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# The effects of *Litsea cubeba* essential oil inhalation on brain wave activity

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*Litsea cubeba* is a dioecious tree found in Asia, including Thailand. It is known for its therapeutic properties in aromatherapy. This study aims to investigate the effect of *L. cubeba* essential oil on EEG recordings. Sweet almond oil was used as the essential oil diluent. Twenty-five healthy participants aged 20–35 were recruited. First, the participants inhaled sweet almond oil; then, after a 7-day washout period, they inhaled 10% of the essential oil via face mask (2 L/min). Brainwaves were recorded using Nicolet EEG v32 from Natus Neurology Company (USA). The absolute powers of 4 main frequency bands (delta, theta, alpha, and beta waves) were compared during three conditions i.e., resting-state, sweet almond oil inhalation and *L. cubeba* inhalation. The results indicated that the absolute powers of alpha (8–12.9 Hz) and beta (13–30) activity increased significantly after *L. cubeba* inhalation. In conclusion, the inhalation of *L. cubeba* essential oil induced effects on the central nervous system (brainwave activity). *L. cubeba* essential oil activated both alpha and beta waves, representing calmness and alertness, respectively. This study has demonstrated the effect of *L. cubeba* essential oil inhalation in complement with well-being and aromatherapeutic applications.

**Keywords**: Brainwaves, Electroencephalography, Essential oil, *Litsea cubeba* oil, Sweet almond oil

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Essential oils are a group of plant secondary metabolites consisting of a mixture of volatile phytochemicals that produce a unique aroma[1]. One of the most popular alternative therapies widely practiced worldwide is aromatherapy, which administers essential oils with pharmaceutical properties as their main agents. Aromatherapy has been practiced extensively for its healing and well-being effects in many Asian countries. Thailand is renowned for traditional Thai medicine, including aromatherapy. One of the aromas or essential oils which has long been used in daily life in Thai society is *Litsea cubeba* essential oil. *L. cubeba* is a small-to-medium-sized, dioecious tree, which is 4-15 meters high locally found in China, Taiwan, Japan, India, Southeast Asia, and Northern Thailand[2]. *L. cubeba* was found to be one of the medicinal plants in Arunachal Pradesh, a North-Eastern state in India. Local inhabitants have used it in ethnomedicinal practices to treat headache, hysteria, paralysis, bone fracture, loss of memory, blood dysentery, digestive disorders, and fever[3,4]. *L. cubeba,* one of the 21 *Litsea* species native to Thailand, grows in the natural evergreen forests located more than 1,200 m above sea level. *L. cubeba* essential oil is a pale yellow liquid with a very lemon like, spicy aroma[5]. Derived from *L. cubeba* fruits and leaves, the essential oil is known for its therapeutic properties, including anticancer activity, as an important ingredient in medicinal products for treating cardiac arrhythmia and inflammatory mediators and for its use in clinical aromatherapy as well as cosmetics[6,7]. The effects of essential oils on the human nervous system have been demonstrated via electroencephalography (EEG) recordings. Iijima *et al*.[8] verified the effects of incense on brain function using EEGs and event-related potential (ERP). The researchers found that alpha 2 activity (10–13 Hz) increased significantly during incense exposure in posterior brain regions, and that the odour could affect the primary olfactory cortex

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and the adjoining emotion- and memory-regulating cortices. They concluded that the odour of incense may trigger strong emotional responses and memories[8]. Results of a previous study conducted on the effect of *Abies sibirica* (Pinaceae) essential oil on arousal levels showed that inhalation of the essential oil increased theta bands in EEGs of the participants. The researchers suggested that volatile constituents of the essential oil might aid recovery from mental fatigue[9]. Sowndhararajan *et al.*[10], evaluated the effects of olfactory stimulation of isomeric aroma compounds on human EEG activity. The inhalation of (+)-limonene increased relative high beta activity significantly, while inhalation of terpinolene increased relative fast alpha activity. The researchers concluded that terpinolene displayed positive effects by decreasing tension while increasing the relaxation and stabilisation brain activity states[10]. Studies on the effects of *L. cubeba* essential oil inhalation are still lacking. Therefore, this study aims to investigate the effects of *L. cubeba* essential oil inhalation on human brainwave activity. Sweet almond oil was used as the essential oil diluent. As an experimental control, the effects of sweet almond oil inhalation were compared to *L. cubeba* essential oil inhalation.

## Methodology

#### Participants

Twenty-five participants were recruited to achieve an adequate experimental sample size, based on Lemeshow (1990) and Sayorwan (2011)[11,12], using a significance level below 0.05 ($Z_\alpha$=1.96), a test power of 80% and a 10% drop-out assumption. The participants were recruited from the general public. The research was conducted at Kanchanabhisek Institute of Medical and Public Health Technology, Thailand.

#### Ethical considerations

The Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University approved this research on 8 February, 2018 (COA No. 034/2561). The clinical trial registry number was TCTR20181108002. In addition, written informed consent from the participants was obtained.

#### Inclusion and exclusion criteria

Healthy male and female participants aged between 20 and 35 years with normal cardiovascular health and a normal body mass index range according to WHO and Asian criteria values[13] were recruited. The inclusion criteria were right-handedness (evaluated by the Edinburgh Handedness Inventory scale)[14] and a normal sense of smell (evaluated by the n-butyl alcohol test)[15]. Exclusion criteria were smoking, upper respiratory infection symptoms, hypertension or cardiovascular disease, history of neurological illness, epilepsy, loss of consciousness for over 30 min and use of nervous system medication or sedative drugs.

The "Odour familiarity five-point Likert scale" form was used to screen the pleasantness of *L. cubeba* essential oil in sweet almond oil. The inclusion criteria were those participants whose pleasantness scores were between 2 and 4. The exclusion criteria were the participants who were allergic to essential oils or had a headache after the essential oil inhalation. Before the experiment, the participants were informed not to use any hairspray, deodorants or perfume, nor to smoke cigarettes or consume any caffeinated drinks. They should not have felt tired or drowsy on the experiment date.

## Materials and Methods

#### Essential oil analysis

*L. cubeba* seed essential oil and sweet almond oil were obtained from Thai–China Flavours and Fragrances Industry Co., Ltd. The chemical composition of *L. cubeba* essential oil was analysed by Trace GC Ultra gas chromatograph equipped with Finnigan DSQ mass spectra detector, Thermo Finnigan (USA).

#### Essential oil delivery

Sweet almond oil was used as carrier oil to dilute the *L. cubeba* essential oil. One milliliter of sweet almond oil or 10% *L. cubeba* essential oil in the sweet almond oil was delivered from oxygen pump system through a plastic tube via face mask in an inhalation set for adults which allowed selective routine air flow (2 L/min).

#### Outcome instruments

Nicolet EEG v32 from Natus Neurology Company (USA) was used for brainwave recordings. The set of 21 electrodes with 1 additional ground were placed, in accordance with the international 10-20 system, at Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4, Pz, T3, T4, T5, T6, O1, O2, A1, and A2 (LOC, ROC for eye movements). The left anterior area (Fp1, F3, F7), the right anterior area (Fp2, F4, F8), left posterior area (P3, T5, O1), right posterior area (P4, T6, O2) and the central (Fz, Cz, Pz) brain regions were evaluated.

**Procedures**

Each experiment was conducted in a quiet, air-conditioned (24±1°C) and pre-ventilated room with 50–65% humidity between 8.00 a.m. and 12.00 p.m. to reduce circadian variation. Each participant was tested separately to avoid contamination. EEG recordings were performed as 5 min eyes opening and 5 min eyes closing for baseline. Sweet almond oil was inhaled for 8 min during eyes closing. After that, the experiment was performed again using 10% *L. cubeba* essential oil in the sweet almond oil inhaled for 8 min during eyes closing.

**Data analysis**

STATA version 14 statistical software was used for data analysis. A paired t-test was used for comparison within participants.

## Results

**The chemical composition of *L. cubeba* essential oil**

The GC/MS analysis showed that the major chemical compounds in the *L. cubeba* essential oil were geranial (41.73%) and neral (33.95%); the rest of the oil comprised several minor compounds, i.e., sylvestrene (10.23%), isocitral<E-> (1.70%) and citronellal (1.66%) and other trace compounds (Table 1).

**Demographic data of the participants**

The average (SD) age, height, weight, and BMI of the 25 participants were 31.32 (±2.77) years, 1.61 (±0.07) m, 53.56 (±6.9) kg and 20.47 (±1.2) kg/m$^2$, respectively.

Table 1 — *Litsea cubeba* essential oil

| S. No. | RT (min) | Name compound | Kovat's Index | Area % |
|---|---|---|---|---|
| 1. | 6.67 | Pinene<alpha-> | 939 | 1.30 |
| 2. | 7.12 | Camphene | 954 | 0.32 |
| 3. | 7.87 | Sabinene | 975 | 0.56 |
| 4. | 7.99 | Pinene<beta-> | 979 | 0.83 |
| 5. | 8.24 | Hepten-2-one<6-methyl-5-> | 985 | 0.94 |
| 6. | 8.38 | Myrcene | 990 | 1.16 |
| 7. | 9.75 | Sylvestrene | 1030 | 10.23 |
| 8. | 9.85 | Cineole<1,8-> | 1031 | 1.53 |
| 9. | 12.48 | Linalool | 1096 | 0.99 |
| 10. | 14.68 | Citronellal | 1153 | 1.66 |
| 11. | 15.17 | Chrysanthenol<cis-> | 1164 | 1.07 |
| 12. | 15.94 | Isocitral<E-> | 1180 | 1.70 |
| 13. | 16.31 | Terpineol<alpha-> | 1188 | 0.44 |
| 14. | 17.90 | Nerol (Z-citral) | 1229 | 0.37 |
| 15. | 18.44 | Neral (E-citral) | 1238 | 33.95 |
| 16. | 19.70 | Geranial | 1267 | 41.73 |
| 17. | 25.95 | Caryophyllene (E-) | 1419 | 1.11 |
| 18. | 27.32 | Humulene<alpha-> | 1454 | 0.11 |

**EEG data analysis**

EEG recordings within the participants who inhaled sweet almond oil compared to the *L. cubeba* essential oil in sweet almond oil were conducted. The mean scores of alpha wave band power in the right anterior, center, left posterior, and right posterior brain regions increased significantly after *L. cubeba* essential oil inhalation ($p<0.05$). In addition, the mean scores of beta wave band power in the left anterior, center, left posterior, and right posterior regions also increased significantly ($p<0.05$). Inhalation of *L. cubeba* essential oil did not affect delta and theta wave band powers (Table 2).

## Discussion

Sweet almond oil is a carrier oil or base oil widely used to dilute essential oil in aromatherapy because it can help the essential oil to be absorbed more evenly; carrier oils are also utilised because undiluted essential oils can cause irritation[16]. In this study, each participant received the sweet almond oil as a control in the first phase, and subsequently received the tested essential oil in dilution with the sweet almond oil. The comparison of EEG recordings between baseline and sweet almond oil inhalation as well as sweet almond oil and the *L. cubeba* essential oil in sweet almond oil was performed within each participant. Sweet almond oil inhalation did not affect the band powers of delta, theta, alpha, and beta waves. During the *L. cubeba* essential oil inhalation, alpha power (8–12.9 Hz) activity increased significantly in most brain regions, including the right anterior, center, left posterior and right posterior regions. In addition, the beta power (13–30 Hz) activities also increased significantly in most brain regions, including the left anterior, center, left posterior, and right posterior regions. An increase in alpha wave activity enhances the perception of calmness and an increase in beta wave activity enhances cognitive skill[17]. The potential of *L. cubeba* essential oil to activate alpha and beta brainwaves has been demonstrated earlier. Morinushi *et al*. [18] reported that chewing flavored gum and inhaling aromatic oil, which increased alpha and beta waves, could affect heightened arousal status as well as high cognition and emotional status. Chen *et al*.[19] concluded that the oral administration of fruit essential oil from *L. cubeba* in mice had potent neuropharmacological effects.

The major component in *L. cubeba* essential oil was citral (75.68%) in two isomers, cis-form (neral 33.95%) and trans-form (geranial 41.73%) (Fig. 1).

Table 2 — EEG parameters during resting, the inhalation of sweet almond oil and *L. cubeba* essential oil

| Area | B | | SO | | LE | | p-value B and SO | p-value SO and LE |
|---|---|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD | | |
| | | | | Delta Power ($\mu V^2$) | | | | |
| Left anterior | 6.42 | 1.85 | 6.47 | 2.07 | 6.41 | 2.70 | 0.875 | 0.798 |
| Right anterior | 6.17 | 1.73 | 6.49 | 2.21 | 6.04 | 2.78 | 0.321 | 0.142 |
| Center | 9.04 | 3.09 | 9.30 | 3.09 | 9.34 | 3.66 | 0.703 | 0.875 |
| Left posterior | 4.17 | 1.48 | 4.21 | 1.17 | 3.95 | 1.16 | 0.847 | 0.119 |
| Right posterior | 4.03 | 1.08 | 4.09 | 1.13 | 4.01 | 1.52 | 0.523 | 0.576 |
| | | | | Theta Power ($\mu V^2$) | | | | |
| Left anterior | 4.63 | 2.12 | 4.65 | 2.15 | 4.71 | 2.01 | 0.824 | 0.848 |
| Right anterior | 5.23 | 3.33 | 5.10 | 2.87 | 5.01 | 2.22 | 0.505 | 0.761 |
| Center | 8.11 | 2.87 | 7.64 | 2.67 | 8.13 | 3.06 | 0.137 | 0.139 |
| Left posterior | 3.86 | 2.27 | 3.79 | 2.23 | 3.68 | 1.80 | 0.583 | 0.734 |
| Right posterior | 3.80 | 3.11 | 3.57 | 1.67 | 3.72 | 1.91 | 0.508 | 0.494 |
| | | | | Alpha Power ($\mu V^2$) | | | | |
| Left anterior | 3.77 | 1.62 | 3.73 | 1.83 | 4.17 | 1.96 | 0.531 | 0.160 |
| Right anterior | 4.88 | 2.70 | 5.03 | 3.62 | 5.62 | 3.91 | 0.612 | 0.019* |
| Center | 10.00 | 5.53 | 9.32 | 5.89 | 11.07 | 6.31 | 0.225 | 0.001* |
| Left posterior | 6.36 | 4.58 | 6.58 | 4.77 | 8.56 | 5.20 | 0.656 | 0.001* |
| Right posterior | 6.56 | 3.46 | 6.79 | 3.34 | 8.05 | 3.34 | 0.529 | 0.013* |
| | | | | Beta Power ($\mu V^2$) | | | | |
| Left anterior | 3.44 | 0.81 | 3.47 | 0.77 | 3.96 | 1.25 | 0.825 | 0.010* |
| Right anterior | 3.66 | 1.19 | 3.57 | 1.10 | 3.83 | 1.18 | 0.575 | 0.216 |
| Center | 4.35 | 1.25 | 4.42 | 1.18 | 4.85 | 1.23 | 0.614 | 0.009* |
| Left posterior | 3.54 | 1.38 | 3.54 | 1.14 | 4.00 | 1.42 | 0.968 | 0.041* |
| Right posterior | 3.96 | 1.17 | 4.13 | 1.47 | 4.66 | 1.55 | 0.425 | 0.024* |

* Significant difference, p-value < 0.05, B= Baseline, SO=Sweet almond oil, LE=*L. cubeba* essential oil

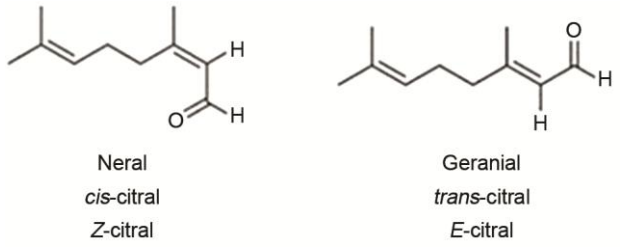


Fig. 1 — Chemical structures of neral and geranial[18]

Neral and geranial are naturally combined components of citral in citrus-aroma essential oils that can be extracted from herbal plants, such as *Cymbopogon citratus* (lemongrass), *Melissa officinalis* (melissa) and *Verbena officinalis* (verbena). They both have a lemon-like odour and taste, and are therefore commonly applied in food, cosmetic and healthcare products[20,21].

The difference in chemical configuration of enantiomeric compounds may affect odour perception and EEG responses[22]. Each essential oil has its own unique odour as well as a particular effect on brain activity.

## Conclusion

The inhalation of *L. cubeba* essential oil induced effects on the central nervous system (brainwave activity). *L. cubeba* essential oil activated both alpha and beta waves, representing calmness and alertness, respectively. This study has demonstrated the effect of *L. cubeba* essential oil inhalation in complement with well-being and aroma therapeutic applications.

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## Conflict of Interest

The researchers declare no conflict of interest.

## Author's Contributions

NN proposed the research objective, conducted this study, collected the EEG recordings, analysed the

EEG data and statistical data, and drafted the manuscript. VS provided insightful knowledge and useful discussion points on brain wave activity, and analyzed the EEG data. WS provided technical and material support. TGF helped draft the manuscript and analyze the statistical data. CP reviewed the literature, made relevant suggestions and revised the discussion. NR gave useful guidance and revised the manuscripts. All of the authors read and approved the final manuscript.

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