in this study. All the data of ANS

parameters, emotional states and EEG recordings showed normal distribution. The mean and SD values of the participants' age, height, weight and BMI were 21.29 (± 1.46) years, 168.63 (± 6.74) cm, 59.63 (± 7.00) kg, 20.89 (± 1.18) kg/m² respectively (Table 1).

Table 1 General characteristics of the participants

Parameters	Number	mean	SD
Age (years)	24	21.29	1.46
Height (cm)	24	168.63	6.74
Weight (kg)	24	59.63	7.00
Body Mass Index (kg/m ²)	24	20.89	1.18

ANS physiological parameters

Table 2 showed the mean and SD values of the ANS parameters during the three phases of the experiment: resting (R), sweet almond oil inhalation (SO) and *d*-camphor inhalation (CH) (6% w/v in sweet almond oil). *d*-Camphor inhalation caused significant changes in most ANS parameters.

Systolic blood pressure, diastolic blood pressure, and the respiratory rate decreased significantly after CH inhalation. Heart rate decreased significantly after sweet almond oil inhalation and CH inhalation. Skin temperature increased significantly after CH inhalation.

Table 2 ANS parameter values during the three experimental periods: resting (R), the sweet almond oil inhalation (SO) and *d*-camphor inhalation (CH)

Parameters	n	R		SO		CH		p-value R and SO	p-value SO and CH
		Mean	SD	Mean	SD	Mean	SD		
Systolic blood pressure (millimeters of mercury)	24	110.00	4.85	110.79	4.91	109.29	3.75	0.103	0.039*
Diastolic blood pressure (millimeters of mercury)	24	68.63	4.05	69.04	3.83	67.42	3.59	0.498	0.027*
Heart rate (beats per minute)	24	82.54	8.27	79.83	10.07	77.46	9.00	0.020*	0.011*
Skin temperature (degrees celsius)	24	31.29	2.14	31.90	1.12	32.38	1.28	0.108	0.037*
Respiratory rate (breaths per minute)	24	18.54	2.87	18.33	2.75	17.08	2.45	0.737	0.025*

* Significant difference, *p*-value < 0.05 by paired *t*-test

Psychological parameters of emotional states

Table 3 showed the mean and SD values of the psychological parameters of emotional states during the three phases of the experiment: resting (R), sweet almond

oil inhalation (SO) and *d*-camphor inhalation (CH).

CH inhalation caused significant changes in the psychological parameters of emotional states. The mean scores of relaxed, and calm feelings increased significantly while the mean scores of

active feelings decreased significantly after CH inhalation. In contrast, the mean scores of stressed feelings increased significantly

after SO inhalation and decreased without statistical significance after CH inhalation.

Table 3 The psychological parameter values of emotional states during the three periods of the experiment: resting (R), sweet almond oil inhalation (SO) and *d*-camphor inhalation (CH)

Parameters	n	R		SO		CH		p-value R and SO	p-value SO and CH
		Mean	SD	Mean	SD	Mean	SD		
1. good	24	5.53	1.60	5.13	1.60	5.76	1.41	0.313	0.079
2. bad	24	2.80	1.37	3.09	1.04	2.79	1.49	0.163	0.265
3. active	24	4.47	1.40	4.43	1.55	3.60	1.38	0.869	0.034*
4. drowsy	24	3.91	1.45	4.49	1.60	4.85	2.02	0.072	0.323
5. fresh	24	4.58	1.72	4.92	1.38	4.63	1.48	0.339	0.371
6. relaxed	24	4.39	1.59	4.73	1.90	5.48	1.75	0.270	0.031*
7. stressed	24	3.86	1.23	4.43	1.33	3.72	1.72	0.029*	0.063
8. frustrated	24	3.51	1.36	3.30	1.31	2.88	1.19	0.320	0.202
9. romantic	24	2.95	1.31	3.32	1.65	3.40	1.48	0.202	0.758
10. annoyed	24	3.09	1.15	2.91	1.20	2.60	1.20	0.306	0.222
11. calm	24	4.22	1.27	4.32	1.32	5.43	1.68	0.685	0.008*
12. disgusted	24	2.46	1.37	2.65	1.18	2.27	0.93	0.285	0.119

* Significant difference, p -value < 0.05 by paired t -test

The data of EEG recordings

The absolute powers of brain activities were calculated during the three experimental phases: resting, sweet almond oil inhalation, and *d*-camphor inhalation. The significant changes in the alpha wave powers were revealed. Table 4 reported the mean and SD values of the absolute powers of brain activities during the three experimental phases: resting (R), the sweet almond oil inhalation (SO) and *d*-camphor

inhalation (CH). The regions of EEG recordings were divided into the left anterior (Fp1, F3, F7), right anterior (Fp2, F4, F8), right posterior (P4, T6, O2), left posterior (P3, T5, O1), and center (Fcz, Cz, Cpz) regions.

CH inhalation increased the alpha wave in all the brain regions i.e., left anterior, right anterior, center, left posterior, and right posterior regions.

Table 4 The brain activities (alpha powers) during the three periods of the experiment: resting (R), sweet almond oil inhalation (SO) and *d*-camphor inhalation (CH)

Brain Regions	Alpha Power (μV^2)						p-value R and SO	p-value SO and CH
	R		SO		CH			
	Mean	SD	Mean	SD	Mean	SD		
Left anterior	3.26	1.53	4.04	1.91	6.10	3.06	0.097	0.013*
Right anterior	3.30	1.66	3.90	2.04	6.01	3.11	0.098	0.004*
Center	4.53	1.98	5.70	2.60	8.06	4.54	0.059	0.015*
Left posterior	4.21	2.04	5.09	2.38	7.10	3.28	0.087	0.008*
Right posterior	4.31	1.86	5.01	1.99	6.51	2.67	0.077	0.029*

* Significant difference, p -value < 0.05 by pair t -test

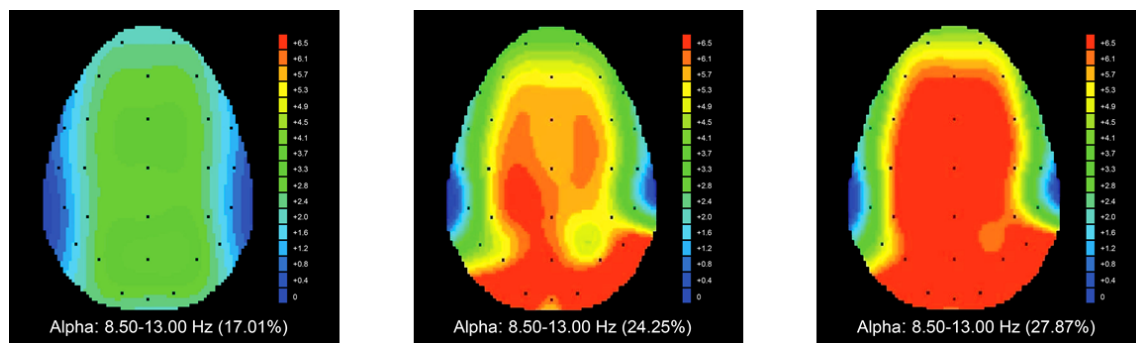


Figure 2. Brain topographical map of the alpha brainwave activity distribution. The red areas indicate an increase of alpha power during sweet almond oil inhalation (3b) and a significant increase of alpha power in all regions during d-camphor inhalation (3c).

DISCUSSION

d-Camphor, a natural product derived from *Cinnamomum camphora*, has a long history of use as antiseptic, analgesic, antipruritic, counterirritant, and rubefacient^{19,20}. In this study, *d*-camphor diluted in sweet almond oil was administered to healthy participants through inhalation during the three phases of the experiment: resting (R), the sweet almond oil inhalation (SO) and *d*-camphor inhalation (CH) (6% W/V in sweet almond oil). The recommended percent of essential oils should range from 2.5% to 10% diluted in based oils²¹. The 6% use of *d*-camphor diluted in sweet almond oil in this study was applied from the results of the participants' satisfaction from a pre-test study. If the percentage of essential oils is too high, the scent will be too strong. In contrast, if the percentage of essential oils is too low, the scent will be too weak. So, the 6% use of *d*-camphor is a safe and appropriate amount that most participants considered the most satisfactory in the pre-test study. The procedures used in this study can prove the effect of *d*-camphor since these procedures have been proven by previous studies^{22,23}, which introduced the standard procedures to investigate the

effects of essential oils on ANS parameters, emotional states, and brain wave activities through EEG recordings. *d*-Camphor was diluted in sweet almond oil since the oil did not cause any significant changes in brain wave activities validated by a recent study by Nida Nuiden et al. (2021)²².

However, the effects of sweet almond oil on ANS and emotional states were confirmed. The findings on ANS parameters in this study showed that SO inhalation induced significant decreases in heart rate which were in line with previous studies by Nida Nuiden (2019)²³ and Thanatuskitti, (2020)²⁴. Moreover, the findings on psychological parameters of emotional states in this study showed that SO inhalation decreased good feelings but increased stressed feelings in healthy participants. The previous study also reported less stressed feelings, less good feelings but more romantic feelings²³. In contrast, another previous study on the effects of inhaled *Limnophila aromatica* essential oil on brain wave activities and emotional states in healthy volunteers found that sweet almond oil inhalation increased good feelings but decreased stressed feelings²⁵.

This pre-test and post-test study design that compared SO and CH inhalation could highlight the effects of *d*-camphor

among healthy participants. In this study, *d*-camphor inhalation caused significant changes in ANS parameters by decreasing systolic blood pressure, diastolic blood pressure, respiratory rate, and heart rate significantly while increasing skin temperature. The results of this study were consistent with previous studies. For instance, Kim et al. (2018) investigated the effect of *Chrysanthemum indicum* Linne with *d*-camphor as a major compound on blood pressure and electroencephalogram in healthy participants. The oil administered through inhalation could induce a decrease in the systolic blood pressure and heart rate but an increase in alpha waves. The researchers concluded that *C. indicum* Linne could help reduce blood pressure and may provide mental and physical relaxation²⁶.

d-Camphor inhalation induced significant changes in psychological parameters of emotional states. CH inhalation increased the mean scores of relaxed, and calm feelings significantly but decreased the mean scores of active feelings decreased significantly. A previous study was conducted to investigate basic emotions induced by odorants including *d*-camphor by analyzing autonomic nervous system (ANS) responses. The participants were asked to complete a hedonic scale to rate the pleasantness or unpleasantness of the odors. The results showed that camphor was ranked intermediate between happiness or surprise as characteristics of the pleasant odorants and sadness²⁷. The sedative effect of *d*-camphor administered through inhalation to mice was conducted using an open field test and the result showed that *d*-camphor could decrease the amount of spontaneous motor activity indicating the sedative effect²⁸.

The data of EEG recordings were collected and interpreted to measure the effects of *d*-camphor inhalation on the central nervous system. The results showed that *d*-camphor inhalation triggered significant changes in alpha wave power

whereas there were no significant changes in the other 3 waves. The alpha wave power increase was found in all the brain regions namely left anterior, right anterior, center, left posterior and right posterior. Alpha waves manifest when humans are in a state of relaxed wakefulness which decreases with concentration, stimulation or visual fixation²⁹. In essence, the increases in alpha brain waves are associated with mental coordination, calmness, attention, and brain consciousness and are highly associated with a reduced stressed level³⁰. As a result, a significant increase in alpha wave in this study caused the participants to feel more relaxed and calmer.

CONCLUSION

d-Camphor seemed to possess sedative effects. According to the results of this study, *d*-camphor could decrease ANS parameters, increase all areas of alpha waves, and induce relaxation, and calmness while reducing stress in healthy participants. The results of this study also recommended that natural *d*-camphor could be inhaled to induce relaxation and calmness. Future research could be conducted on the effects of *d*-camphor inhalation on other groups of participants who have experienced stressors or stressful situations in work or daily life to provide more health-related scientific data and knowledge. Then, the general public can apply the use of *d*-camphor safely and properly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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