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Effects of inhaled essential oils on central nervous system, autonomic nervous system, emotional states and reaction time: randomized crossover study

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EFFECTS OF INHALED ESSENTIAL OILS ON CENTRAL NERVOUS SYSTEM, AUTONOMIC NERVOUS SYSTEM, EMOTIONAL STATES AND REACTION TIME: RANDOMIZED CROSSOVER STUDY

Miss Pakamon Thanatuskitti

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ผลของน้ำมันระเหยต่อระบบประสาทส่วนกลาง ระบบประสาทอัตโนมัติ อารมณ์ และเวลาปฏิกิริยา: การศึกษาแบบสุ่มไขว้กลุ่ม

น.ส.ภคมน ธนทัศกิตติ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Thesis Title	EFFECTS OF INHALED ESSENTIAL OILS ON CENTRAL NERVOUS SYSTEM, AUTONOMIC NERVOUS SYSTEM, EMOTIONAL STATES AND REACTION TIME: RANDOMIZED CROSSOVER STUDY
By	Miss Pakamon Thanatuskitti
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ภคมน ธนทัศกิตติ : ผลของน้ำมันระเหยต่อระบบประสาทส่วนกลาง ระบบประสาทอัตโนมัติ อารมณ์และเวลาปฏิกิริยา: การศึกษาแบบสุ่มไขว้กลุ่ม. (EFFECTS OF INHALED ESSENTIAL OILS ON CENTRAL NERVOUS SYSTEM, AUTONOMIC NERVOUS SYSTEM, EMOTIONAL STATES AND REACTION TIME: RANDOMIZED CROSSOVER STUDY) อ.ที่ปรึกษาหลัก : ชนิดา พลานุเวช , อ.ที่ปรึกษาร่วม : วรสิทธิ์ ศิริพรพาณิชย์,วินัย สยอวรรณ

สุคนธบำบัดหมายถึงการใช้กลิ่นของน้ำมันระเหยมาใช้รักษาอาการต่าง ๆ ของร่างกาย การสูดดมน้ำมันระเหยนั้นมีประสิทธิผลในการรักษามนุษย์ทั้งในด้านจิตวิทยาและสรีรวิทยา ถึงแม้ว่าการศึกษาวิจัยเกี่ยวกับประสิทธิผลของการสูดดมน้ำมันระเหยที่ผ่านมามีจำนวนมาก แต่ยังขาดการศึกษาผลต่อระบบประสาทส่วนกลางและเวลาปฏิกิริยา ดังนั้นการวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของน้ำมันระเหย ได้แก่ น้ำมันดอกส้ม (*Citrus aurantium* Linn.) น้ำมันใบอบเชย (*Cinnamomum zeylanicum* Blume), และน้ำมันผักแขยง (*Limnophila aromatica* Merr.) ที่มีต่อระบบประสาทส่วนกลาง (คลื่นสมอง) ระบบประสาทอัตโนมัติ (อัตราการเต้นของหัวใจ ความดันโลหิต อัตราการหายใจ และอุณหภูมิผิวหนัง) สภาวะอารมณ์และเวลาปฏิกิริยา การวิจัยครั้งนี้เป็นการศึกษาเชิงทดลองแบบสุ่มไขว้กลุ่ม อาสาสมัครทุกคนได้แสดงความยินยอมเป็นลายลักษณ์อักษรก่อนเข้าร่วมการวิจัย อาสาสมัครที่มีสุขภาพดีจำนวน 72 คน ที่เข้าร่วมงานวิจัยในครั้งนี้ได้รับการคัดเลือกจากบุคคลทั่วไปและถูกแบ่งออกเป็น 3 ชุดการทดลอง (อาสาสมัคร 24 คนต่อชุดการทดลอง) โดยแต่ละชุดแบ่งตามน้ำมันระเหยจำนวน 3 ชนิด ที่ใช้ในการศึกษาครั้งนี้ซึ่งใช้วิธีการสุ่มแบบง่ายโดยการจับฉลาก อาสาสมัครแต่ละคนจะได้ดมน้ำมัน 2 ชนิด (ได้แก่น้ำมันระเหยและน้ำมันอัลมอนด์ซึ่งใช้เป็นน้ำมันพา) โดยเว้นระยะห่างกัน 1 สัปดาห์ ขณะที่ให้ดมน้ำมันแต่ละชนิดอาสาสมัครจะถูกบันทึกคลื่นสมองของระบบประสาทส่วนกลาง โดยเครื่อง Nicolet EEG v32 ถูกบันทึกพารามิเตอร์ต่างๆของระบบประสาทอัตโนมัติโดยเครื่องวัดสัญญาณชีพรุ่น BIOM7000 ให้อาสาสมัครทำแบบสอบถามประเมินตนเองของ Geneva Emotion และ Odor Scale ในฉบับภาษาไทยเพื่อวัดสภาวะอารมณ์และความรู้สึกต่อกลิ่นของอาสาสมัคร ศึกษาผลของเวลาปฏิกิริยาด้วยโปรแกรม Deary-Liewald วิเคราะห์ข้อมูลโดยใช้สถิติทดสอบ paired t-test ที่ระดับนัยสำคัญที่ .05 ผลการศึกษาพบว่า หลังจากสูดดมน้ำมันดอกส้ม อาสาสมัครมีความรู้สึกผ่อนคลายและโรแมนติกเพิ่มขึ้นในขณะที่มีความรู้สึกเครียดลดลงด้วย หลังจากการสูดดมน้ำมันใบอบเชยอาสาสมัครรู้สึกผ่อนคลายสงบเพิ่มขึ้นและเครียดลดลง หลังจากสูดดมน้ำมันผักแขยงอาสาสมัครรู้สึกผ่อนคลายเพิ่มขึ้น ในด้านพารามิเตอร์ของระบบประสาทอัตโนมัติของน้ำมันระเหยทั้ง 3 ชนิด พบว่ามีผลการเปลี่ยนแปลงที่ลดลงอย่างมีนัยสำคัญทางสถิติ ส่วนผลต่อระบบประสาทส่วนกลางพบว่า น้ำมันดอกส้มทำให้คลื่นทีต้าและคลื่นแอลฟาเพิ่มขึ้น คลื่นเบต้าลดลง น้ำมันใบอบเชยทำให้คลื่นแอลฟาเพิ่มขึ้นและคลื่นเบต้าลดลง น้ำมันผักแขยงทำให้คลื่นแอลฟาและธีต้าเพิ่มขึ้น น้ำมันดอกส้มและน้ำมันผักแขยงสามารถลดเวลาปฏิกิริยาอย่างง่ายได้อย่างมีนัยสำคัญทางสถิติ น้ำมันใบอบเชยลดเวลาปฏิกิริยาอย่างง่ายได้แต่ไม่มีนัยสำคัญทางสถิติ น้ำมันระเหยทั้ง 3 ชนิด ไม่มีผลต่อเวลาปฏิกิริยาแบบมีตัวเลือกได้อย่างมีนัยสำคัญทางสถิติ การสูดดมน้ำมันดอกส้ม น้ำมันใบอบเชยและน้ำมันผักแขยงสามารถกระตุ้นให้เกิดการผ่อนคลายต่อระบบประสาทอัตโนมัติ สภาวะอารมณ์เชิงบวกและการผ่อนคลายของสมอง

สาขาวิชา	วิทยาศาสตร์สาธารณสุข	ลายมือชื่อนิสิต ....
ปีการศึกษา	2562	ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
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KEYWORD: ESSENTIAL OIL NEROLI OIL CINNAMON LEAF OIL RICE PADDY HERB OIL EMOTIONAL STATES AUTONOMIC NERVOUS SYSTEM BRAIN WAVE REACTION TIME

Pakamon Thanatuskitti : EFFECTS OF INHALED ESSENTIAL OILS ON CENTRAL NERVOUS SYSTEM, AUTONOMIC NERVOUS SYSTEM, EMOTIONAL STATES AND REACTION TIME: RANDOMIZED CROSSOVER STUDY. Advisor: Assoc. Prof. CHANIDA PALANUVEJ, Ph.D. Co-advisor: Asst. Prof. Vorasith Siripornpanich, M.D., Ph.D.,Asst. Prof. Winai Sayorwan, Ph.D.

The odors of essential oils used to cure various symptoms refer to aromatherapy. The effects of inhaled essential oil on humans are therapeutically effective due to both the psychological and physiological effects. A large body of previous studies have been conducted on the effects of essential oil inhalation. However, the studies conducted on the effects on central nervous system and reaction time task are still lacking. Thus, the purpose of this study was to evaluate the effects of selected essential oils including neroli oil *(Citrus aurantium* Linn.*),* cinnamon leaf oil *(Cinnamomum zeylanicum* Blume*),* and rice paddy herb oil *(Limnophila aromatica* Merr.) on central nervous system (brain wave activity), autonomic nervous system (heart rate, blood pressure, respiratory rate, skin temperature), emotional states and reaction time. This study was an experimental study with a randomized crossover design. Individuals who volunteered to participate in this study submitted a written consent form before participating in the study. Seventy-two healthy volunteers were recruited from general public and assigned into 3 sessions (24 participants/session) based on these 3 essential oils administered in this study using a simple random sampling method enclosed in envelopes. All the participants received 2 interventions (essential oil and carrier oil) separated by a 1-week washout period. The essential oil and the sweet almond oil were administered while ANS parameters were recorded using BIOM7000 Patient Monitor. Nicolet EEG v32 was used to record brain activities and the self-evaluated questionnaire on emotional states in Thai version of The Geneva Emotion and Odor Scale was used to measure the subjective feelings of the participants. The participants were instructed to rapidly complete the simple and choice reaction tasks on computer screen by the Deary-Liewald reaction time program. Data were analyzed using paired t-test. A value of $p < 0.05$ was considered statistically significant. After the neroli oil inhalation, the participants felt more relaxed, romantic and less stressed. After the cinnamon leaf oil inhalation, the participants felt more relaxed, calmer and less stressed. After the rice paddy oil inhalation, the participants felt more relaxed. All 3 essential oils had statistically significant attenuated effects on ANS parameters. Neroli essential oil increased theta, and alpha waves as well as decreased beta wave. Cinnamon leaf oil increased alpha wave and decreased beta wave. Rice paddy herb oil decreased theta wave but increased alpha wave. The neroli essential oil and the rice paddy herb oil statistically significantly decreased the time spent on the simple reaction time tasks. The cinnamon leaf oil tended to reduce the time spent on the simple reaction time tasks. All 3 essential oils had no significant effects on correct answers as well as the response times for the choice reaction time tasks. The inhalation of neroli oil, cinnamon leaf oil, and rice paddy herb oil could induce the relaxing effects on autonomic nervous system, positive emotional states and the relaxation of brain state.

Field of Study: Public Health Sciences — Student's Signature ....
Academic Year: 2019 — Advisor's Signature ....
Co-advisor's Signature ....
Co-advisor's Signature ....

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CHAPTER I

INTRODUCTION

Background and rationale

Essential oil is a secondary metabolite of plant material that has been extracted by steam or water distillation or by expression. It is a complex mixture of various aromatic compounds and these mixed compounds provide the characteristic odor. The oil is mainly characterized by the genetic of each cultivar and environmental condition [1, 2]. Currently, it is widely known that the odors of essential oils can be used to cure various symptoms which is referred to an aromatherapy. In Latin, aroma means sweet odor, while aroma in Greek means spice. Therapy in both Latin and Greek means curing or healing. The specialists mentioned that the word intends the oils to be used only in way which conforms to the meaning of therapy through aroma, that is not by massage or other ways of application but only by inhalation [3]. The effects of inhaled essential oil on humans are thought to be therapeutically effective due to both the psychological and physiological effects. The physiological effect acts directly on the physical organism while the psychological effect acts *via* the sense of smell or olfactory system. The latter effects are believed to act *via* the limbic system, particularly the amygdala and hippocampus and in turn may cause a physiological effect. The physiological effect is controlled by two major parts of the nervous system, the central nervous system and the peripheral nervous system. Primarily, functions of the central nervous system, brain and spinal cord receive and process sensory information and regulate body movements. Alternatively, the peripheral nervous system is the nervous tissues outside brain and spinal cord, including the cranial and spinal nerves. The peripheral

nervous system is divided into the somatic nervous system, relating to muscular activities, and the autonomic nervous system that controls visceral structures such as glands and organs. Functionally, the autonomic nervous system regulates the internal and relatively involuntary responses that are associated with emotions [4-6].

Measurement of the odor effects on physiology can be divided into two different forms of arousal, the autonomic arousal (heart rate, blood pressure, skin temperature and respiratory rate) and the cortical arousal (brain wave activity). A decrease of the autonomic arousal and the cortical arousal is interpreted in term of a relaxing effect of odors. In contrast, an increase of the arousal is interpreted in term of a stimulating effect of odors [7]. For example, Ylang-Ylang oil showed significant decreases in blood pressure and heart rate [8] whereas rosemary oil caused a considerably significant increase in respiratory rate as well as systolic and diastolic blood pressure [9]. Electroencephalography has been widely used for studying brain activity associated with higher mental functions in humans [10]. Electroencephalography showed that alpha waves, which are associated with relaxation, increased in the presence of relaxing odors such as lavender oil and citronella oil [11-12]. On the other hand, jasmine oil and rosemary oil have been reported that both essential oils increased beta waves for the presence of stimulating effect [9,13]. The odor of essential oil has several effects on psychological well-being, emotions and behaviors. Emotional states questionnaire and the Deary-Liewald reaction time task [14-15] are selected for measurement of psychological effect in this investigation.

In Thailand, the investigations of the effects of essential oils were previously performed in three magnitudes i.e. EEG, autonomic nervous system and emotional

states effects of the jasmine oil, lavender oil, citronella oil and rosemary oil inhalation [9,11-12]. Up to now, there are no investigations of the effects of inhaled essential oil on the reaction time. The literature reviews suggest that this study seems to be the first experimental research in Thailand to examine the four magnitudes. In addition, a randomized crossover design has been used in this study. Neroli oil, cinnamon leaf oil and rice paddy herb oil have been selected in this investigation for the reason that all of them are commonly used in Thailand. Neroli oil is the essential oil in *Citrus* spp. It is popular genus used for aromatherapy in several countries including Thailand [16]. Cinnamon leaf oil is commonly used for aromatherapy and it is active ingredient in various remedies. Moreover, it has a variety of dosage forms such as ointment or inhaler medicine [17]. Rice paddy herb is a Thai local vegetable, which is used for cooking due to its characteristic odor [18]. However, there are no investigations about effects on central nervous system and reaction time task of three essential oils. Thus, the purpose of this study is to evaluate the effects of selected essential oils on central nervous system (brain wave activity), autonomic nervous system (heart rate, blood pressure, respiratory rate, skin temperature), emotional states and reaction time.

Research questions

What are the effects of selected essential oils inhalation on physiological and psychological functions?

Research hypothesis

Each essential oil inhalation can selectively affect central nervous system (brain wave activities), autonomic nervous system (heart rate, blood pressure, respiratory rate, skin temperature), emotional states and reaction time.

Objectives

1. To study the effects of selected essential oils on central nervous system by electroencephalography.

2. To study the effects of selected essential oils on autonomic nervous system by monitoring of heart rate, blood pressure, respiratory rate and skin temperature.

3. To study the effects of selected essential oils on emotional states by questionnaire.

4. To study the effects of selected essential oils on reaction time by the Deary-Liewald reaction time task.

5. To study the chemical composition of selected essential oils.

Benefits of the study

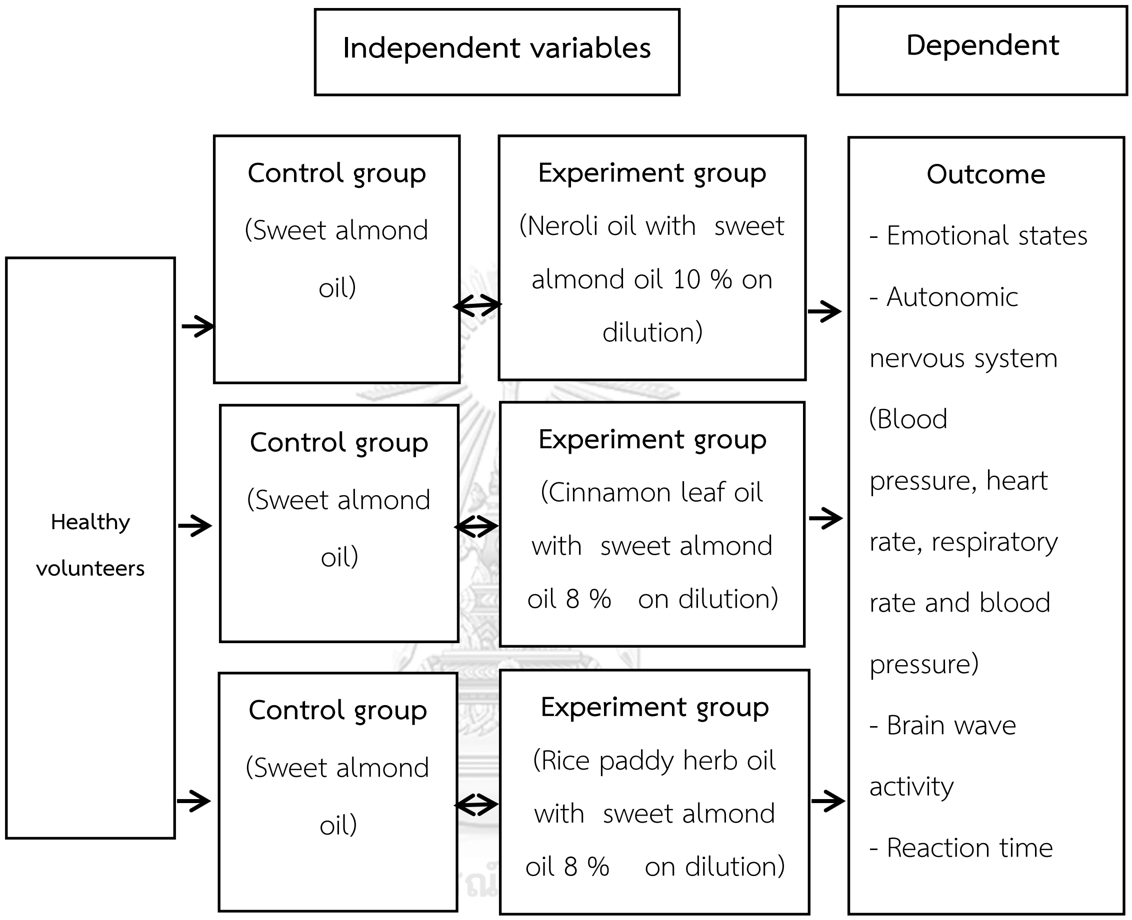
1. The essential oils can be used as alternative and complementary medicine for stimulating or relaxing effects.

2. This research reveals the physiological and psychological effects of neroli oil, cinnamon leaf oil and rice paddy herb oil that can be useful for relaxation.

3. This study design can be applied to further research to study any other essential oils to evaluate its effects on central nervous system (brain wave activity), autonomic nervous system (heart rate, blood pressure, respiratory rate, skin temperature), emotional states and reaction time.

Conceptual Frameworks

The conceptual frameworks of this study are scoped in the chart as shown below.



CHAPTER II
LITERATURE REVIEW

The human olfactory system

Human can detect the scent more than 1000 types by olfactory system. The human olfactory system is the sensory system used for olfaction or the sense of smell [19]. There are two distinct parts of olfactory system i.e. a main olfactory system and an accessory olfactory system. The main olfactory system is used for detecting volatile, airborne substances, while the accessory olfactory system is used for fluid-phase stimuli. Behavioral evidence indicates that most stimuli detected by the accessory olfactory system are pheromones. The scent molecules can enter olfactory system through a primary and secondary pathway. The primary pathway is through the nose, and the secondary pathway is the space between the nasal cavity and the mouth through the mouth *via* the pharynx. The nasal cavity is lining with the olfactory mucosa which is totally different in diverse species. The olfactory mucosa is composed of two parts, the olfactory epithelium and olfactory glands that function in secreting mucus for lining epithelium. The olfactory epithelium in mucosa area which is only a few centimeters wide contains more than 100 million olfactory receptor cells [20-21].

The essential oil can enter the body by directly trapped on the mucus of the nasal cavity. The scent molecule is attached with the smell receptor in dendrite of the olfactory neuron. After that, it is shifted into the nerve on the other side of the mucous patch, which sends the scent information in to the olfactory bulb, lateral olfactory tract, olfactory cortex and limbic system respectively. The essential oil cannot directly enter to the brain, but a neural translation of the complex essential

oil is transmitted through the olfactory system instead. The millions of nerves pass through the ethmoid bone to the olfactory bulb and combine into two large nerves that turn to the limbic brain in the inner center of the head. The right nerves turn to the amygdala, a center for emotions in the limbic system. There, the odorant information is connected to the emotions of the situation. The nerve data are sent from the amygdala to the hippocampus, a center for short-term memory about 3 years in the limbic system. Thus, the scent, mood, and memory are intermingled, as they are stored in the limbic system. Then, the data go on to the hypothalamus for long-term memory. They are passed through the pituitary or master gland and ultimately the whole endocrine system. This is a very primary version of how essential oil affects the moods, memory, and hormonal system. Nevertheless, the limbic system is triggered; the interconnecting by hypothalamus and the pituitary bodies is stimulated, leading to the responses of the central nervous system, autonomic nervous system, mood state and endocrine system. [22-24]

In conclusion, any processes sending impulse directly to the brain affect body and mind as shown in Figure 1 and Figure 2.

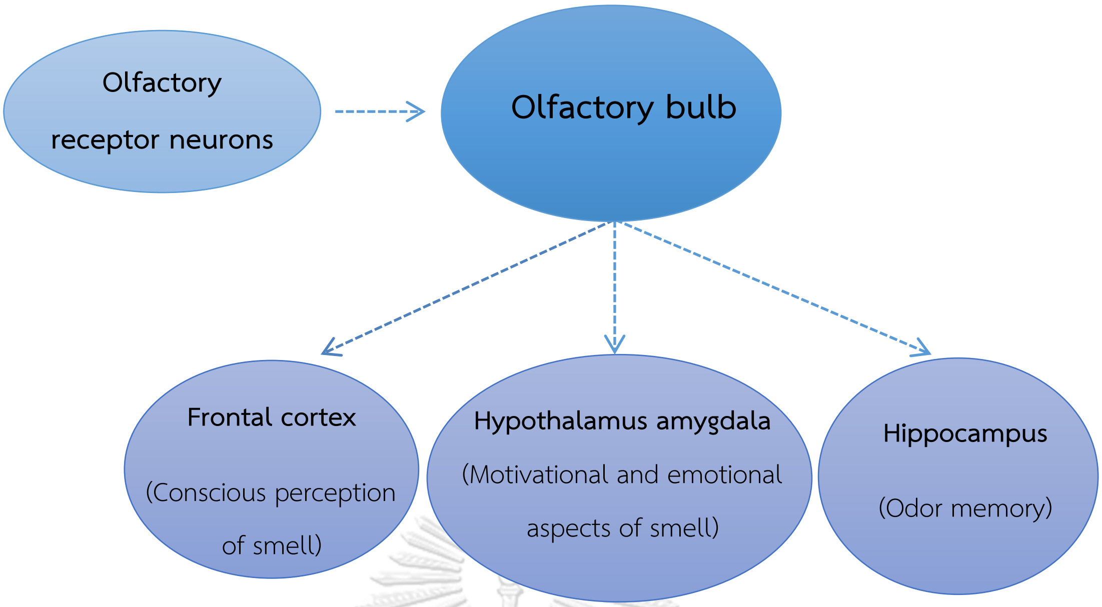


Figure 1 Summarization of the olfactory pathway [25]

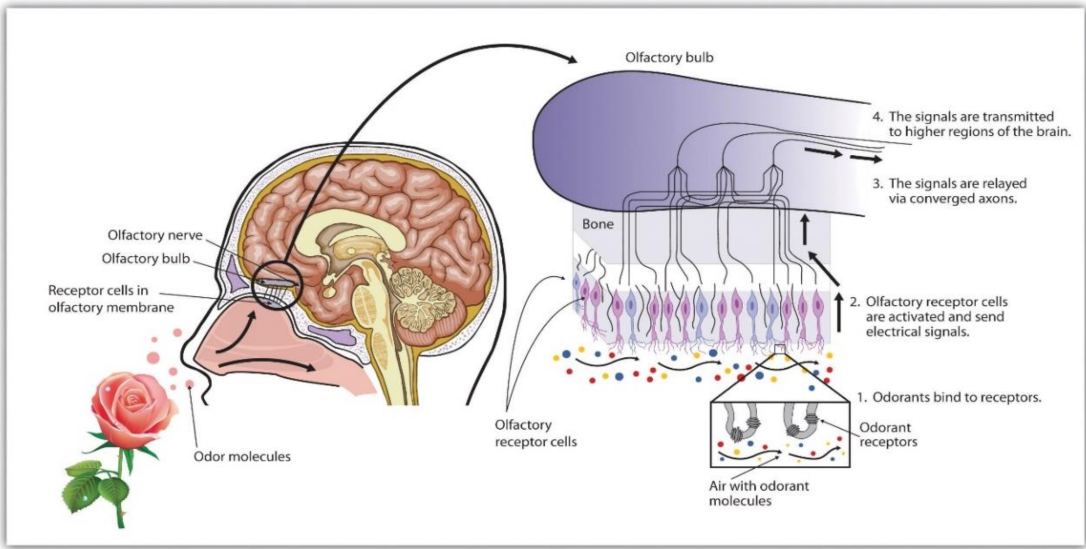


Figure 2 The human olfactory receptors [25]

Human nervous system

The most complex system of any organisms is human nervous system, which probably includes over 100 billion nerve cells and each nerve cell can have up to 10,000 connections to other nerve cells. This means that a nerve impulse as

electrochemical signal to or from the brain can move along 10^{15} paths. Two main divisions of the nervous system are divided into the central nervous system and the peripheral nervous system. Investigators made this division based on where nervous tissue is located in the body centrally or away from the center (peripherally). Together, the central nervous system and the peripheral nervous system control sensory input, combination, and motor output. The central nervous system, which consists of the brain and spinal cord, integrates and processes information sent by nerves.

The peripheral nervous system contains nerves that transport sensory information to the central nervous system and nerves that send information from the central nervous system to the muscles and glands. The peripheral nervous system is divided in two types i.e. sensory (afferent) and motor (efferent) neurons. The sensory nervous system carries sensory information to the central nervous system. The motor nervous system brings instructions from the central nervous system out to the muscles and glands. Functionally, the motor nervous system can be classified into somatic and autonomic nervous system according to voluntary and involuntary control. The skeleton muscles are controlled by the somantic nervous system. The glandular secretions, the functioning of the smooth and cardiac muscles are controlled by the autonomic system. The sympathetic and parasympathetic parts of the autonomic system often work in opposition to each other to determine the autonomic procedures of the body. The autonomic procedures, such as heartbeat and peristalsis, are those that do not require or involve conscious control [26-28].

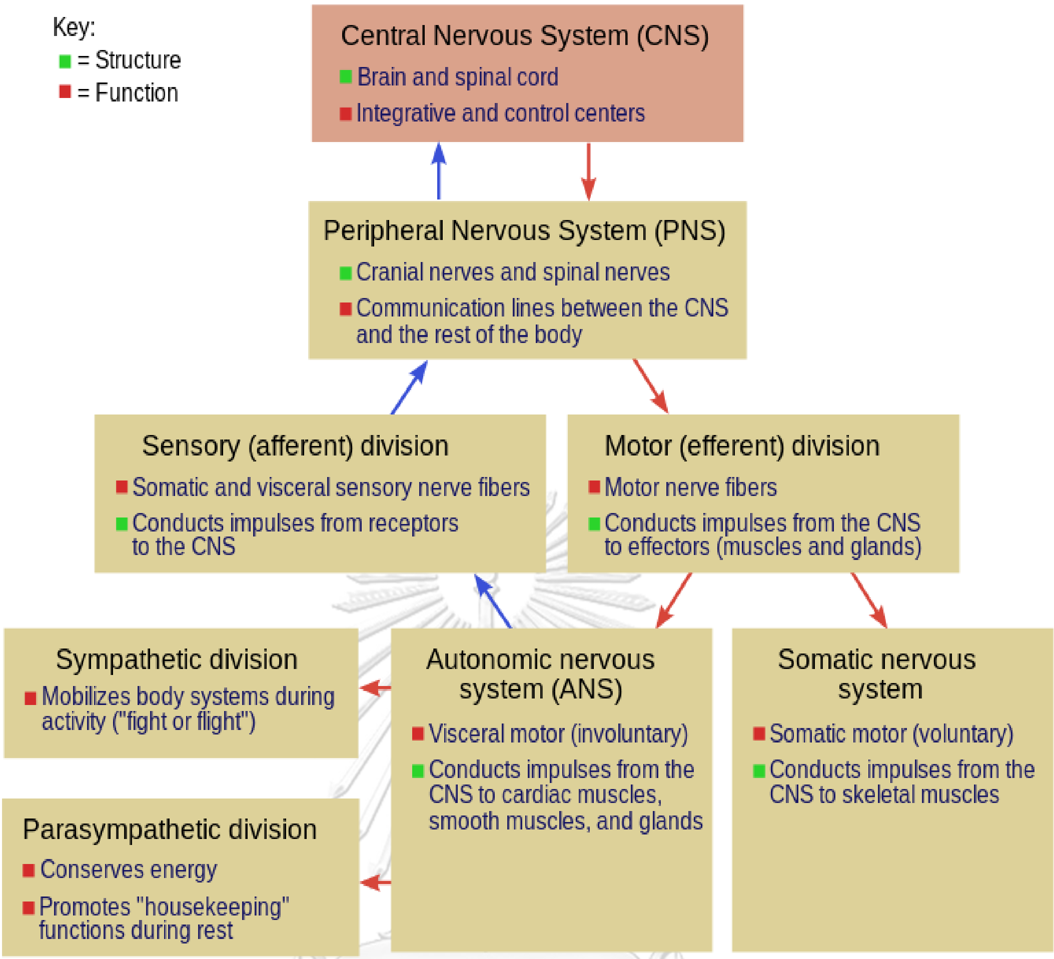


Figure 3 The organization of the human nervous system [27]

Effect of essential oil on physiological activities

Odors can affect the physiological activities by performing through nervous system stimulation and direct performing through organ, or tissue *via* an effector receptor. Nervous system is the main function that controls the physiological properties in living organism. The measurement of the nervous system can be measured in different ways upon the mechanism. The effect of odors can be measured by different types of arousal: a) the cortical arousal using brain wave activities b) the autonomic arousal by obtaining the heart rate, respiratory rate, body

temperature and blood pressure. The changes of physiological effects can be referred to the sedative, relaxing or stimulating effects of the odors [29].

Effect of essential oil on electroencephalogram (EEG)

EEG signal is derived from the voltage (microvolt) in cerebral cortex which harmonized and aggregated at the scalp surface of electrodes. The difference of the voltage from reference and measuring electrode can be used to obtain the difference of the signal over a certain time range. The neuron signal is derived in terms of electrical signal as an analogue voltage. The analogue signal is digitalized, amplified by gaining and filtering and displayed as the biological signal. The digital signal is further used as the frequency measured in Hertz (Hz) which can divide brain wave into four types as mentioned below [30-32].

Alpha wave

Alpha rhythm (8-12.9 Hz) occurs during wakefulness which can be measured at the posterior regions of head over the occipital region. Alpha wave can be found in physical relaxation and the relative mental activity state. It is present when humans are awake and relaxed.

Beta wave

Beta rhythm (13-30 Hz) is associated with wakefulness. Beta wave can be found in thinking and active attention function.

Theta wave

Theta rhythm (4-7.9 Hz) is associated with intuition. It is activated during dream and a deep meditation state. Theta is also associated with creative thinking, recalls, intuition and allows people to tap into their inner genius.

Delta wave

Delta rhythm (0.5-3.9 Hz) appears during deep sleep.

The effects of odor inhalation can be evaluated by EEG measurement. The amplitude and frequency of brain wave are expressed as the EEG rhythm including alpha, beta, delta, and theta waves which can be detected during odor inhalation *via* cortical activity. It has been shown that beta wave is dominant when people are engaged in reading, concentrated deliberation, highly emotional and other tense mental states. In addition, alpha wave can also be found in the same condition with beta wave, but alpha wave is more potent in the mental relaxation state. It could be suggested that brain waves continuously reflect human psychological level, levels of consciousness, and arousal level. Lorig and Schwartz studied the effects aromas including eucalyptus, lavender, spiced apple and vehicle control. They found that the studied essential oil affected the alpha and theta wave distributions [33]. Furthermore, the study in Japan demonstrated the variations of EEG after inhalation of the essential oil using a reading experiment. They also found that lavender, cineol, jasmine, and sandalwood had a relaxing effect by increasing alpha activity. Moreover, the jasmine odor could increase beta activity which might be referred to energizing effect [34].

Nakagawa *et al.* [35] studied methyl jusmonate, cineol aromas and jasmine lactone odors. Methyl jusmonate and cineol aromas were shown to be an alpha and theta waves enhancement by decreasing the EEG activity which might be affected by stimulating effect while jasmine lactone odor enhanced alpha and theta activities which possibly presented a relaxing effect.

From several investigations, the researchers reported the influences of odor on changes of EEG activities. Tonoike *et al* [36 suggested that pleasant odors could increase alpha activity. In addition, Lorig [37] supported that the effects of lavender and jasmine odor which tended to be pleasant odors on brain wave activity were also consistent when participants inhaled them by increasing the alpha activity. Their results strongly suggested that the pleasant odors might increase the alpha rhythm in encephalograph activity.

Furthermore, Ekman *et al* [38] found that negative emotions were related to right frontal lobe while happiness excited more left frontal lobe activity. Bensafi [39] found that the output of alpha waves was significantly reduced in the right compared with the left frontal brain region when volunteers were stimulated with a pleasant odor (vanilla). Sayowan *et al* demonstrated the effects of the four aroma types compared with base oil. They found that jasmine, lavender, rosemary and citronella oil could alter alpha, beta and theta activities. Jasmine oil showed the increasing beta wave. Lavender oil increased theta and alpha activities. In addition, the alpha activity was reduced by rosemary oil, but the beta activity was increased in the anterior region landmark. Citronella oil reduced the power of alpha and beta waves [9, 11-13]. In conclusion, the effects of aromas on EEG depended on two factors: the characteristics and pleasantness of essential oil.

Effect of essential oil on autonomic nervous system

Effect of essential oil on heart rate

In general psychophysiology, heart rate is used for measuring heart activity. Stress can increase heart rate whereas heart rate may be changed in an anxious state. Depression can change the heart rate. The autonomic nervous system can

innervate the heart. The autonomic nervous system is subdivided into sympathetic nervous system and parasympathetic nervous system. Both systems reduce the heart activities which may be referred to the influences of heart rate, particularly affecting the pumping function of myocardial muscle.

Yamaguchi *et al*. [40] investigated the effect of lemon and rose odors in order on the changes of heart rate after inhalation. They found that rose odors can statistically decreased heart rate, but lemon odor led to increase it. They concluded that lemon odor had a stimulating effect but rose odor possessed a relaxing effect. Kikuchi *et al*. [41] also investigated lemon and rose odors. In contrary, they found that lemon odor decreased heart rate, and rose odor also decreased it, which represented a sedative effect. Nagai *et al*. [42] examined sweet fennel oil. They found that it also suppressed heart rate. In addition, Hongratanaworakit *et al*. [43] investigated the effects of sweet orange oil on behavioral activities. They observed the vital response on olfactory stimulation. From their results, the sweet orange oil significantly increased the physiological and behavior activities. Similar to the study of Shiina *et al.* [44], they examined the effect of lavender oil on coronary circulation and serum cortisol which was referred to stressful state hormone. After the experiment, they found that it increased the vasodilatation significantly and decreased the cortisol level statistically. The researchers suggested that lavender oil could hold a promise to be a relaxing agent with additional effect on coronary circulation. Brauchli *et al* [45] reported the difference between pleasant and unpleasant odors which affected autonomic variable. They found that heart rate increased after inhalation of valeric acid (unpleasant), and decreased during phenylethyl alcohol inhalation (pleasant).

Effect of essential oil on blood pressure

Blood pressure is a vital sign leading to the physiological variables. Blood pressure was used as general index for optimizing the cardiovascular function. Blood pressure can be categorized into two categories: systolic and diastolic blood pressure. The systolic blood pressure occurs when the ventricle of the heart contracts while diastolic blood pressure refers to the relaxation state of the ventricle. Blood pressure change is regulated by various factors. Blood volume and peripheral resistance also cause blood pressure change.

Royal Sussex County Hospital studied the physiological changes in foot massage combination with lavender oil and transdermal absorption of sandalwood oil. Their study observed the blood pressure, heart rate and respiratory rate changes of patients in an intensive care unit. The researchers found that the blood pressure decreased compared to the placebo group [46]. Heuberger *et al.* [47] examined the effects of chiral fragrances on blood pressure. The chiral fragrances which comprised of limonene and carvone increased blood pressure led to subjective restlessness and alertness. The researchers concluded that chiral fragrances had a stimulating effect.

Heuberge *et al.* [48] studied effects of citronella oil compared to base oil. Their findings suggested that citronella oil decreased blood pressure significantly after inhalation. In addition, Hongratanaworakit *et al*., [49] investigated the effect of ylang-ylang oil. From their results, ylang-ylang oil was shown to be a harmonized agent by increasing subjective attention and decreasing blood pressure. In contrary, another research investigated the transdermal absorption of bergamot and lavender combination oil. The researchers found that the combination oil decreased the blood pressure of the subjects in their study significantly [50].

Effect of essential oil on skin temperature and breathing rate

The skin temperature and respiratory rate can be influenced by cognitive and emotional psychological effects. It is well-known that stress can activate the response of sympathetic nervous system by reducing skin temperature and increasing respiration rate. In previous research, the experimental animals had been used for studying the physiological change in stressfulness by observing the muscular and cardiovascular activity. The physiological effects of stress were found as lowering skin temperature, increasing heart rate, respiration rate and muscle tension. These physiological changes were accompanied with the psychological states. The stressfulness can activate the parasympathetic system by returning the blood flow into peripheral area which results in the increase in peripheral skin temperature, the decrease of breathing rate and induces relaxation [51-52]. According to a study conducted by Hongratanaworakit *et al*. [8], they have demonstrated that ylang-ylang oil after transdermal absorption caused a significant increase of skin temperature. In addition, transdermal absorption of the mixture of bergamot oil and lavender oil caused a significant decrease of breathing rate but an increase in skin temperature. Furthermore, Hongratanaworakit *et al*. [43] have demonstrated that sweet orange oil after transdermal absorption caused significant decreases of breathing rate but no significant effects of the sweet orange oil on skin temperature have been reported.

Effect of essential oil on emotional state

In all cultures, odor is an influential elicitor of sentiments. In the last few decades, a growing scientific literature has recognized numerous demonstrative effects of scents. The odor experience is indissolubly related to odor hedonic tone (pleasantness-unpleasantness) and, thus, is likely to affect temper. For instance,

pleasant odors tend to induce optimistic tempers, whereas unpleasant odors tend to induce undesirable tempers [53]. Several experiments also reported that odors decrease effects on cognition and behavior that are similar to those created by emotional stimuli in other perceptual modalities. In addition, odor experiences have been shown to provoke changes in physiological parameters, such as heart rate or electrical conductivity of the skin, which are directly involved in the emotional responses [54]. Sattely-Miller [55] assessed whether the daily use of pleasant smelling colognes could elevate mood in men by the Profile of Mood States (POMS) questionnaire which could be divided into six factors: tension-anxiety, depression-dejection, anger-hostility, vigor-activity and fatigue-inertia. The first two days of the study provided the baseline information for each participant. For both the baseline and the remainder of the study, the POMS was completed twice each day. Statistical significance was found for the vigor factor, with the fragrance condition having significantly higher scores than the placebo condition. Lehrner *et al.* [56] examined the ability of orange odor to reduce anxiety and improve mood in dental patients while they were waiting for a dental treatment. The participants were assigned to either a control condition (where they waited with no odor present) or to a scent condition (where ambient orange scent was diffused into the waiting room). Compared to the control group, the orange scent group reported a lower level of nervousness, a more optimistic temper, and a higher level of stillness. Burnett and Strapp [57] presented the participants with the scent of lavender, rosemary, or water. Both rosemary and lavender aromas were related with subordinate mean scores on the fatigue-inertia subscale of POMS. Sayowan *et al*. demonstrated the effects of the four aroma types i.e., jasmine, lavender, rosemary and citronella oil compared with

base oil. The positive emotions including the feeling of well-being, active, renewed and idealistic have been augmented by jasmine oil. On the other hand, the negative emotion for example the feeling of drowsy decreased significantly. The subjects in the lavender oil group were considered to be more energetic, and relaxed than the subjects just gasping base oil. After the inhalation of rosemary oil, the subjects were found to become more energetic. Citronella oil was reported to make the subjects feel in a better mood and fresher after inhalation [9, 10-12].

Reaction time task

Reaction time task is a method which is basically and impliedly used for psychological manipulations. It has been used as a psychological task for a century. There are several different reaction time tasks using in psychological performance. There are two common reaction time tasks which are used to evaluate the psychological activities, simple reaction time and choice reaction time. Simple reaction time refers to the response in single stimulus. In addition, choice reaction time is complicated response in multiple stimuli. The central tendency and intra individual variability are the measurements which are derived from simple reaction time [58].

The essential oils have several effects on psychological well-being, emotions and behaviors. The Deary-Liewald reaction time task is one of reaction time tasks appropriately and widely used for measuring the psychological effects. The Deary-Liewald reaction time task is selected in this research because this task is easy to use. It is a program available for free and a computer-based reaction time task with no special software. The Deary-Liewald reaction time task was developed and tested

on 150 participants, aged from 18 to 80, alongside another widely used reaction time device and tests of fluid and crystallized intelligence and processing speed. The task's parameters could be performed as expected with respect to age and intelligence differences. The parameters of this task were reliable, and had very high correlations with the other task [59-60].

The Deary-Liewald reaction time task can be classified into simple reaction time (SRT) and choice reaction time (CRT). SRT is the task in which the participants are informed to click the button on mouse to respond to a single stimulus rapidly. The incentive to the response is appearance of a diagonal cross within the square. SRT involved eight practice trials and twenty test trials. For the CRT, there are four types of stimuli and participants have to click the button on mouse quickly that corresponds to the correct response. Four white squares are positioned in a horizontal line across approximately the middle of the computer screen, set against a blue background. CRT involves eight practice trials and forty test trials. Four keys on a standard computer keyboard correspond to the different squares. The z key corresponds to the square on the far left; the x key to the square second from the left; the comma key to the square second from the right and the full-stop key to the square on the far right. The stimulus to respond is the appearance of a diagonal cross within one of the squares. A cross appears randomly in one of the squares and participants are asked to respond as quickly as possible by pressing the corresponding key on the keyboard. Each cross remains on the screen until one of the four keys is pressed, after which it disappears and another cross appears shortly after. The computer program calculates the mean, median, variance, and standard deviation of the response times of the participants [14].

Schmoll *et al.* determined the mean and the SD of two modalities of reaction time, namely complex reaction time and simple reaction time on 15 patients (age range 59-87 years, mean 75.4 years) with bilateral cataract who performed the reaction time task before and after surgery on one eye. The results demonstrated that responses became both quicker and more consistent following surgery, with statistically significant improvements in the CRT mean (p=.016) and the CRT variability (p=.055), which were not due to a learning effect or improved vision [15].

Essential oils

1. Neroli Oil [61-69]

Neroli oil is obtained through the steam distillation of fresh flowers of Citrus *aurantium* L. The average is 0.12% w/w. A clear essential oil with has a pale yellow or yellow orange with a sweet fresh green odor.

Botanical name: *Citrus aurantium* Linn.

Common name: Bitter orange, Sour orange, Seville orange and bigarade orange

Thai name: -

Family: Rutaceae

Genus: *Citrus*

Plant description

"Small trees. Branches with spines up to ca. 8 cm. Petiole obovate, 1–3 × 0.6–1.5 cm, base narrow; leaf blades dark green, thick. Inflorescences racemes, with few flowers or flowers solitary. Flowers perfect or male by ± complete abortion of pistil; buds ellipsoid to subglobose. Calyx lobes 4 or 5. Petals 2–3.5 mm in diam.

Stamens 20–25, usually basally connate into bundles. Fruit orange to reddish, globose to oblate, surface coarse; pericarp thick, sometimes difficult to remove; sarcocarp with 10–13 segments, acidic and sweet or sometimes bitter. Seeds numerous, large, with ridges; embryo(s) solitary to numerous; cotyledons milky white".

Location

The sour orange originated in the South Sea Islands, Fiji, Samoa, and Guam.

Usage

For aromatherapy, inhaling neroli oil is very relaxing and can decrease depression or stress and it is effective in treating headaches, neuralgia, vertigo and muscle spasm. On the skin, neroli oil can help to regenerate skin cells and is rejuvenating oil useful to prevent ugly scar tissue.

Pharmaceutical effect

Pharmacological research of neroli oil was found to have antimicrobial activity. This oil was effective in both gram positive and gram negative bacteria especially against *Pseudomonas aeruginosa*. Moreover, it exhibited a very strong fungal agent compared with the standard antibiotic (Nystatin). Neroli oil showed the potential antioxidant activity by ABTS assay (IC50 values of 672 mg L^{-1}) and DPPH-H assay (53.98%).

Several studies in animal models of anxiety have demonstrated the therapeutic potential of neroli oil. The results showed that inhaled neroli oil could help reduce anxiety levels, decrease blood pressure, relieve anxiety and improve sleep quality.

Emotional effects

Neroli oil may have relaxing, balancing and sedative effects.

Toxicological information

This oil may cause serious eye irritation or damage, skin dermatitis and repeated contact may cause allergic dermatological reaction, gastro intestinal irritation when human swallows this oil. Respiratory tract irritation may happen depending on the amount of inhalation.

2. Cinnamon leaf oil [70-76]

Cinnamon leaf oil is obtained by steam distillation from the leaves of *Cinnamomum verum*. The average yield was 1.6 % w/w. A clear liquid is brown to red brown with a warm sweet - spicy odor.

Botanical name: *Cinnamomum verum* J.S. Presl syn.
Cinnamomum zeylanicum Blume

Common name: Cinnamon, Ceylon and Cinnamon tree

Thai name: อบเชย (Aob - Choey)

Family: Lauraceae

Genus: *Cinnamomum*

Plant description

"Evergreen small trees, up to 10 m tall. Bark black-brown, inner bark with cinnamic aldehyde flavor. Young branchlets gray, somewhat tetragonous, white-maculate. Buds sericeous-puberulent. Leaves usually opposite; petiole ca. 2 cm, glabrous; leaf blade greenish white abaxially, green and shiny adaxially, ovate or ovate-lanceolate, 11-16 × 4.5-5.5 cm, leathery or subleathery, glabrous on both surfaces, triplinerved, midrib and lateral veins elevated on both surfaces, transverse

veins and veinlets reticulate, conspicuously foveolate abaxially, base acute, margin entire, apex acuminate. Panicle axillary or terminal, 10-12 cm; peduncle and rachis sericeous-puberulent. Flowers yellow, ca. 6 mm. Perianth tube obconical; perianth lobes 6, oblong, subequal, gray puberulent outside. Fertile stamens 9; filaments hairy near base, those of 3^{rd} whorl each with 2 glands, others glandless; anthers 4-celled; cells of 1st and 2nd whorls introrse but those of 3^{rd} whorl extrorse. Ovary ovoid, 10-15 mm, glabrous; style short; stigma discoid. Fruit ovoid, 10-15 mm, black when mature; perianth cup in fruit cupuliform, dilated, dentate, teeth truncate or acute at apex."

Location

Cinnamon tree is native to Sri Lanka and India; also cultivated in many countries in Asia such as China and Taiwan.

Usage

Cinnamon barks and leaves are widely used as spice and flavoring agent in foods and for various applications in medicine (to treat stomachache). It can be a mouth purifier and breath freshener. For Aromatherapy, this oil is used for warming, energizing, focusing and revitalizing.

Pharmaceutical activities

The antioxidant, antifungal and antibacterial potentials of volatile oils of *Cinnamomum zeylanicum* Blume leaf were investigated in several studies. In antifungal investigations, using agar well diffusion method, the leaf volatile oils have been found to be highly effective against all the tested fungi except *Aspergillus ochraceus* including *Penicillium citrinum Aspergillus flavus Aspergillus niger, Aspergillus terreus, Penicillium* citrinum and *Penicillium viridicatum*.

Cinnamon leaf into petroleum ether and ethanol exhibited complete inhibition (100%) of spore germination in both the pathogenic dematiaceous moulds ***(****Alternaria solani* and *Curvularia lunata****)*** (5). This oil was effective in inhibiting the growth of various isolates of bacteria including Gram-positive (*Staphylococcus aureus*), and Gram-negative (*E.coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Vibrio parahaemolyticus and Samonella typhymurium*).

The essential leaf oil from cinnamon has shown excellent activity for the scavenger activity against DPPH at concentrations which are lower than the concentrations of eugenol, butylated hydroxytoluene and butylated hydroxyanisole.

Toxicological information

This oil may cause serious eye irritation or damage, skin dermatitis and repeated contact may cause allergic dermatological reaction, gastro intestinal irritation when human swallows this oil, respiratory tract irritation may happen depending on the amount of inhalation.

3. Rice paddy herb oil [77-82]

Rice paddy herb oil is obtained from the fresh aerial parts of *Limnophila aromatica* by hydrodistillation.

Botanical name: *Limnophila aromatica* Merr.

Common name*:* Rice paddy herb

Thai name: ผักแขยง *(*Phak- Ka-Yeang*)*

Family: Scrophulariaceae

Genus: *Limnophila*

Plant description

"Annuals or perennials. Stems 30-70 cm, simple to much branched, glabrous or glandular, base decumbent and rooting from nodes. Leaves opposite or in whorls of 3, sessile, ovate-lanceolate to lanceolate-elliptic, 1-5 X 0.3-1.5 cm, base semiamplexicaul, margin crenate and serrate; veins pinnate. Flowers solitary in leaf axils or in terminal or axillary racemes. Pedicel 0.5-2 cm, glabrous or glandular. Bracteoles linear to linear-lanceolate, 1.5-2 mm. Calyx 4-6 mm, glabrous or glandular pubescent, with raised veins in fruit. Corolla white, blue-purple, or pink, 1-1.3 cm, sparsely and finely glandular, inside white villous. Style apex dilated; stigma short, 2-lamellate. Capsule ovoid, ca. 6 mm."

Location

Rice paddy herb is native to Southeast Asia (Thailand, Laos, Philippines, Vietnam, India, Indonesia, Japan and Korea), where it grows in hot temperatures and mostly in watery environments, particularly in flooded rice fields.

Usage

Rice paddy herb is used in Asia cuisine, eaten raw or steamed. It is sour, slight bitter. The plant is extensively used in Asian indigenous system of medicine for menstrual problems, wounds, dysentery, fever, elephantiasis, galactagogue, aperient, appetizer, digestive and carminative.

Pharmaceutical effects

The essential oil of *Limnophila aromatic* had been reported for antioxidant activities, achieved by scavenging abilities on DPPH and nitric oxide as well as inhibition of lipid peroxidation. Moreover, it had strong antibacterial activity in *B. cereus* and *S. aureus.*

Toxicological information

This oil may cause serious eye irritation or damage, skin dermatitis and repeated contact may cause allergic dermatological reaction, gastro intestinal irritation when human swallows this oil, respiratory tract irritation may happen depending on the amount of inhalation.

CHAPTER III

MATERIALS AND METHODOLOGY

Study site and duration

This study was conducted at Kanchanabhisek Institute of Medical and Public Health Technology, Klong Kwang-Jao Fueng road, Ratniyom, Sainoi, Nontaburi. It was performed during May 2018 to December 2018.

Research design

A randomized experimental crossover design was used in the study. All the participants received 2 interventions (essential oil and carrier oil) separated by a 1-week washout period. The research design was an experimental study that was divided into three sessions according to the number of materials in this study including neroli oil *(Citrus aurantium* Linn.*),* cinnamon leaf oil *(Cinnamomum zeylanicum* Blume*)*, and rice paddy herb oil *(Limnophila aromatica* Merr.*)*.

The researcher had 3 research assistants. The roles of the research assistants were recruiting participants, making appointments with participants, setting up data collection tools and equipment, collecting the data and facilitating the data collection process in general. All the assistants were trained on research protocol so that the research protocol was precise and accurate for each participant.

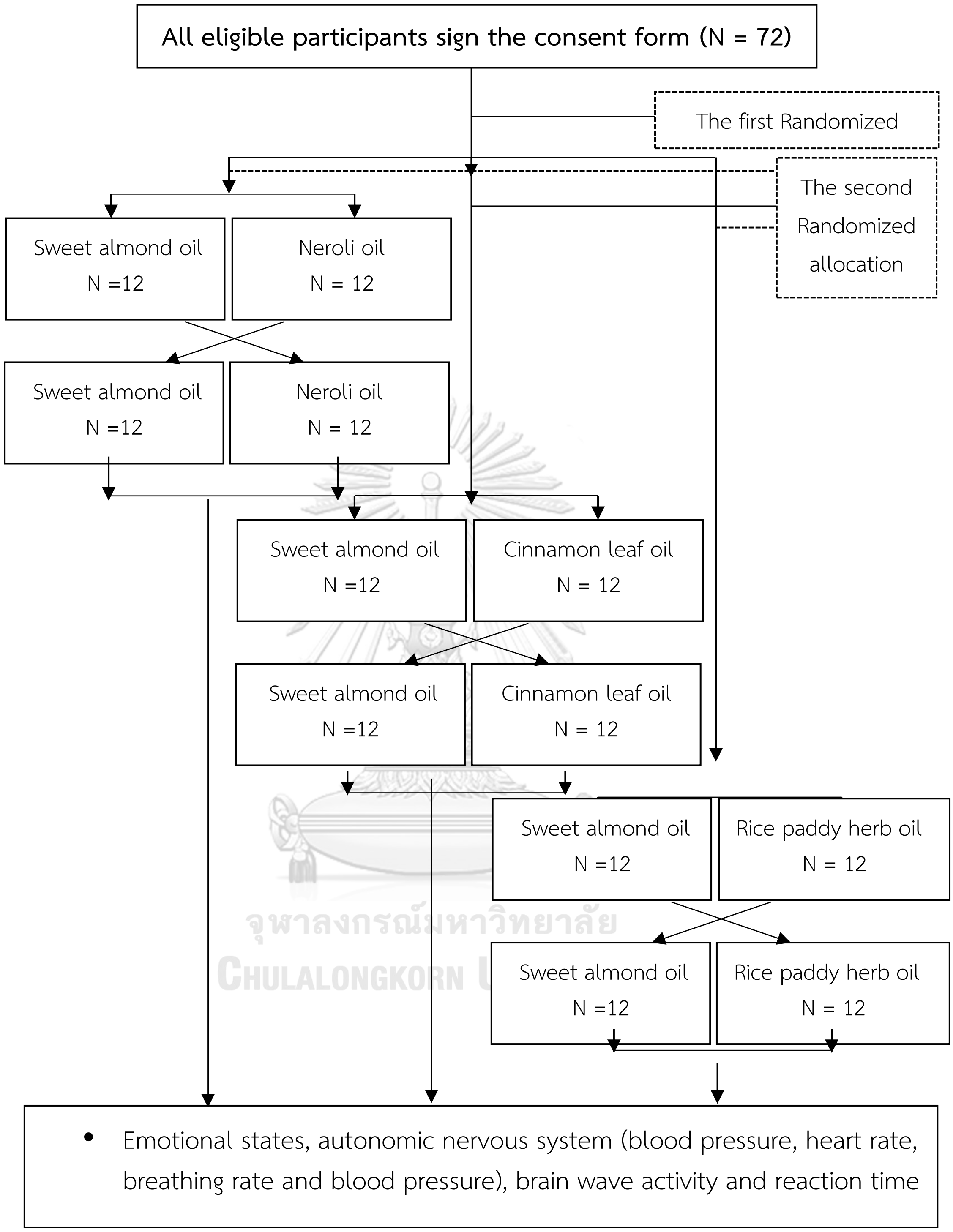


Figure 4 The Flow chart of this study

Participants

All the three sessions consisted of 72 participants (24 participants/session) who were recruited from general public between 9.00 a.m. and 4.00 p.m. at Kanchanabhisek Institute of Medical and Public Health Technology, Klong Kwang-Jao Fueng road, Ratniyom, Sainoi, Nontaburi. The participants were healthy and confirmed by a modern general physician.

Before the experiment, the researcher asked all potential participants about their history of allergic reactions from essential oils. The tests of allergic reaction and pleasantness were done by asking each potential participant to inhale the various concentrations of essential oil diluted with sweet almond oil. During and after the experiment, a team of researchers monitored the participants closely all the time. If any allergic symptom was observed, the experiment would be stopped immediately and a medical doctor (Vorasit Siripornpanich, MD) would examine and treat the participant. If severe condition was observed, the subject would be sent to hospital.

Inclusion criteria

1. Males or females aged between 18 and 25 years old.

2. The participants were right-handed that was confirmed by Edinburge Handedness Inventory test [83].

3. The participants had normal sense of smell that was confirmed by "n-butyl alcohol method test" before the experiment. This test measured the lowest concentration of a stimulus that could be distinguished between n-butyl alcohol and water. The normal participant could separate two odors at concentration lower than Step 6 (5.48×10^{-3} v/v) of n- butyl alcohol in water [84, 85].

4. The participants had normal blood pressure (systolic blood pressure should not be higher than 140 mmHg, diastolic blood pressure should not be higher than 90 mmHg).

5. Their heart rate was in normal rhythm (should not be higher than 90).

6. Their body mass index was between 18.25 – 22.9 Kg/M^2.

7. The participants were non-smokers.

Exclusion criteria

1. The participants had upper respiratory diseases, otorhinolaryngological infection, neurological diseases, hypertension or cardiovascular disease and physical conditions that could affect the sense of smell.

2. The participants had the history of neurological illness, epilepsy, loss of consciousness longer than 30 minutes and abnormal brain wave which was detected by electroencephalogram.

3. The participants who were allergic to essential oils.

4. The participants were taking CNS medication.

5. Each potential participant was asked to inhale the various concentrations of selected essential oil diluted with sweet almond oil (carrier oil) to fill out the pleasantness form "Odour familiarity five-point Likert scale". The participants who indicated oil pleasantness in the target level range of 1 and 5 were excluded from this study.

Discontinuation criteria

1. The participant could not follow the protocol

2. The participant required withdrawing

Sample size

The sample size in this study was calculated from the previous study of jasmine oil that affected the brain wave activity. The sample was calculated from the two-sample crossover group formula. This study used technical sample size calculation by computer G*Power 3.1 [13].

- Type II error 0.05
- Power of test at 80%
- Effect size 1.2 *(*13)

There were 20 participants (N=20) in each group. To account for the expecting dropouts during the experiment and ensure the study confidence, more than 20% of the total number of the participants was added. Thus, the participants are 24 for each essential oil. The total number of the participants was 72 for 3 essential oils. Each participant received carrier oil treatment and essential oil in carrier oil treatment.

Sampling technique

Two randomized allocations were used in this study. For the first randomized allocation, all eligible participants were randomly assigned to each session by choosing the drawing lots. After that, each participant was randomly assigned for the second time to receive first oil between essential oil and carrier oil by choosing the drawing lots.

Materials

Three types of pure essential oils and one carrier oil were used in this study as follows:

1. Neroli oil *(Citrus aurantium* Linn.*)*
2. Cinnamon leaf oil *(Cinnamomum zeylanicum* Blume*)*

3. Rice paddy herb oil *(Limnophila aromatica* Merr.)

4. Sweet almond oil *(Prunus dulcis* (Mill.) D.A. Webb)

Cinnamon leaf oil and sweet almond oil were purchased from Thai China Flavors and Fragrances industry.

Limnophila aromatic Merr. was collected from The Northeast of Thailand and was authenticated by Associate Professor Dr. Nijsiri Ruangrungsi. The voucher specimen was deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Rice paddy herb oil was obtained from the fresh aerial parts of *Limnophila aromatica* by hydrodistillation for 2 hours using a Clevenger apparatus and the average yield of the essential oil was recorded.

Essential oil analysis

All essential oils were analyzed by gas chromatography/mass spectrometry (GC/MS) using Thermo Finnigan Trace GC Ultra equipped with the Finnigan DSQ MS detector.

The chemical components of the essential oil were identified by matching their mass spectra and retention indices with Adam Essential MS library, and the amount of each component was computed as the percentage of peak area ratio.

Essential oil administration

In this study, pure sweet almond oil was used as diluent. The oil was delivered from an oxygen pump system through plastic tube *via* a face mask that permitted selective routine air flow (2 L/min). The concentration of neroli oil was 10 % v/v in sweet almond oil, cinnamon leaf oil was 8 % v/v in sweet almond oil and rice paddy herb oil was 8 % v/v in sweet almond oil.

Outcome measurement

Four outcome measurements were used in this study as follows:

1. Emotional states recording

In this research, the questionnaire procedure was derived from a conceptual model that proposed the aspects of mood. The Geneva Emotion and Odor Scale (GEOS) [86] described the participative affective feelings induced by 5 factors as follows:

- Pleasant feeling was mainly related to happiness and wellbeing, with a noteworthy association to ecstatic feeling as reflected by the term “feel good” was used in this research.
- Unpleasant feeling was mainly related to disgust and irritation, but it also emphasized other irritating feelings. In this research, the selected words were “feel bad, uncomfortable, disgusted and frustrated, stressed.”
- Sensuality reflected the role of olfaction in social interaction and, in particular, in socio-sexual behaviors, expressed by the terms “sensual” or “desire”. The selected words used in this research were “romantic”.
- Relaxation was strongly associated with soothing effects, at the point that certain odors might induce meditative feelings. In this research, the selected words including “relaxed, serene and drowsy” were used in this research.

- Refreshing was mainly associated with effects of stimulation and purification as well as physiological responses, that could be expressed by the terms of “refresh and energetic”.

The questionnaire was verified by advisor, co advisor, specialist in Thai interpreter and physiologist. The 10 millimeter visual analog scale was chosen in this study to assess the emotion condition (Appendix A). The measure reliability was done by 20 participants in preliminary study and calculated for Cronbach’s α value. The measure with Cronbach’s α value was at 0.752.

2. Autonomic nervous system recording

Four ANS parameters including skin temperature, respiratory rate, heart rate and blood pressure were recorded simultaneously and in real time. All the parameters were measured using BIOLIGHT M7000 Multi parameter patient monitor (figure 5) with the participant seated in a semi-reclining chair, quiet, air-conditioned (24 ± 1°C), 40-50% humidity. All tests were performed between 08.30 AM and 12.30 AM to minimize circadian variation of the autonomic nervous system. Each participant was separated to avoid mutual distraction during testing. The room was ventilated with fresh air for at least 15 minutes between the participants. The following parameters were used.

- **Heart rate and respiratory rate**

  The heart rate and breathing rate were measured every 1 minute. The electrode leads were connected in three positions (Modified Lead I, II, III): the left infraclavicular fossa, the right infraclavicular fossa and the left anterior axillary line below the bottom rib. Respiratory measurement was influenced by movement of chest and abdomen in

the left infraclavicular fossa and the left anterior axillary line below the bottom rib as shown in figure 6.

- **Blood pressure**

  Blood pressure was measured every 2.5 minute. Systolic blood pressure and diastolic blood pressure were measured on the left arm as shown in figure 6.

- **Skin temperature**

  Skin temperature was measured every 1 minute. The sensor was placed in middle of the back of a non-dominant hand and fixed with non-caustic adhesive tape as shown in figure 6.

Figure 5 BIOLIGHT M7000 Multi parameter patient monitor

Figure 6 Autonomic nervous system recording

3. Electroencephalographic recording

The power spectra of the respective frequency bands were recorded. They were interpreted by fast fourier transformation (FFT) and expressed as Delta (0 –3.99 Hz), Theta (4–7.99 Hz), Alpha (8–12.99 Hz) and Beta (13–29.99 Hz).

The researchers used Nicolet EEG v32 from Natus Neurology Company, USA (figure 7) to provide the set of 21 electrodes with 1 additional ground which 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. LOC and ROC were placed for eye movements. EEG jelly was inserted into each electrode to keep the impedance below 10 kohm all the time. Additional reference electrodes were applied to measure for electrical activity

at the ear lobes (A1 and A2), behind auricles and for detection of eye movements. The areas of interest were grouped into 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 (FCz, Cz, CPz) brain regions.

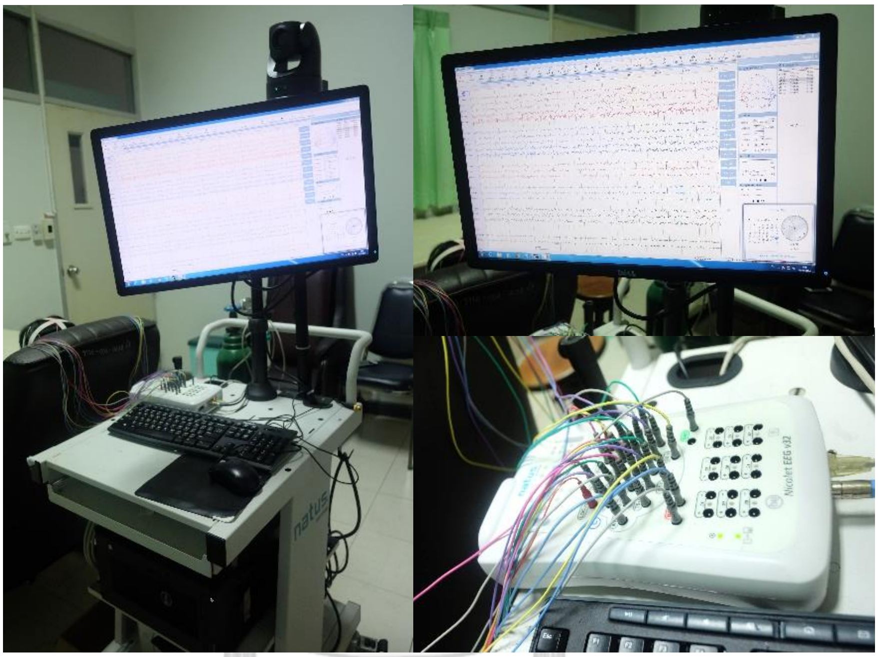


Figure 7 Nicolet EEG v32

4. Reaction time recording

The Deary-Liewald reaction time task was used in this research reaction time. This task was computer-based reaction time task and was divided to simple reaction time (SRT) and choice reaction time (CRT).

For the SRT, the participants were requested to click quickly the button on mouse for response to a single stimulus. The stimulus to respond was the appearance of a diagonal cross within the square. Each time a cross appeared. SRT involved eight practice trials and twenty test trials.

For the CRT, there were four stimuli and participants had to click quickly the button on mouse that corresponded to the correct response. Four white squares were positioned in a horizontal line across approximately the middle of the computer screen, set against a blue background. CRT involved eight practice trials and forty test trials. Four keys on a standard computer keyboard corresponded to the different squares. The z key corresponded to the square on the far left; the x key to the square second from the left; the comma key to the square second from the right and the full-stop key to the square on the far right. The stimulus to respond was the appearance of a diagonal cross within one of the squares. A cross appeared randomly in one of the squares and the participants were asked to respond as quickly as possible by pressing the corresponding key on the keyboard. Each cross remained on the screen until one of the four keys was pressed, after which it disappeared and another cross appeared shortly after.

The computer program calculated the mean, median, variance, and standard deviation of the response times of the participants.

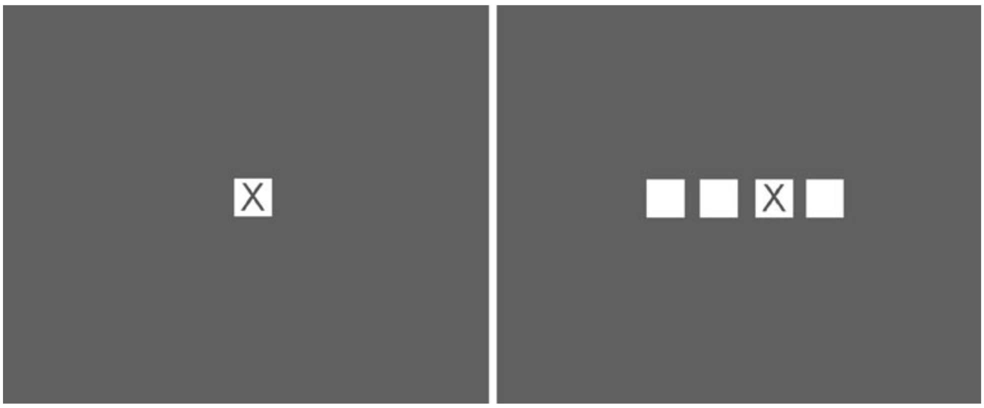


Figure 8 Screen shots of the Deary-Liewald task for the simple reaction time task (left) and the choice reaction time task (right) [14]

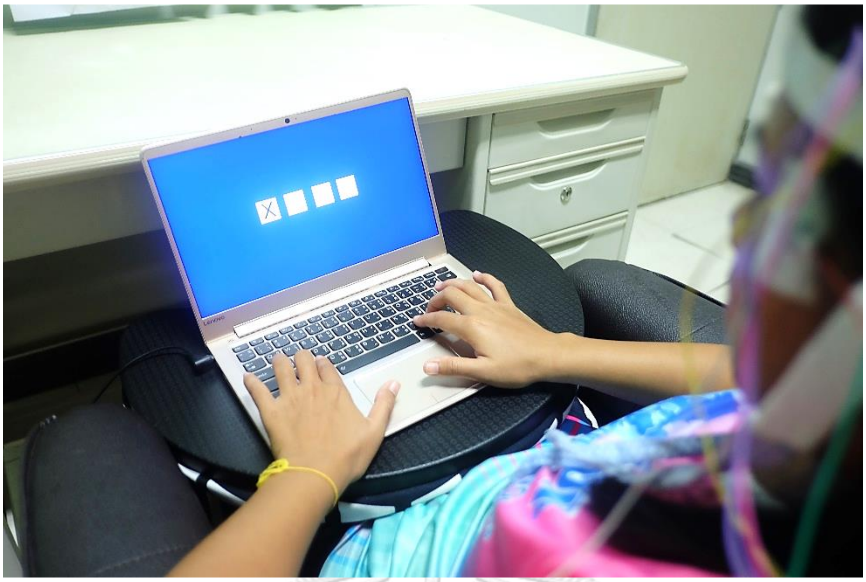


Figure 9 Reaction time recording

Instruments

Screening session

- Health status questionnaire
- Edinburge Handedness Inventory test
- Score sheet for odor test (Butanol Thereshold)

Emotional states recording

- The modified questionnaire that proposed the aspects of mood based on The Geneva Emotion and Odor Scale (GEOS) (86)

Autonomic nervous system measurement

- BIOLIGHT M7000 Multi-parameter patient monitor – BIOM7000
- ECG conductive Adhesive electrodes
- Case record autonomic nervous form
- Comfortable armchair
- 70% alcohol

Electroencephalographic recording

- Nicolet EEG v32 from Natus Neurology Company, USA
- Weaver Ten 20 conductive paste
- Weaver Nuprep skin prep gel
- 15 inch USB cable (connection EEG acquisition computer – Amplifier)
- Serial cable (connection Stimulation computer – Amplifier)
- Comfortable armchair

Reaction time recording

- CPU and monitor for The Deary-Liewald reaction time program

Experimental procedures

Screening session

1. Participants had to sign consent form before the screening process after they listened to the information about procedure and risks of the research. The information on the procedure and risks were given in Thai.

2. The personal health of all the participants was evaluated by using questionnaire (Appendix B). Their weight, height and blood pressure were measured and recorded.

3. The Edinburgh Handedness Inventory test was chosen to evaluate the handedness of the participants (Appendix C).

4. The olfactory ability was evaluated by n-butyl alcohol method test (Appendix D) by using these following steps:

    - The butanol solution was prepared in the concentration levels of 0 (4% v/v) to 11 ($2.25x10^{-5}$ v/v) and water solution in the bottles.

- The participants were asked to identify the bottle containing the odorant; the initial concentration was level 9 ($2.03x10^{-4}$ v/v).
- After each correct response, the concentration of butanol was decreased by a factor of 3 (level 10 ($6.77x10^{-5}$ v/v), 11 ($2.25x10^{-5}$ v/v)).
- After each incorrect response, the concentration of butanol was increased by a factor of 3 until the participant either achieved 5 correct responses or failed to correctly identify the bottle with level 0 (4% v/v).
- The detection threshold was recorded as the concentration at which the participant correctly identified the butanol on 5 consecutive trials. The scores related the participant's threshold to a normal participant population.

Autonomic nervous system and emotional states

1. Before the experiment, the participants had to shampoo their own hair. Hair spray, antiperspirants or perfumes were not allowed, and they were advised to have no consumption of alcohol, cigarettes or caffeinated drinks. They should not feel fatigued or drowsy on the day of the experiment.

2. Participants had to listen to the instructions before the ANS recordings. The instructions were given in Thai. The instruction details were given to the participant prior to the experiment.

3. The participants were asked to sit in the separate section to the ANS acquisition unit, room temperature at 24 ± 1°C, relative humility at 50-65 % in a comfortable chair. Each participant was applied the EEG instruments for 5 minutes.

4. In the first process, ANS activity was recorded when participant felt comfortable for 10 minutes. After that, the participant in both groups was requested to evaluate emotional states by questionnaire.

5. In the second process, the intervention (the essential oil or the sweet almond oil) was applied. ANS activity was recorded for 10 minutes. After that, the participant was requested to evaluate their emotional states by the same questionnaire.

6. ANS and emotional state recording were analyzed.

Electroencephalographic recording and reaction time recording

1. Before the experiment, the participants had to shampoo their own hair. Hair spray, antiperspirants or perfumes were not allowed, and they were advised to have no consumption of alcohol, cigarettes or caffeinated drinks. They should not feel fatigued or drowsy on the day of the experiment.

2. Participants had to listen to the instructions before the EEG recordings. The Instructions were provided in Thai and given to the participants before the experiment.

3. The participant was asked to sit in the separate section to the EEG acquisition unit, in a comfortable chair under the condition, room temperature at 24 ± 1°C, relative humility at 50-65%. Each participant was applied the EEG instruments for 30 minutes.

4. In the first process, EEG activity was recorded during eyes opened and eyes closed condition for 10 minutes to set up baseline when the participant felt comfortable.

5. In the second process, the intervention (the essential oil or the sweet almond oil) was applied for 8 minutes while the EEG activity were recorded during the eyes closed simultaneously.

6. In the third process, the reaction time of the participant was evaluated. The participant was instructed to click button on mouse whenever target picture appeared randomly on computer screen.

7. EEG recording and reaction time were analyzed.

Ethical consideration

Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University granted this study an ethics approval on 2 July, 2018 with ethics number COA No. 154/2018. The informed consent was obtained from each participant after the full explanation of the study.

Data analysis

The data were analyzed with SPSS version 22 (licensed for Chulalongkorn University). The statistical significance was set at probability level less than .05 ($p < .05$). Descriptive statistic explained demographic characteristics such as percentage, mean and standard deviation of participants' data. The data in this study were normally distributed, and the statistics were used as Table 1.

Table 1 Descriptive statistics and Inferential statistics

Variables	Descriptive statistics	Inferential statistics
The comparison between sweet almond oil and essential oil groups ● Emotional scores	Mean ± SD	Pair T-test
The comparison between sweet almond oil and essential oil groups ● Blood pressure (BP) ● Heart rate (HR) ● Respiratory rate (BR) ● Skin temperature (ST)	Mean ± SD Mean ± SD Mean ± SD Mean ± SD	Pair T-test Pair T-test Pair T-test Pair T-test
The comparison between sweet almond oil and essential oil groups ● The absolute power of each brain wave	Mean ± SD	Pair T-test
The Comparison between sweet almond oil and essential oil groups ● Simple reaction time ● Choice reaction time	Mean ± SD Mean ± SD	Pair T-test Pair T-test

CHAPTER IV

RESULTS

A randomized experimental crossover design was used in the study. All the participants received 2 interventions (essential oil and carrier oil) separated by a 1-week washout period. The study investigated the effects of inhaled essential oils on central nervous system, autonomic nervous system, emotional states and reaction time. The study was divided into three sessions according to the selected essential oils including neroli oil (*Citrus aurantium* Linn.), cinnamon leaf oil (*Cinnamomum zeylanicum* Blume), and rice paddy herb oil (*Limnophila aromatica* Merr). Seventy-two healthy volunteers were recruited from general public and assigned into 3 sessions (24 participants/session) based on these 3 essential oils administered in this study by using a simple random sampling method enclosed in envelopes.

This study was conducted at Kanchanabhisek Institute of Medical and Public Health Technology, Klong Kwang-Jao Fueng road, Ratniyom, Sainoi, Nontaburi. The participants were healthy and confirmed by a modern general physician. The healthy participants aged between 18 and 25 years old from both genders were willing to take part in this study. Their heart rate was in normal rhythm. They had normal blood pressure. Their body mass indices were between 18.25 – 22.9 Kg/M^2 which were in normal range according to WHO and Asian criteria values. They were non-smokers and did not take any CNS acting medication nor sedative drugs. They did not suffer from upper respiratory infection, neurological diseases, hypertension and cardiovascular disease, epilepsy and loss of consciousness longer than 30 minutes. They were right-handed individuals with a normal sense of smell.

The participants in this study did not have abnormal brain wave detected by the analysis of EEG. They were not allergic to essential oils. The participants who indicated oil pleasantness of 1, 5 would be excluded from the research program. No participants were excluded or quit from the research program. All the participants could follow the protocol procedures.

The results were divided into 3 sections of 3 essential oils which consisted of 6 parts. Each section included the chemical components of each essential oil, the characteristics of the participants, the emotional state responses, the ANS parameters, the EEG parameters and the reaction time.

Neroli essential oil

Chemical components

The chemical composition of neroli essential oil was analyzed by GC/MS. Figure 10 showed the GC chromatogram of neroli essential oil. The results showed that the main compounds of neroli oil were 37.27% of linalool, 18.34% of pinene <beta->, 14.81% of nerolidol<Z-> and 10.31% of limonene (Table 2).

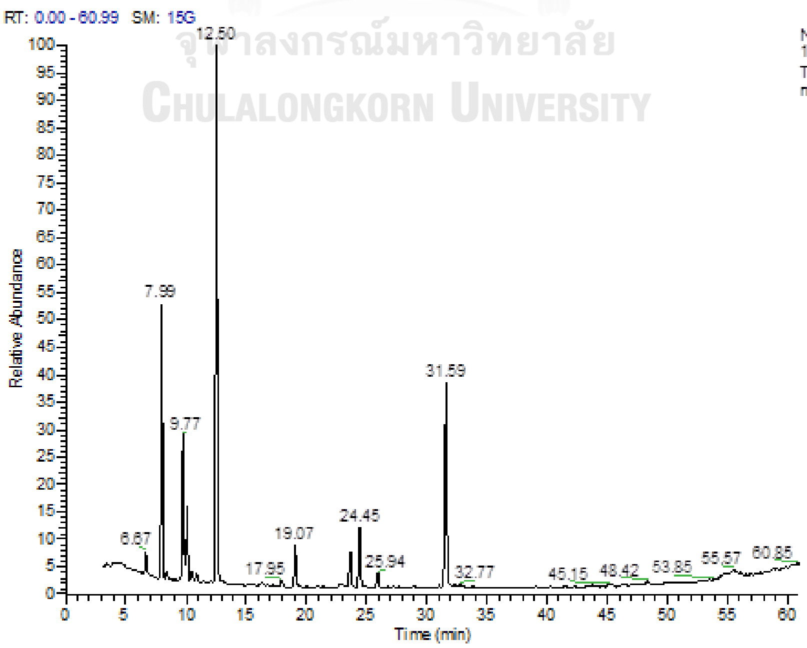


Figure 10 The GC chromatogram of neroli essential oil

Table 2 The chemical composition of the neroli essential oil identified by GC/MS

Retention Time (min)	Chemical component	Kovat's Index	Peak Area %
6.67	Pinene<alpha->	939	1.67
7.99	Pinene<beta->	979	18.34
9.77	Limonene	1029	10.31
10.06	Ocimene<(Z)-beta->	1037	5.14
10.45	Ocimene<(E)-beta->	1050	0.71
10.89	Terpinene<gamma->	1059	0.66
12.50	Linalool	1096	37.27
19.07	Linalool acetate	1257	3.01
23.64	Neryl acetate	1361	2.67
24.45	Geranyl acetate	1381	4.35
25.94	Caryophyllene(E-)	1419	1.07
31.59	Nerolidol<Z->	1532	14.81

Characteristics of the participants

A total number of 24 healthy volunteers consisting of 3 males and 21 females aged 19.83 ± 0.56 years with normal BMI of 20.31 ± 1.57 kg/m^2 were recruited. All of them were right-handed. The mean and SD values of the participants' height and weight were 1.59 ±0.06 m and 51.29 ±6.16 kg.

Emotional state responses

Table 3 showed the effects on emotional state response. Each participant inhaled the sweet almond oil (SO) and the neroli essential oil diluted in sweet almond oil (NO). The self-evaluated emotional state questionnaires were filled out by all the participants. The effects were compared within each participant. For SO inhalation, all the feelings were not affected. For NO inhalation, the relaxed and romantic feelings increased significantly (p=.000 and p=.016 respectively). The stressed feelings decreased significantly (p = .000). The comparison of emotional

state responses between SO and NO inhalation showed that the changes of the relaxed and romantic feelings in NO were statistically significant and higher than those in SO (p = .012 and p = .040 respectively). The changes of the stressed feelings in NO were statistically significant and lower than those in SO (p = .000).

Table 3 The emotional state scores between sweet almond oil and neroli essential oil inhalation

Parameter	Sweet almond oil (SO) n = 24 Mean (SD)			Neroli essential oil (NO) n = 24 Mean (SD)			P-value between SO and NO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
1) good	4.45 (2.00)	4.55 (2.29)	.761	4.21 (1.97)	4.26 (1.94)	.868	.920
2) bad	1.54 (1.51)	1.42 (1.60)	.573	1.62 (2.09)	1.67 (1.83)	.850	.570
3) active	2.62 (2.08)	2.89 (2.06)	.405	2.94 (2.02)	2.72 (2.06)	.492	.234
4) drowsy	2.91 (2.21)	3.80 (2.24)	.092	2.33 (2.14)	2.73 (1.16)	.368	.405
5) fresh	3.61 (2.34)	3.19 (2.47)	.304	3.22 (1.97)	3.42 (2.02)	.579	.206
6) relaxed	3.97 (1.73)	4.22 (2.22)	.536	3.35 (1.80)	5.44 (2.34)	.000*	.012*
7) stressed	2.13 (2.03)	2.21 (2.5)	.694	2.58 (2.07)	0.70 (0.90)	.000*	.000*
8) frustrated	1.94 (2.16)	1.63 (1.98)	.271	1.26 (1.75)	1.29 (1.63)	.893	.374
9) romantic	1.57 (1.61)	1.41 (1.48)	.711	1.63 (1.78)	2.87 (2.30)	.016*	.040*
10) annoyed	1.56 (2.31)	1.12 (1.84)	.228	1.44 (2.01)	1.14 (1.54)	.093	.735
11) calm	3.91 (2.01)	3.92 (2.55)	.983	3.24 (2.19)	3.70 (2.03)	.333	.442
12) disgusted	0.53 (0.58)	0.61 (1.01)	.719	0.69 (0.87)	0.89 (1.03)	.197	.604

* Significant difference, p-value < .05

ANS parameters

The effects on ANS were displayed in Table 4. The ANS parameters were recorded at rest and SO or NO inhalation. The effects were compared within each participant. For SO inhalation, systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate and skin temperature were not affected (p > .05). For NO inhalation, systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate statistically significantly decreased (p = .005, p = .003, p = .000, p = .003 respectively). The comparison of ANS changes between RO and NO inhalation showed that the neroli essential oil had statistically significant attenuated effects on the systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate (p = .034, p = .006, p = .032, p = .012 respectively) but no significant effects on skin temperature.

Table 4 ANS parameters between sweet almond oil and neroli essential oil inhalation

Parameter	Sweet almond oil (SO) n = 24 Mean (SD)			Neroli essential oil (NO) n = 24 Mean (SD)			P-value between SO and NO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
Systolic blood pressure (mmHg)	103.27 (8.09)	102.75 (6.33)	.748	108.08 (11.71)	100.96 (8.21)	.005*	.034*
Diastolic blood pressure (mmHg)	63.72 (6.77)	62.61 (7.13)	.361	67.97 (10.82)	61.35 (4.45)	.003*	.006*
Heart rate (bpm)	75.13 (9.07)	73.59 (10.14)	.263	78.05 (10.45)	70.19 (9.51)	.000*	.032*
Respiratory rate (bpm)	18.62 (3.60)	19.07 (4.15)	.362	19.34 (2.35)	17.97 (2.30)	.003*	.012*
Skin temperature (ºC)	29.39 (5.17)	30.19 (4.15)	.063	31.13 (4.54)	31.38 (2.30)	.750	.586

* Significant difference, p-value < .05

EEG parameters

The effects on EEG parameters were displayed in Table 5. The EEG parameters were recorded at rest and SO or NO inhalation. The effects were compared within each participant. For SO inhalation, there were no significant changes in the band powers of delta, theta, alpha and beta of all brain regions. For NO inhalation, the theta power of center, left and right posterior brain regions increased significantly (p = .022, p = .027, p = .004 respectively). The alpha power of left and right anterior, center, left and right posterior brain regions increased significantly (p = .002, p = .007, p = .000, p = .011, p = .009 respectively). The beta power of left and right anterior brain regions decreased significantly (p = .011, p = .010 respectively). The comparison of EEG parameters between SO and NO inhalation showed that there were significant changes in the power of theta, alpha and beta waves. The power of theta over center, left and right anterior brain regions increased significantly (p = .023, p = .039, p = .000 respectively). There were statistically significant increases in the alpha power of all regions namely left anterior, right anterior, center, left and right posterior brain regions (p = .004, p = .007, p = .015, p = .026, p = .023 respectively). There were statistically significant decreases in the beta power of most regions namely left anterior, right anterior, and left posterior brain regions (p = .029, p = .006, p = .039 respectively).

Table 5 Brainwave power between sweet almond oil and neroli essential oil inhalation

Area	Sweet almond oil (SO) n = 24 Mean (SD)			Neroli essential oil (NO) n = 24 Mean (SD)			P-value between SO and NO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
Delta power (μV^2)							
Left anterior	6.75 (1.54)	6.29 (1.40)	.123	6.40 (1.70)	6.09 (1.53)	.321	.658
Right anterior	6.82 (1.48)	6.53 (1.66)	.440	6.80 (1.66)	6.49 (1.95)	.224	.965
Center	11.11 (2.87)	10.79 (2.86)	.596	9.54 (3.75)	8.50 (2.62)	.147	.459
Left posterior	3.63 (1.71)	3.78 (1.60)	.566	3.41 (1.25)	3.11 (1.34)	.312	.322
Right posterior	2.30 (1.19)	1.97 (0.63)	.124	2.81 (1.72)	2.54 (1.68)	.507	.895
Theta power (μV^2)							
Left anterior	5.22 (3.40)	5.11 (3.90)	.713	5.27 (3.32)	5.94 (4.03)	.319	.264
Right anterior	5.31 (3.97)	5.35 (4.96)	.904	5.26 (3.33)	5.95 (4.27)	.260	.317
Center	9.38 (2.69)	8.57 (3.59)	.325	6.85 (3.90)	9.81 (4.18)	.022*	.023*
Left posterior	3.34 (1.97)	3.39 (1.63)	.821	3.31 (1.37)	4.95 (3.43)	.027*	.039*
Right posterior	2.25 (1.59)	2.30 (1.42)	.856	3.01 (2.74)	4.94 (3.45)	.004*	.000*

Alpha power (μV^2)							
Left anterior	6.08 (4.90)	5.89 (4.98)	.756	4.51 (2.66)	7.54 (5.27)	.002*	.004*
Right anterior	6.49 (5.80)	6.40 (6.09)	.884	4.80 (3.13)	8.21 (6.99)	.007*	.007*
Center	6.59 (1.78)	6.67 (4.45)	.930	6.44 (4.39)	10.37 (4.35)	.000*	.015*
Left posterior	6.99 (5.05)	7.19 (5.55)	.788	5.04 (2.76)	8.43 (5.28)	.011*	.026*
Right posterior	4.69 (2.96)	4.98 (3.96)	.670	4.74 (3.12)	7.51 (5.97)	.009*	.023*
Beta power (μV^2)							
Left anterior	3.41 (1.07)	3.37 (0.99)	.805	3.73 (1.13)	2.78 (0.94)	.011*	.029*
Right anterior	3.33 (0.93)	3.51 (1.20)	.215	3.92 (1.15)	3.20 (1.16)	.010*	.006*
Center	4.23 (1.11)	4.28 (0.81)	.858	4.99 (1.49)	4.53 (1.25)	.161	.331
Left posterior	3.37 (1.15)	3.69 (1.43)	.188	3.82 (1.24)	3.55 (1.34)	.369	.039*
Right posterior	2.59 (1.43)	2.84 (1.34)	.503	3.55 (1.50)	3.12 (1.31)	.166	.190

* Significant difference, p-value < .05

Reaction Time

The effects on reaction time were demonstrated in Table 6. The simple reaction time and choice reaction time between sweet almond oil and neroli essential oil inhalation were compared within each participant. Simple reaction time was represented in SRT mean (seconds) and choice reaction time was classified into 2 types: correct count (items) from 40 items and correct mean (seconds). For the simple reaction time, the neroli essential oil inhalation took statistically significantly less time to respond than the sweet almond oil ($p = .042$). For the choice reaction

time, the neroli essential oil inhalation showed no significant improving effects on correct answers as well as the response times compared to the sweet almond oil.

Table 6 The simple reaction time and choice reaction time between sweet almond oil and neroli essential oil inhalation

Reaction time parameter	Sweet almond oil (SO) n = 24 Mean (SD)	Neroli essential oil (NO) n = 24 Mean (SD)	P-value between SO and NO
	Simple reaction time (SRT)		
SRT Mean (s)	314.82 (24.13)	297.70 (31.07)	.042*
	Choice reaction time (CRT)		
Correct count (%)	98.44 (2.06)	99.06 (1.44)	.283
Correct mean (ms)	493.72 (51.13)	483.69 (52.31)	.477

* Significant difference, p-value < .05, ms = milliseconds

Cinnamon leaf essential oil

Chemical components

The chemical composition of cinnamon leaf essential oil was analyzed by GC/MS. Figure 11 showed the GC chromatogram of cinnamon leaf essential oil. The results showed that the main compounds of cinnamon leaf essential oil were 85.15% of eugenol, 4.36% of benzyl benzoate, 3.29% of eugenol acetate and 2.50% of caryophyllene(E-) (Table 7).

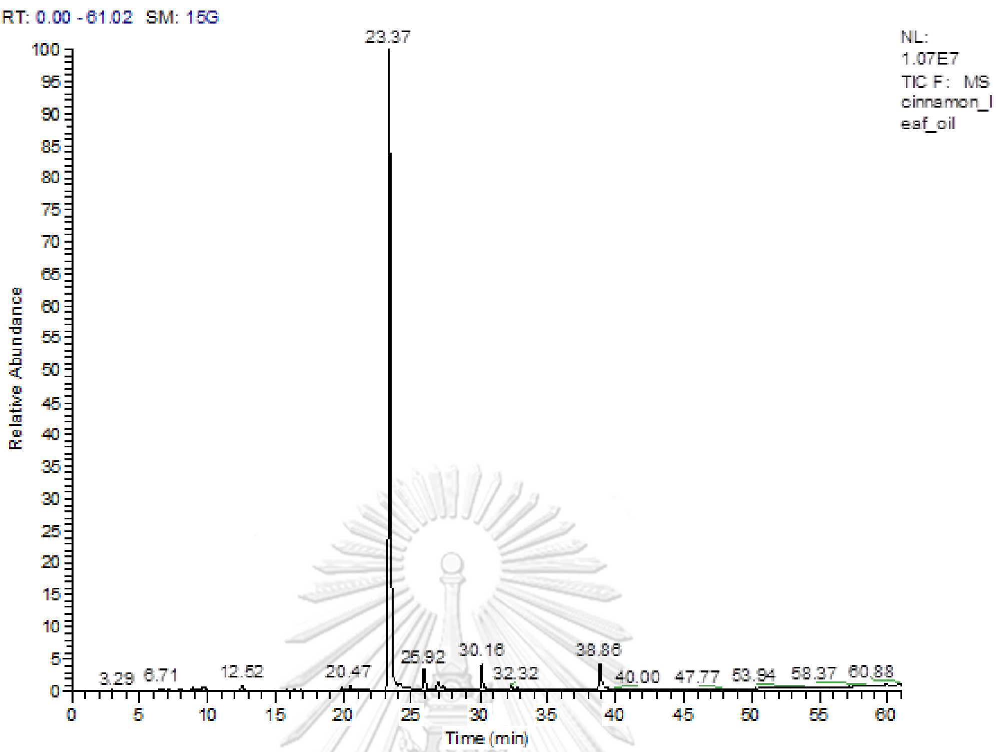


Figure 11 the GC chromatogram of cinnamon leaf essential oil

Table 7 The chemical composition of cinnamon leaf essential oil identified by GC/MS

Retention Time (min)	Chemical component	Kovat's Index	Peak Area (%)
6.71	Pinene<alpha->	939	0.29
8.92	Phellandrene<alpha->	1002	0.26
9.63	Cymene<ortho->	1026	0.25
12.50	Linalool	1096	0.61
20.45	Safrole	1287	0.55
23.39	Eugenol	1359	85.15
24.14	Copaene<alpha->	1376	0.65
25.92	Caryophyllene(E-)	1419	2.50
26.91	Cinnamyl acetate<E->	1446	1.12
27.29	Humulene<alpha->	1454	0.47
30.14	Eugenol acetate	1522	3.29
32.32	Caryophyllene oxide	1583	0.49
38.84	Benzyl benzoate	1760	4.36

Characteristics of the participants

A total number of 24 healthy volunteers consisting of 3 males and 21 females aged 19.71 ± 1.10 years with normal BMI of 20.57 ± 2.21 kg/m^2 were recruited. All of them were right-handed. The mean and SD values of the participants' height and weight were 1.62 ±0.06 m and 53.96 ±4.95 kg.

Emotional state responses

Table 8 showed the effects on emotional state response. Each participant inhaled the sweet almond oil (SO) and the cinnamon leaf essential oil diluted in sweet almond oil (CO). The self-evaluated emotional state questionnaires were filled out by all the participants. The effects were compared within each participant. For SO inhalation, most feelings were not affected but the drowsy feelings increased significantly (p = .001). For CO inhalation, the drowsy, relaxed, romantic and calm feelings increased significantly (p= .020, p= .001, p= .003 and p= .000 respectively). The stressed feelings decreased significantly (p = .005). The comparison of emotional state responses between SO and CO inhalation showed that the changes of the relaxed and calm feelings in CO were statistically significant and higher than those in SO (p = .006 and p = .018 respectively). The changes of the stressed feelings in CO were statistically significant and lower than those in SO (p = .026).

Table 8 The emotional state scores between sweet almond oil and cinnamon leaf essential oil inhalation

Parameter	Sweet almond oil (SO) n = 24 Mean (SD)			Cinnamon leaf essential oil (CO) n = 24 Mean (SD)			P-value between SO and CO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
1) good	5.17 (1.87)	5.25 (1.96)	.802	4.85 (2.23)	5.26 (2.07)	.243	.210
2) bad	1.42 (1.68)	1.27 (1.45)	.584	1.18 (1.25)	1.21 (1.56)	.904	.612
3) active	3.95 (1.71)	3.78 (2.29)	.675	3.89 (2.26)	4.15 (2.29)	.555	.221
4) drowsy	2.18 (1.88)	3.60 (2.39)	.001*	2.44 (1.81)	3.58 (2.66)	.020*	.591
5) fresh	4.47 (1.74)	4.17 (2.20)	.513	4.24 (2.12)	4.40 (2.25)	.674	.362
6) relaxed	5.03 (1.69)	4.87 (2.44)	.734	4.28 (2.33)	6.06 (2.35)	.001*	.006*
7) stressed	1.77 (1.67)	1.73 (1.79)	.884	2.27 (2.20)	1.03 (1.30)	.005*	.026*
8) frustrated	1.51 (1.50)	1.29 (1.53)	.287	1.52 (1.49)	1.22 (1.43)	.189	.760
9) romantic	2.88 (1.80)	3.60 (2.65)	.142	1.98 (1.71)	3.50 (2.29)	.003*	.065
10) annoyed	1.23 (1.66)	1.06 (1.37)	.217	1.02 (1.29)	0.93 (1.42)	.348	.661
11) calm	4.37 (1.57)	4.57 (2.14)	.573	3.38 (2.21)	5.76 (2.30)	.000*	.018*
12) disgusted	0.74 (1.13)	0.89 (1.23)	.245	0.74 (1.09)	1.01 (1.55)	.115	.389

* Significant difference, p-value < .05

ANS parameters

The effects on ANS were displayed in Table 9. The ANS parameters were recorded at rest and SO or CO inhalation. The effects were compared within each participant. For SO inhalation, systolic blood pressure, diastolic blood pressure, heart rate, respiratory rate and skin temperature were not affected ($p > .05$). For CO inhalation, heart rate statistically significantly decreased ($p = .006$). The comparison

of ANS changes between SO and CO inhalation showed that the cinnamon leaf essential oil had statistically significant attenuated effects on heart rate (p = .034).

Table 9 ANS parameters between sweet almond oil and cinnamon leaf essential oil inhalation

Parameter	Sweet almond oil (SO) n = 24 Mean (SD)			Cinnamon leaf oil (CO) n = 24 Mean (SD)			P-value between SO and CO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
Systolic blood pressure (mmHg)	101.17 (6.70)	99.39 (6.97)	.115	101.25 (8.72)	100.68 (8.08)	.234	.285
Diastolic blood pressure (mmHg)	60.13 (4.80)	60.13 (4.50)	.991	60.53 (6.23)	60.38 (5.36)	.867	.883
Heart rate (bpm)	80.11 (8.26)	79.33 (8.47)	.407	85.44 (11.09)	81.54 (8.54)	.006*	.034*
Respiratory rate (bpm)	19.74 (4.49)	19.40 (4.55)	.585	19.13 (3.09)	18.95 (4.69)	.813	.853
Skin temperature (°C)	32.31 (2.28)	31.89 (2.64)	.572	31.69 (2.52)	32.07 (2.77)	.445	.435

* Significant difference, p-value < .05

EEG parameters

The effects on EEG parameters were displayed in Table 10. The EEG parameters were recorded at rest and SO or CO inhalation. The effects were compared within each participant. For SO inhalation, there were no significant changes in the band powers of delta, theta, alpha and beta of all the brain regions. For CO inhalation, the alpha power of left and right anterior brain regions increased significantly (p = .004, p = .023 respectively). The beta power of left anterior, left and right posterior brain regions decreased significantly (p = .038, p = .021, p = .003, p = .011 respectively). The comparison of EEG parameters between SO and CO inhalation showed that there were significant changes in the power of alpha and beta waves. There were statistically significant increases in the alpha power of most regions

namely left anterior, right anterior and right posterior brain regions (p = .002, p = .007, p = .003 respectively). There were statistically significant decreases in the beta power of few regions namely left and right posterior brain regions (p = .039, p = .018 respectively).

Table 10 Brainwave power between sweet almond oil and cinnamon leaf essential oil inhalation

Area	Sweet almond oil (SO) n = 24 Mean (SD)			Cinnamon leaf essential oil (CO) n = 24 Mean (SD)			P-value between SO and CO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
Delta power (μV^2)							
Left anterior	7.47 (2.28)	7.06 (2.05)	.275	6.69 (2.06)	6.54 (2.10)	.615	.583
Right anterior	7.84 (2.51)	7.79 (2.41)	.861	6.67 (2.25)	6.62 (2.05)	.916	.994
Center	9.72 (4.07)	9.20 (2.93)	.337	9.58 (3.99)	9.31 (3.17)	.777	.806
Left posterior	4.16 (1.41)	3.98 (1.17)	.390	4.77 (2.14)	4.41 (2.00)	.492	.746
Right posterior	3.51 (1.40)	3.41 (1.15)	.634	5.16 (2.78)	4.75 (3.15)	.379	.537
Theta power (μV^2)							
Left anterior	4.52 (1.89)	4.48 (1.81)	.735	4.41 (1.40)	4.07 (1.29)	.209	.344
Right anterior	5.06 (1.91)	5.09 (1.90)	.899	4.80 (1.76)	4.99 (1.96)	.708	.781
Center	8.91 (4.39)	8.53 (3.06)	.397	6.89 (3.10)	7.14 (2.66)	.720	.517
Left posterior	3.82 (1.97)	3.52 (1.68)	.084	3.57 (1.89)	3.78 (2.32)	.693	.408
Right posterior	3.00 (1.50)	2.93 (1.34)	.668	3.68 (2.05)	3.84 (2.55)	.801	.715

Alpha power (μV²)							
Left							
anterior	5.60	5.07	.254	5.02	7.14	.004*	.002*
	(4.28)	(4.18)		(4.64)	(3.29)		
Right							
anterior	6.29	5.50	.115	6.58	8.87	.023*	.007*
	(3.97)	(4.06)		(6.19)	(4.59)		
Center	10.29	9.49	.255	9.82	10.41	.623	.319
	(4.97)	(5.65)		(4.83)	(5.83)		
Left							
posterior	7.46	6.89	.556	8.24	8.27	.981	.732
	(4.22)	(5.39)		(5.30)	(6.72)		
Right							
posterior	7.48	7.14	.676	7.23	10.88	.001*	.003*
	(2.93)	(4.12)		(6.38)	(4.93)		
Beta power (μV²)							
Left							
anterior	3.50	3.51	.948	3.85	3.54	.038*	.089
	(1.25)	(1.27)		(1.68)	(1.44)		
Right							
anterior	3.71	3.67	.811	3.80	3.50	.083	.237
	(1.29)	(1.13)		(1.57)	(1.44)		
Center	4.72	4.63	.641	4.80	4.50	.355	.495
	(1.58)	(1.42)		(1.75)	(1.69)		
Left							
posterior	3.78	3.82	.857	4.13	3.12	.021*	.039*
	(1.60)	(1.34)		(1.43)	(1.42)		
Right							
posterior	3.74	3.77	.928	4.35	3.30	.003*	.018*
	(1.47)	(1.33)		(2.21)	(1.51)		

* Significant difference, p-value < .05

Reaction Time

The effects on reaction time were demonstrated in Table 11 The simple reaction time and choice reaction time between sweet almond oil and cinnamon leaf essential oil inhalation were compared within each participant. Simple reaction time was represented in SRT mean (seconds) and choice reaction time was classified into 2 types: correct count (items) from 40 items and correct mean (seconds). For the simple reaction time, the cinnamon leaf essential oil inhalation took less time to respond than the sweet almond oil inhalation. For the choice reaction time, the

cinnamon leaf essential oil inhalation showed no significantly effects on correct count and respond time.

Table 11 The simple reaction time and choice reaction time between sweet almond oil and cinnamon leaf essential oil inhalation

Reaction time parameter	Sweet almond oil (SO) n = 24 Mean (SD)	Cinnamon leaf essential oil (CO) n = 24 Mean (SD)	P-value between SO and CO
		Simple reaction time (SRT)	
SRT Mean (s)	316.36 (37.66)	304.96 (26.33)	.267
		Choice reaction time (CRT)	
Correct count (%)	97.60 (5.13)	98.44 (2.42)	.312
Correct mean (ms)	470.21 (59.29)	463.28 (51.26)	.572

* Significant difference, p-value < .05, m = milliseconds

Rice paddy herb essential oil

Chemical components

The chemical composition of rice paddy herb essential oil was analyzed by GC/MS. Figure 12 showed the GC chromatogram of rice paddy herb oil. The results showed that the main compounds of rice paddy herb oil were 48.95% of limonene/sylvestrene, 18.04% of caranone <cis-4->, 14.84% of perilla aldehyde, 8.42% of caranone <trans-4-> and 2.79% of pinene (Table 11).

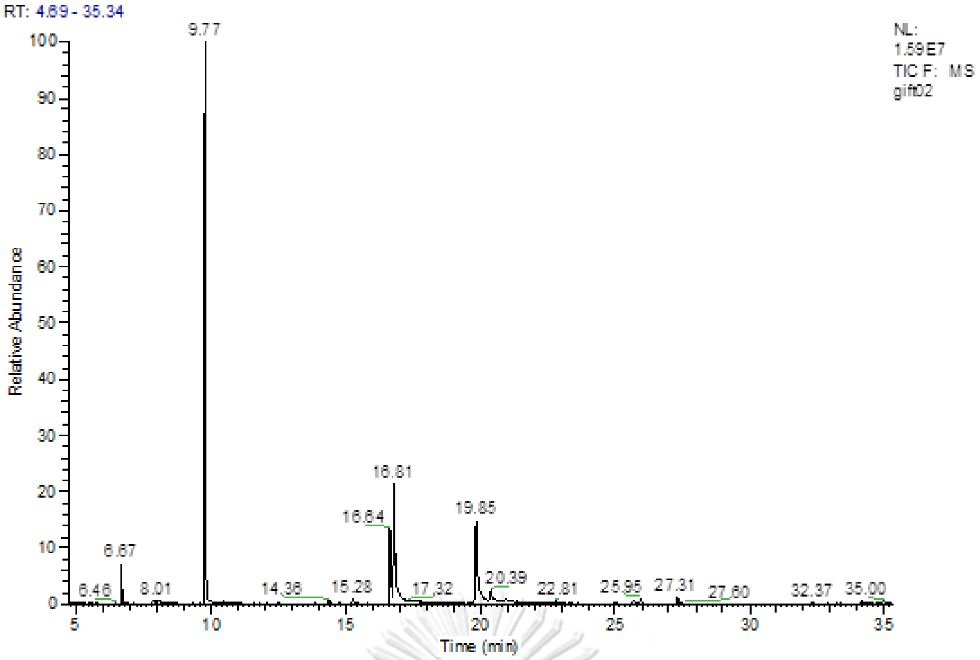


Figure 12 the GC chromatogram of rice paddy herb essential oil

Table 12 The chemical composition of rice paddy herb oil identified by GC/MS

Retention time (min)	Chemical component	Kovat's Index	Peak area (%)
6.67	Pinene<alpha->	939	2.79
7.87	Sabinene	975	0.25
8.01	Octen-3-ol<1->	979	0.75
8.38	Myrcene	990	0.22
9.77	Limonene/Sylvestrene	1030	48.95
10.45	Ocimene<(E)-beta->	1050	0.21
12.52	Linalool	1096	0.20
14.36	Camphor	1146	0.35
15.28	Borneol	1169	0.69
16.64	Caranone<trans-4->	1196	8.42
16.81	Caranone<cis-4->	1200	18.04
19.85	Perilla aldehyde	1271	14.84
20.39	Menth-1-en-9-ol<para->	1295	1.75
20.99	Limonene aldehyde	1328	0.32
25.69	Dihydro carveol acetate<neoiso->	1359	0.22
25.95	Caryophyllene(E-)	1419	0.52
27.31	Humulene<alpha->	1454	0.74
32.37	Cadinene<alpha->	1538	0.21
34.18	Eudesmol<10-epi-gamma->	1623	0.27
35.00	Eudesmol<gamma->	1632	0.26

Characteristics of the participants

A total number of 24 healthy volunteers consisting of 5 males and 19 females aged 19.67 ± 0.76 years with normal BMI of 20.03 ± 2.32 kg/m^2 were recruited. All of them were right-handed. The mean and SD values of the participants' height and weight were 1.61 ±0.08 m and 52.25 ±10.41 kg.

Emotional state responses

Table 12 showed the effects on emotional state response. Each participant inhaled the sweet almond oil (SO) and the rice paddy herb essential oil diluted in sweet almond oil (RO). The self-evaluated emotional state questionnaires were filled out by all the participants. The effects were compared within each participant. For SO inhalation, the feelings of stress decreased significantly (p= .046). For RO inhalation, the feelings of relaxation and calmness increased significantly (p=.002 and p=.009 respectively). The comparison of emotional state responses between RO and SO inhalation showed that the changes of the relaxation feelings in RO were statistically significant and higher than those in SO (p = 0.049).

Table 13 The emotional state scores between sweet almond oil and rice paddy herb essential oil inhalation

Parameter	Sweet almond oil (SO) n = 24 Mean (SD)			Rice paddy herb essential oil (RO) n = 24 Mean (SD)			P-value between SO and RO change
	Rest	Intervention	P-value	Rest	Intervention	P-value	
1) good	4.78 (2.31)	5.51 (1.92)	.114	4.86 (2.52)	5.55 (2.73)	.151	.957
2) bad	1.34 (2.28)	0.92 (1.29)	.312	1.14 (1.08)	0.86 (1.11)	.391	.730
3) active	3.44 (2.44)	3.21 (2.19)	.612	3.31 (1.98)	3.79 (2.23)	.180	.167
4) drowsy	1.94 (1.78)	2.88 (2.53)	.096	2.43 (2.35)	2.14 (2.05)	.639	.116
5) fresh	3.92	3.90	.984	3.81	4.53	.258	.172

	(2.16)	(1.93)		(1.84)	(2.38)		
6) relaxed	4.33	4.61	.554	3.85	5.78	.002*	.049*
	(1.76)	(2.03)		(1.76)	(2.86)		
7) stressed	1.17	0.94	.046*	1.37	1.09	.365	.872
	(1.27)	(1.12)		(1.58)	(1.54)		
8) frustrated	0.96	0.88	.714	1.18	1.41	.595	.523
	(0.87)	(1.32)		(1.40)	(2.00)		
9) romantic	1.55	2.16	.130	1.97	2.58	.116	.986
	(1.58)	(2.18)		(2.06)	(2.16)		
10) annoyed	0.85	0.52	.110	0.77	0.95	.639	.141
	(1.14)	(0.62)		(1.27)	(1.57)		
11) calm	3.48	3.68	.713	3.82	5.28	.009*	.171
	(1.78)	(2.26)		(2.10)	(2.50)		
12) disgusted	0.39	0.40	1.00	0.50	0.51	.923	.978
	(0.48)	(0.59)		(0.54)	(0.60)		

* Significant difference, p-value < .05

ANS parameters

The effects on ANS were displayed in Table 13. The ANS parameters were recorded at rest and SO or RO inhalation. The effects were compared within each participant. For SO inhalation, systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate were not affected ($p > .05$). The skin temperature statistically significantly increased ($p = .006$). For RO inhalation, systolic blood pressure, diastolic blood pressure and heart rate statistically significantly decreased ($p = .006$, $p = .019$, $p = .000$ respectively). Skin temperature statistically significantly increased ($p = .003$). The comparison of ANS changes between RO and SO inhalation showed that the rice paddy herb essential oil had statistically significant attenuated effects on the systolic blood pressure, diastolic blood pressure and heart rate ($p = .017$, $p = .021$, $p = .001$) respectively but no significant effects on respiratory rate and skin temperature.

Table 14 ANS parameters between sweet almond oil and rice paddy herb essential oil inhalation

Parameter	Sweet almond oil (SO) n = 24 Mean (SD)			Rice paddy herb oil (RO) n = 24 Mean (SD)			P-value between SO and RO change
	Rest	Intervention	P-value	Rest	Intrvention	P-value	
Systolic blood pressure (mmHg)	103.01 (7.23)	102.23 (7.33)	.571	105 (7.83)	99.53 (7.80)	.006*	.017*
Diastolic blood pressure (mmHg)	61.64 (8.15)	61.36 (6.77)	.801	68.34 (12.95)	61.92 (5.52)	.019*	.021*
Heart rate (bpm)	79.67 (8.20)	78.93 (7.40)	.364	80.06 (8.58)	75.29 (7.59)	.000*	.001*
Respiratory rate (bpm)	19.15 (2.85)	19.11 (3.57)	.951	19.79 (4.48)	19.33 (3.98)	.371	.554
Skin temperature (ºC)	31.73 (1.30)	32.28 (1.19)	.006*	31.57 (1.09)	32.73 (2.03)	.003*	.133

* Significant difference, p-value < .05

EEG parameters

The effects on EEG parameters were displayed in Table 14. The EEG parameters were recorded at rest and SO or RO inhalation. The effects were compared within each participant. For SO inhalation, there were no significant changes in the band powers of delta, theta, alpha and beta of all brain regions. For RO inhalation, the alpha power of right anterior, center and right posterior brain regions increased significantly (p = .040, p = .015, p = .017 respectively). The comparison of EEG parameters between RO and SO inhalation showed that there were significant changes in the power of theta and alpha waves. The power of theta over right anterior brain regions decreased significantly (p = .031). There were statistically significant increases in the alpha power of most regions namely left

anterior, right anterior, center and right posterior brain regions (p = .037, p = .039, p = .015, p = .046 respectively).

Table 15 Brain wave power between sweet almond oil and rice paddy herb essential oil inhalation

Area	Sweet almond oil (SO) n = 24 Mean (SD)			Rice paddy herb oil (RO) n = 24 Mean (SD)			P-value between SO and RO change
	Rest	Intervention	P-vlue	Rest	Intervention	P-value	
Delta power (μV^2)							
Left anterior	6.10 (1.94)	6.22 (1.57)	.674	6.11 (1.52)	5.94 (2.16)	.688	.540
Right anterior	6.54 (2.06)	6.42 (1.69)	.672	6.98 (1.49)	6.79 (2.08)	.638	.886
Center	8.70 (2.34)	8.60 (1.66)	.837	7.80 (3.13)	8.37 (3.98)	.422	.413
Left posterior	3.72 (1.24)	3.89 (1.29)	.385	3.78 (1.02)	3.72 (1.38)	.729	.393
Right posterior	3.49 (1.81)	3.46 (1.37)	.931	3.23 (1.35)	3.33 (1.39)	.659	.760
Theta power (μV^2)							
Left anterior	3.88 (1.94)	3.69 (1.70)	.118	3.84 (2.40)	4.93 (3.55)	.130	.066
Right anterior	4.31 (2.16)	4.09 (1.81)	.134	4.26 (2.35)	5.30 (3.36)	.093	.031*
Center	7.91 (3.74)	7.57 (3.27)	.478	6.92 (2.42)	7.54 (5.45)	.620	.433
Left posterior	2.90 (1.43)	2.89 (1.30)	.954	3.05 (1.86)	3.12 (1.85)	.839	.820
Right posterior	2.80 (2.11)	2.67 (1.52)	.723	3.05 (1.86)	2.60 (1.28)	.240	.564

			Alpha power (μV²)				
Left anterior	3.69	3.44	.411	4.44	6.02	.068	.037*
	(1.91)	(1.69)		(2.86)	(4.09)		
Right anterior	4.07	3.83	.521	4.74	6.15	.040*	.039*
	(2.18)	(1.94)		(2.58)	(4.29)		
Center	4.07	3.83	.885	8.40	11.14	.015*	.015*
	(2.18)	(1.94)		(4.25)	(5.94)		
Left posterior	5.28	5.34	.942	6.58	7.76	.157	.369
	(3.00)	(4.50)		(3.82)	(5.36)		
Right posterior	5.48	5.41	.925	6.41	8.81	.017*	.046*
	(3.07)	(4.29)		(4.07)	(5.26)		
			Beta power (μV²)				
Left anterior	3.37	3.21	.191	3.38	3.20	.175	.860
	(1.40)	(1.54)		(1.02)	(0.97)		
Right anterior	3.42	3.33	.667	3.34	3.29	.748	.894
	(1.39)	(1.89)		(1.14)	(1.08)		
Center	4.84	4.46	.101	4.50	4.60	.683	.166
	(1.35)	(1.10)		(1.44)	(1.35)		
Left posterior	3.37	3.45	.714	3.92	3.54	.178	.209
	(0.99)	(1.35)		(1.71)	(1.32)		
Right posterior	3.44	3.26	.558	3.45	3.83	.427	.326
	(1.94)	(1.73)		(1.60)	(2.11)		

* Significant difference, p-value < .05

Reaction Time

The effects on reaction time were demonstrated in Table 15. The simple reaction time and choice reaction time between sweet almond oil and rice paddy herb essential oil inhalation were compared within each participant. Simple reaction time was represented in SRT mean (seconds) and choice reaction time was classified into 2 types: correct count (items) from 40 items and correct mean (seconds). For the simple reaction time, the rice paddy herb oil inhalation statistically significantly

decreased the responded time than the sweet almond oil (p = .043). For the choice reaction time, the rice paddy herb essential oil inhalation showed no effects on correct answers as well as the response times.

Table 16 The simple reaction time and choice reaction time between sweet almond oil and rice paddy herb essential oil inhalation

Reaction time parameter	**Sweet almond oil (SO)** n = 24 Mean (SD)	**Rice paddy herb oil (RO)** n = 24 Mean (SD)	**P-value between SO and RO**
	Simple reaction time (SRT)		
SRT Mean (s)	324.63 (48.36)	298.70 (27.13)	.043*
	Choice reaction time (CRT)		
Correct count (%)	96.88 (3.78)	97.60 (2.50)	.338
Correct mean (ms)	469.08 (49.13)	459.00 (56.37)	.562

* Significant difference, p-value < .05, ms = milliseconds

CHAPTER V
DISCUSSION AND CONCLUSION

In experimental research, the odor effects can be measured on physiology and psychology. Physiology can be divided into different forms of arousal: the autonomic arousal (heart rate, blood pressure, skin temperature and respiratory rate) and the cortical arousal (brain wave activities). In this study, ANS parameters were used to measure the effects of essential oil inhalation on physiology of the participants. Brain wave activities through EEG recordings namely delta, theta, alpha, beta were collected to measure the effects of essential oil inhalation on physiology of the participants. The essential oil inhalation could affect psychology through subjective feelings of the participants which was measured by emotional states questionnaire in terms of good, bad, active, drowsy, fresh, relaxed, stressed, frustrated, romantic, annoyed, calm and disgusted feelings. Reaction time task by the Deary-Liewald reaction time program, which is an easy-to-use, reliable, available for free and can run both tasks without any special software is a method basically used to measure psychological effects. It is divided into simple reaction time and choice reaction time. Simple reaction time refers to the response in single stimulus but choice reaction time refers to complicated response in multiple stimuli [14-15]. In this study, the physiological parameters included ANS parameters and brain wave activities through EEG recordings and the psychological parameters involved subjective feelings of the participants measured by emotional states questionnaire and reaction time tasks were investigated. The study was divided into three sessions according to the selected essential oils including neroli oil (*Citrus aurantium* Linn.),

cinnamon leaf oil (*Cinnamomum zeylanicum* Blume), and rice paddy herb oil (*Limnophila aromatica* Merr.). Seventy-two healthy volunteers were recruited from general public and assigned into 3 sessions (24 participants/session) based on these 3 essential oils administered in this study using a simple random sampling method enclosed in envelopes.

The crossover design

Three essential oils selected in this study were diluted with the sweet almond oil to obtain the appropriate concentrations for inhalation. Hence, the effects from the sweet almond oil inhalation were studied as control. The repeated measurement was designed for the same participant who was received the sweet almond oil and the essential oil diluted in sweet almond oil by inhalation. To minimize the selection bias that might be obtained from the order of the inhalation sequence, the crossover study was performed. Each participant received both treatments whose sequence order was determined by randomization such as treatment A and then B or treatment B and then A [87]. The advantage of randomization is that it reduces bias related to confounding variables. So, randomized clinical trials are recognized as the 'gold standard' used to investigate the effectiveness of the treatment. Crossover research is different from the parallel group trial because it requires each participant to receive 2 sequential treatments in a random order separated by a washout period. In crossover research, each participant serves as his or her own control and can be used for within and between group comparisons. Since each participant is his or her own control, cross-over research needs smaller sample sizes compared to a parallel group trial design [88]. Concerning the limitation, the carry-over effects between treatments should be

considered in cross-over research. So, a washout period is necessary and must be long enough to eliminate such effects [89]. This study separated the 2 treatments by a 1-week washout period to eliminate the carry-over effects of the essential oil inhalation.

The effects of the neroli essential oil inhalation

The chemical constituents of the neroli essential oil used in this study was determined by GC/MS. Linalool was major compound (37.27%) followed by 18.34% pinene <beta->, 14.81% nerolidol<Z->, nerolidol<E-> and 10.31% limonene. The chemical composition was similar to the previous study by Sarrou et al. (29.14% linalool, 19.8% β-pinene and 12.04% limonene) [90]

The study revealed that the neroli essential oil could affect ANS parameters and functions of the brain. After neroli oil inhalation, systolic blood pressure, diastolic blood pressure, heart rate and respiratory rate statistically significantly decreased. Previous research by Choi et al (2014) was also conducted on the effects of the neroli essential oil inhalation on menopausal symptoms, stress and estrogen among postmenopausal women. The results showed that the group inhaling the neroli essential oil had significantly lower systolic blood pressure and lower diastolic blood pressure compared to the control group inhaling sweet almond oil. The researchers concluded that neroli oil essential oil inhalation could improve their quality of life, sexual desire and reduce blood pressure. [91] The essential oil consists of various chemical compounds which can distinctively interact with the brain receptors [48]. The odor molecules in essential oils could influence both parts, the sympathetic nervous system and the parasympathetic nervous system, whose functions work opposite to each other [92]. The sympathetic nervous system

determines the functions of blood pressure. So, the decrease in blood pressure and heart rate could represent a decrease in physiological arousal.

The effects of the neroli essential oil on brain wave activities showed that there were significant changes in the power of theta, alpha and beta waves. The theta power over center, left and right posterior brain regions increased significantly and the alpha power of all regions namely left and right anterior, center, left and right posterior brain waves increased significantly. There were statistically significant decreases in the beta power of left anterior, right anterior, and left posterior brain regions. These results indicated the relaxing effects of the neroli essential oil inhalation on brain wave activities. Alpha, theta and delta powers increasing have been found in relaxation state [93]. Neurofeedback, a type of biofeedback which uses EEG signals as feedback to enable self-regulation of the brain activity and eventually control one's mind and body has been increasingly used, especially alpha neurofeedback. The training program by listening relaxing music affected on soothing the participants, as could be observed from the increase in alpha power and decrease in physiological parameters (systolic blood pressure, diastolic blood pressure and heart rate) after the training [94].

For the emotional state, the standard questionnaire was used for self-evaluation to rate subjective feelings of the participants. The comparison of emotional state responses between sweet almond oil and neroli oil inhalation showed that the participants felt more relaxed and romantic as well as less stressed after neroli oil inhalation. The neroli oil could improve the emotional states and reduce stress of the participants. Previous studies reported similar results on psychological parameters affected by the neroli essential oil inhalation. Heydari et al.

(2019) determined the effect of aromatherapy using *Rosa Damascena* and *Citrus Aurantium* blossom on mental, physical symptoms and social functions of females with premenstrual syndrome. The participants were divided into 3 groups: *Rosa Damascena group, Citrus Aurantium* blossom group and sweet almond oil as control group. The results indicated that the scores of physical symptoms were decreased in all three groups but they were not significant in *Citrus Aurantium* blossom group. The scores of mental symptoms including anger, anxiety, depression decreased in all three groups and they were significant in *Citrus Aurantium* blossom group. The researchers suggested that *Citrus Aurantium* or neroli essential oil could be effective in improving the symptoms of premenstrual syndrome. [95] Sayorwan et al. (2012) conducted the effects of lavender oil inhalation on emotional states, autonomic nervous system and brain electrical activity. Lavender essential oil with linalool as a major compound was administered by inhalation to healthy participants. The results revealed that lavender essential oil could increase the power of theta and alpha brain activities. The researchers proposed the relaxing effects with the increases of alpha wave activities after lavender essential oil inhalation. [11]

The reaction time study demonstrated that the neroli essential oil inhalation could improve performance by reducing reaction time spent on responding to click button on mouse whenever target picture appeared randomly on computer screen. Many researches revealed the alpha band activity and improvement in performance on perceptual tasks after training or experience with stimuli related to those tasks. The faster the reaction time was correlated to the lower delta and the higher alpha bands [96-97].

The effects of the cinnamon leaf essential oil inhalation

The chemical constituents of the cinnamon leaf essential oil used in this study was determined by GC/MS. Eugenol was major compound (85.15%) followed by 4.36% benzyl benzoate, 3.29% eugenol acetate and 2.50% caryophyllene(E-). The chemical composition was similar to the previous reports of Singh et al. (87.3% eugenol, 3.6% bicyclogermacrene) [98] and Joshi (60.24 to 89.82% eugenol across a period of 1 year) [99]. Therefore, these odor effects were based on the eugenol major type of cinnamon leaf essential oil.

The study revealed that the cinnamon leaf essential oil affected on one of the ANS parameters. Heart rate statistically significantly decreased after the cinnamon leaf essential oil inhalation. The effects on brain wave activities showed that there were significant changes in the power of alpha and beta waves. There were statistically significant increases in the alpha power of most regions namely left anterior, right anterior and right posterior brain regions. There were statistically significant decreases in the beta power values of few regions namely left and right posterior brain regions. The self-evaluated emotional states indicated that the cinnamon leaf essential oil could increase the relaxed and calm feelings. Additionally, it could decrease the stressed feeling significantly. Masago et al. [100] determined the effects of inhalation of essential oils including eugenol on EEG activity and sensory evaluation. EEG changes among healthy participants were recorded after the essential oil inhalation. The participants were asked to complete self-evaluated emotional states questionnaires. The order of high contribution were lavender, eugenol, chamomile and sandalwood. The eugenol inhalation could cause significant lessening of alpha 1. After the eugenol inhalation, the participants rated

themselves to have comfortable feelings including restful, stimulating, pleasant, refreshing. Based on their results, the researchers concluded that alpha 1 decreased when the essential oils the participants inhaled were evaluated as comfortable but alpha 1 did not change when the essential oil they inhaled were evaluated as uncomfortable.

The results demonstrated none of statistically significant effects of cinnamon leaf essential oil on simple reaction time and choice reaction time.

The study of eugenol on central nervous system effects in rat and mouse models found that eugenol possessed antinociceptive, anticonvulsant and sedative effect whereas it didn't have any effect on models of motor co-ordination and anxiety i.e. it was neither anxiogenic nor anxiolytic [101]. Furthermore, Wang *et al*. reported the anxiolytic effects of natural small-molecule phenols including eugenol on rats and mice behavioral experiments and hippocampus CA1 region might be involved in the anxiolytic mechanism of these molecules [102].

The effects of the rice paddy herb oil inhalation

The chemical constituents of the rice paddy herb essential oil used in this study was determined by GC/MS. Limonene/sylvestrene was major compound (48.95%) followed by 18.04% cis-4-caranone and 14.84% perilla aldehyde. The chemical composition was similar to the previous report of Tucker *et al*. (53.12% limonene and 12.19% cis-4-caranone) [103]. but different from the reports of Bhuiyan *et al*. (39.21% Z-Ocimene and 17.24% Terpinolene) [104]. as well as Vairappan and Nagappan (30.06% Sabinene and 14.06% Terpinen-4-ol). Therefore, these odor effects were based on the limonene major type of rice paddy herb essential oil. [105].

The study revealed that the rice paddy herb essential oil could affect ANS function of the brain. The systolic, diastolic blood pressure and heart rate after the rice paddy herb oil inhalation statistically significantly decreased. The effects on brain wave activities showed that there were significant changes in the power of theta and alpha waves. The theta power at right anterior brain region increased significantly. There were statistically significant increases in the alpha power of most regions namely left anterior, right anterior, center and right posterior brain regions. These results indicated the relaxing effects of rice paddy herb oil inhalation. The effects on decreasing ANS and increasing theta and alpha waves were also found by the lavender essential oil inhalation, a well-known relaxing odor [11,106]. The self-evaluated emotional state indicated that the rice paddy herb essential oil increased relaxedness whilst lavender oil increased relaxedness, freshness, active feeling as well as decreased drowsiness [11]. However, this study demonstrated that the rice paddy herb essential oil inhalation could improve performance by reducing simple reaction time.

The simple reaction time experiment is to measure the elapsed times between a single response to a single stimulus by clicking button on mouse whenever a diagonal cross within the square appeared randomly on computer screen. The reaction time test is recognized for its clinical relevance in relation to cognitive function and health. The Paced Auditory Serial Addition test demonstrated that reaction time was affected by age, but not sex differences [11]. Besides the simple reaction time test, the choice reaction time test was also performed to detect the decision and the response to four stimuli. In addition to reaction time, the correct items that the participants chose which referred to rapid identification were

also measured in choice reaction time task. Usually, mean reaction time of simple reaction is less than mean reaction time of choice reaction experiments [107]. Longer reaction time is assumed to be indicative of more complex processing requirements and/or the degree of integrity of the central nervous system [108]. All selected essential oils in this research statistically significantly improved only simple reaction time. However, the correct items and the reaction times in choice reaction time task seemed to be better by essential oil intervention but were not statistically significant.

In this study, the outcome measurements on EEG recordings were divided into 4 brain waves including delta, theta, alpha and beta waves. Further studies may analyze the outcome measurements on EEG recordings by subdividing these brain waves namely alpha 1 and 2. So, the results can be interpreted more accurately. Further studies may offer various concentration levels of each essential oil to observe more obvious and different effects of the concentration levels.

In summary, neroli, cinnamon leaf and rice paddy herb essential oils were revealed for relaxing effects on brain state according to blood pressure and heart rate reduction; mood and performance improvement; alpha and theta waves increasing as well as beta wave decreasing.

REFERENCES

REFERENCES

1. Buchbauer, G. et al., Handbook of essential oils: Science, technology and applications. The United States of America: Taylor and Francis Group, 2010.

2. Hongratanaworakit, T., Essential oils and aromatherapy. Faculty of pharmacy, Srinakharinwirot University, Bangkok, 2012.

3. Price, S. and Price, L., Aromatherapy for health professional. China: Elsevier, 2007.

4. Cristina, E.D., Understanding True Aromatherapy: Understanding Essential oils. Home Health Care Mag&Prac, 2004. 16(6): 474-479.

5. Hongratanaworakit, T., Physiological effects in aromatherapy. Songklanakarin J sci Technical, 2004. 26: 118-125.

6. Andresassi, J. L., Psychophysiology: human behavior and Physiological Response. New Jersey: Lawrence Erlbaum Associated, 2000.

7. Harze, S., Sakai, K. and Gozu, Y., Effect of fragrance inhalation on sympathetic activity in normal adults. The Japanese Journal of Pharmacology, 2002. 90: 247-253.

8. Hongratanaworakit, T. and Buchbauer, G., Evaluation of the Harmonizing Effect of Ylang-Ylang Oil on Humans after Inhalation. Planta Med. 2004; 70: 632-636.

9. Sayowan, W. et al., Effects of inhaled rosemary oil on subjective feelings and activities of the nervous system. Scientia Pharmaceutica, 2013. 81: 531-542.

10. Niedermeyer, E., Dasilva, L.F., Electroencephalography: Basic Principles, Clinical Application and Related Fields. 5th ed. Philadelphia: Lippincott Williams Wilkins, 2005.

11. Sayowan, W. et al., The effects of lavender oil Inhalation on emotional states, autonomic nervous system, and brain electrical activity. The Journal of Medical Association of Thailand, 2012. 95 (4): 1-9.

12. Sayowan, W. et al., The harmonizing effects of citronella oil on mood states and brain activities. Journal of Health Research, 2012. 26(2): 69-75.

13. Sayowan, W. et al., The effects of jasmine oil inhalation on brain wave activies and emotions. Journal of Health Research, 2013. 27(2): 73-77.

14. Deary Ian J., Liewald D., and Nissan J., A free, easy-to-use, computer-based simple and four-choice reaction time programme: The Deary-Liewald reaction time task Behavior Research Methods, 2011. 43: 258-268.

15. Schmoll C. et al., Reaction time as a measure of enhanced blue-light mediated cognitive function following cataract surgery. British Journal of Ophthalmology, 2011. 5(2): 50-56.

16. Patin, R., The most commonly used essential oils for aromatherapy massage in Thai spa business. Master's Thesis, Cosmetic Science, School of Cosmetic Science, Mae Fah Luang

University, 2007.

17. Lawless, J., The encyclopedia of essential oils: The complete guide to the use of aromatic oils in aromatherapy, herbalism, health and well-being, 2002.

18. Nanasombat, S. and Teckchuen, N., Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. Journal of Medicinal Plants Research, 2009. 3(5): 443-449.

19. Laurent G. A systems perception on early olfactory coding. Science. 1999. 286: 723-728.

20. Kay, M L., and Stopfer, M. Information processing in the olfactory system of insects and vertebrates. Seminar in cell & Development Biology, 2006. 17: 433-442.

21. Hayar, A., Karnup, S. and Shipley M.T., External tufted cells: A major excitatory element that coordinal glomerular activity. The journal of neuroscience, 2004. 29: 6676-6685.

22. Liberless, S.D. and Buck, L.B., A second class of chemosensory receptor in the olfactory epithelium. Nature, 2006. 442: 645-650.

23. Wilson, D.A. and Stevenson, R.J. The fundamental role of memory in olfactory perception. Trends Neurosci, 2003. 26(5): 243-247.

24. Bary, W.A. and Janet, M.Y., Olfaction: Diverse species conserved principles. Neuron, 2005. 48: 417-430.

25. Noble prize in physiology or Medicine. [Online]. 2004. Available from http://www.Nobel prize. org. [2012, 10 January].

26. Finger, T.E., Silver, L.W. and Restrepo, D. 2nd ed. The neurobiology of taste and smell. New York: A John Wielly & Sons Inc Publication, 2000.

27. Tortora, G.J., Derrickson,. Principle of Anatomy and Physiology (15th edition) J. Wiley, 2016.

28. Guyton, A.C. and Hall, J.E., Textbook of Medical Physiology. 10th ed. United stated of America: W.B. Saunders, 2000.

29. Stern, R.M., Ray, W.J., and Quigley, K.S., Psychopyhsiological recording, Oxford University Press, New York, 2001.

30. Cristina, E.D., Understanding True Aromatherapy: Understanding Essential oils. Home Health Care Mag & Prac, 2004. 16(6): 474-479.

31. Niedermeyer, E., Da silva, L.F., Electroencephalography: Basic Principles, Clinical Applications, and Related Fields. 5th ed. Philadelphia: Lippincott Williams Wilkins, 2005.

32. Teplan, M., Fundamentals of EEG Measurement. Measurement Science Review, 2002. 2 (2): 1-10.

33 Lorig, T.S. and Schwartz, G.E., Brain and odor I: Alteration of human EEG by odor administration. Psychobiology, 1998. 16: 281-289.

34. Sugano, H., Effect of odors on mental function. Chemical Senses, 1989. 14: 303-310.

35. Nagakawa, M., Nagai, H. and Inui, T., Evolction of drowsiness by EEGs-Odors controlling drowsiness. Fragrance J, 1992. 20(10): 68-72.
36. Tonoike, M., Yamaguchj, M. and Kaetsu, I., Olfactory Cognitive Response Using Odorant Odd-ball Paradigm by Magnetoencephalography. Journal of Temporal Design in Architecture and the Environment, 2003. 3(1): 43-53.
37.T.S., Huffman, E., DeMartino, A. and DeMarco, J., The effects of low concentration odors on EEG activity and behavior. The Journal of Psychophysiology, 1991. 5: 69–77
38. EK Man, P., Davidson, R. J. and Friesen, W. V., The Duchenne smile: emotional expression and brain physiology II. Japavs Soc Psychol, 1990. 58(2): 342-353.
39. Bensafi, M. *et al*., Asymmetry of pleasant vs. unpleasant odor processing during affective judgment in humans. Neuroscience Letters, 2002. 3(16): 309-313.
40 Yamaguchi, H., Effect of odor on heart rate. In: physiological effect in aromatherapy. Songklanakarin J sci Technical, 1990. 26: 119.
41. Kikuchi, A. et al., Effect of odors in cadiac reponse patterns in a reaction time task. Chem.Senses, 1991. 16: 183.
42. Nagai, H. et al., Effect of odors on humans II: Reducting effects stress and fatigue. chem.sensse, 1991. 16: 198.
43. Hongratanaworakit, T. and Bruchbauar, G., Autonomic and emotional response after transdermal of sweet orange oil in humans: Placebo controlled trial. Int J Essential oil Therapeutic, 2007. 1: 29-34.
44. Shiina, Y. et al., Relaxation effects of lavender aromatherapy improve coronary flow velocity reserve in healthy men evaluated by transthoracic Doppler echocardiography. International Journal of cardiology, 2007. 10: 1-5.
45. Brauchli, P. et al., Electrocortical and autonomic alternation by administration of a pleasant and unplesant odor. Chem.Sense, 1995. 20: 505-515.
46. Woolfson, A. and Hewitt, D., Intensive aromacare. Int J Aromatherapy, 1992. 4(2): 12-13.
47. Hongratawaworahik, T., Heuberger, E.and Buchbauer, G., Evaiution of Effect of East Indian sandalwooloil and α-santalol on Human after transdermal absorption. Planta Med, 2004, 70(1): 3-7.
48. Heuberge E. et al., Effect of chiral Fragrances on Human Autonomic Nervous System Parameters and self Evalulation. Chem.Sens, 2001. 26: 281-295.
49. Hongratanaworwhit, T., Effects of Transdermal Essential oil on Physiological and Behavioral changes in Healthy Volunteers. Recent Progress in Medicinal Plants, 2006. 11: 225-235.
50. Kerdtep, P. and Watbamrungsakul, W., Influent of bergamot oil and lavender oil on mental, emotion and physical condition. Special project in pharmacy, faculty of pharmacy Srinakarineirot University, 2003.

51. Hongratanaworakit, T., Essential oil and aromatherapy. Bangkok: Witoonkanpok press, 2010.

52. Dye, J. 1996. First step in aromatherapy. 1st ed. Cambridge: St. Edmundsbury press, 1996.

53. Raudenbush, B., Positive effects of odorant administration on humans review. (n.p.), 2005.

54. Diego, M. A. et al., Aromatherpy positively affects mood EEG patterns of alertness and math computations. Int J Neurosci, 1998. 96(3): 217-224.

55. Mellier, D., Bezard, S. and Caston, J., Expioretory studies of intersensory olfactiory-pain relationships. Enfance, 1991. 1: 98-111.

56. Lehrner, J., Eckersberger, C. and Walla, P., Ambient odor of orange in a dental office reduces anxiety and improves mood in female patients. Physiology &Behavior, 2000. 71: 83-86.

57. Burnett, k.M., Softerbech, L.A. and Strapp, C.M., Psychological Reports, 2004. 95: 707-722.

58. Hultsch, D.F. et al., Variability in reaction time performance of younger and older adults. J Gerontol B Psychol Sci Soc Sci, 2002. 57(2): 101-15.

59. Hiruma T., Yabe H., Sato Y., Sutoh T. and Kaneko S., Differential effects of the hiba odor on CNV and MMN. Biological Psychology, 2002. 61: 321-333.

60. Dominika, D. et al., Age Differences in Intra-Individual Variability in Simple and Choice Reaction Time: Systematic Review and Meta- Analysis. PLoS ONE, 2012. 7(10): 1-23.

61. Battaglia, S., the Complete Guide to Aromatherapy, the International Center of Holistic Aromatherapy, Brisbane, Australia, 2003.

62. Lawless, J., The encyclopedia of essential oils. The complete guide to the use of aromatic oils in aromatherapy, herbalism, health and well-being, 2002.

63. Ammar, A.H. et al., Chemical composition and in vitro antimicrobial and antioxidant activities of Citrus aurantium l. flowers essential oil (Neroli oil). Pak J Biol Sci, 2012. 15(21): 1034-1040.

64. Sarrou, E., Chatzopoulou, P., Dimassi-Theriou, K. and Therios, I., Volatile Constituents and Antioxidant Activity of Peel, Flowers and Leaf Oils of Citrus aurantium L. Growing in Greece. Molecules, 2013. 18: 10640-10647.

65. Pultrini, A.M., Galindo, L.A., Costa, M., Effects of the essential oil from *Citrus aurantium* L. in experimental anxiety models in mice. Life Sciences, 2006. 78: 1720-1725.

66. Goes, T.C., Antunes, F.D., Alves, P.B., Teixeira-Silva, F., Effect of sweet orange aroma on experimental anxiety in humans. J Altern Complement Med, 2012 18: 798-804.

67. Ying-Ju, C. et al., Inhalation of neroli essential oil and its anxiolytic effects in animals. Proceedings of Measuring Behavior, 2008.

68. In-Hee, K., Essential Oil Inhalation on Blood Pressure and Salivary Cortisol Levels in Prehypertensive and Hypertensive Subjects. Evidence-Based Complementary and Alternative Medicine, 2012 10: 1-9.

69. Mi-Yeon, C., Eun-Sil M., Myung-Haeng, H. and Myeong-Soo L., Effects of Aromatherapy on the Anxiety, Vital Signs, and Sleep Quality of Percutaneous Coronary Intervention Patients in Intensive Care Units. Evidence-Based Complementary and Alternative Medicine, 2012. 10: 1-6.

70. Singh, G. et al., A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and Chemical Toxicology, 2007. 45: 1650–1661.

71. Linda, S.M. et al., Antimicrobial Activities of Cinnamon Oil and Cinnamaldehyde from the Chinese Medicinal Herb *Cinnamomum cassia* Blume. The American Journal of Chinese Medicine, 2007. 34(3): 511–522.

72. Mishra, A. K. et al., Inhibitory activity of Indian spice plant *Cinnamomum zeylanicum* extracts against *Alternaria solani* and *Curvularia lunata*, the pathogenic dematiaceous moulds. Annals of Clinical Microbiology and Antimicrobials, 2009. 8: 9.

73. Wang, R., Wang, R. and Yang, Bao., Extraction of essential oils from five cinnamon leaves and identification of their volatile compound compositions. Innovative Food Science and Emerging Technologies, 2009. 10: 289-292.

74. Mathew S. and Abraham T.E., In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. Food and Chemical Toxicology, 2006. 44: 198–206.

75. Chericoni, S., Prieto, J.M., Iacopini, P., Cioni, P. and Morelli, I., In Vitro Activity of the Essential Oil of Cinnamomum zeylanicum and Eugenol in Peroxynitrite- Induced Oxidation Processes. J. Agric. Food Chem, 2005. 53: 4762-4765.

76. Schmidt, E., Composition and Antioxidant Activities of the Essential Oil of Cinnamon (*Cinnamomum zeylanicum Blume*) Leaves from Sri Lanka. Jeobp, 2006. 9 (2): 70-182.

77. Nanasombat, S. and Teckchuen, N., Antimicrobial, antioxidant and anticancer activities of Thai local vegetables. Journal of Medicinal Plants Research, 2009. 3(5): 443-449.

78. Chowdhury, J. U., Bhuiyan, M. N. and Begum, J., Constituents of Volatile Oils from *Limnophila aromatica* and *Adenosma capitatum*. Bangladesh J. Sci. Ind. Res, 2001. 46 (3): 385-388.

79. Sribusarakum, A., Bunyapraphatsara, N., Vajragupta, O. and Watanabe H., Antioxidant activity of *Limnophila aromatica* Merr. Thai Journal of Phytopharmacy, 2004. 11(2): 54-60.

80. Brahmachari, G., *Limnophila* (Scrophulariaceae): Chemical and Pharmaceutical Aspects. The Open Natural Products Journal, 2008, 1*:* 34-43.

81. Prajapati, N.D., Purohit, S.S., Sharma, A.K. and Kumar, T., A Hand book of Medicinal Plants, Agrobios: India, 2003. 316-317.

82. Ambasta, S. P., The Useful Plants of India, (Editor in chief), Publications & Information Directorate, CSIR: New Delhi, 1986.

83. Hummel,T., Mohummation,P. and kobal, G., Handedness is a Determining factor in Latinized olfactory discrimination, Chemical Sense. 1998. 23: 541-544.

84. Doty, L.R., Mckeown, A.D. and Lee, W.W., A study of the test-retest reliability of ten olfactory tests, Chemical Sense. 1995. 20: 645-656.

85. Cain, S.W., Testing olfaction in a clinical setting. Ear, Nose and Throat Journal,1989. 68: 316-328.

86. Cheva, C. et al., Mappiny the semantic space for the subjective experience of emotional response to odors, Chemical Sense, 2009. 34: 49-62.

87. SVERDLOV, Oleksandr; ROSENBERGER, William. Randomization in clinical trials: can we eliminate bias? Clinical Investigation, 2013. 3(1): 37-47.

88. SENN, Stephen S. Cross-over trials in clinical research. John Wiley & Sons, 2002.

89. WELLEK, Stefan; BLETTNER, Maria. On the proper use of the crossover design in clinical trials: part 18 of a series on evaluation of scientific publications. Deutsches Ärzteblatt International, 2012, 109(15): 276.

90. SARROU, Eirini, et al. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of Citrus aurantium L. growing in Greece. Molecules, 2013, 18(9): 10639-10647.

91. Choi, S. Y., et al. Effects of inhalation of essential oil of Citrus aurantium L. var. amara on menopausal symptoms, stress, and estrogen in postmenopausal women: a randomized controlled trial. Evidence-Based Complementary and Alternative Medicine, 2014, 2014.

92. Chang, K. M., & Shen, C. W. Aromatherapy benefits autonomic nervous system regulation for elementary school faculty in Taiwan. Evidence-Based Complementary and Alternative Medicine. 2011; 1-7.

93. Pittayapruek, p., boonyahotra, v., sittiprapaporn, P., Electroencephalographic studies of inner wisdom meditation training program. In: *2017* 14th International Conference on Electrical Engineering/Electronics, Computer, Telecommunications and Information Technology (ECTI-CON). IEEE, 2017. 21-24.

94.Phneah, S. W., Nisar, H. EEG-based alpha neurofeedback training for mood enhancement. Australasian physical & engineering sciences in medicine, 2017, 40(2): 325-336.

95. Heydari, N., et al. The effect of aromatherapy on mental, physical symptoms, and social functions of females with premenstrual syndrome: A randomized clinical trial. Journal of family medicine and primary care, 2019, 8(9): 2990.

96. Bays, B. C., et al. Alpha-band EEG activity in perceptual learning. Journal of vision, 2015, 15(10): 7-7.

97. Vecchio, F., et al. Prestimulus Interhemispheric Coupling of Brain Rhythms Predicts Cognitive–Motor Performance in Healthy Humans. Journal of cognitive neuroscience, 2014, 26(9): 1883-1890.

98. Singh, G., et al. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food and chemical toxicology, 2007, 45.9: 1650-1661.

99. Joshi, R. K. Chemical disparity in the oil from leaves of Cinnamomum zeylanicum Blume. Flavour and Fragrance Journal, 2019.

100. Masago, R., et al. Effects of inhalation of essential oils on EEG activity and sensory evaluation. Journal of Physiological Anthropology and Applied Human Science, 2000, 19.1: 35-42.

101. Sharma, m., rauniar, G. P., DAS, B. P. Experimental study of various central nervous system effects of eugenol in mice and rats. Health Renaissance, 2012, 10(3): 208-214.

102. Wang, X., et al. Support for natural small-molecule phenols as anxiolytics. Molecules, 2017, 22.12: 2138.

103. Tucker, A O., et al. Volatile leaf and stem oil of commercial Limnophila chinensis (Osb.) Merrill ssp. Aromatica (Lam.) Yamazaki (Scrophulariaceae). Journal of Essential Oil Research, 2002, 14.3: 228-229.

104. Bhuiyan, M. N. I., Akter, F., Chowdhury, J. U., & Begum, J. Chemical constituents of essential oils from aerial parts of Adenosma capitatum and Limnophila aromatica. Bangladesh Journal of Pharmacology, 2010, 5(1): 13-16.

105. Vairappan, C. S., & Nagappan, T. Major volatile hydrocarbons of rice paddy herb, Limnophila aromatica Lam. Merr as possible chemotaxonomic marker. Journal of Tropical Biology & Conservation (JTBC), 2014, 11.

106. Lee, I. Effects of inhalation of relaxing essential oils on electroencephalogram activity. International Journal of New Technology and Research, 2016, 2(5).

107. Nikhil Ingole., Satish Pimpale and Veena krishnanand, To study simple and four-choice reaction time in light vehicle drivers and non-drivers in urban area. International Journal of Recent Scientific Research, 2016, 7(9): 13323-13330.

108. Boisgontier, M. P., Wittenberg, G. F., Fujiyama, H., Levin, O., & Swinnen, S. P. (). Complexity of central processing in simple and choice multilimb reaction-time tasks. PloS one, 2014, 9 (2), 90457.

APPENDICES

APPENDIX A

Emotional Record

Emotional Record

Date................................

ในนาทีนี้ท่านมีความรู้สึกตามหัวข้อต่อไปนี้อย่างไรให้ท่านทำเครื่องหมายลงบนเส้นจากน้อยไปหามาก

รู้สึกดี (Good)

รู้สึกไม่ดี (Bad)

รู้สึกกระปรี้กระเปร่า (Active)

รู้สึกเฉื่อยชาง่วงซึม (Drowsy)

รู้สึกสดชื่น (Fresh)

รู้สึกผ่อนคลาย (Relaxed)

รู้สึกเครียด (Stressed)

รู้สึกอึดอัด (Frustrated)

รู้สึกเคลิ้มเคลิ้มรัญจวนใจ(Romantic)

รู้สึกหงุดหงิด (Annoyed)

รู้สึกจิตใจสงบนิ่ง (Calm)

รู้สึกรังเกียจขยะแขยง (Disgusted)

ท่านมีอาการข้างเคียงหลังดมกลิ่นหรือไม่ระบุ...

Have you ever had these symptoms after inhalation?...

APPENDIX B

The personal health questionnaire

แบบสอบถามข้อมูลสุขภาพ (ภาษาไทย)

Date.................................

โปรดตอบแบบสอบถามต่อไปนี้ ตามข้อมูลที่เป็นจริง ตรงกับตัวท่านมากที่สุด

1. ข้อมูลส่วนบุคคล

เพศ..............................อายุ.................... น้ำหนัก..........................กก.ส่วนสูง.........................ซม

ผลการวัด อุณหภูมิ.................. องศา ชีพจร..................ครั้ง/นาที หายใจ......................ครั้ง/นาที ความดันโลหิต ....มม.ปรอท ดัชนีมวลกาย....................................kg/m^2

เบอร์โทรศัพทที่สามารถติดตอไดสะดวก........................

2. ขอมูลดานสุขภาพ

1. ทานมีโรคประจำตัวดังตอไปนี้ หรือไม

- โรคทางระบบประสาท ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคลมชัก ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคติดเชื้อตางๆ ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคติดเชื้อของระบบทางเดินหายใจ ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคหอบหืด ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคภูมิแพ ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคไซนัส ☐ เปน ☐ไมเปน ☐ไมทราบ ☐ ไม่แน่ใจ
- โรคความดันโลหิต ☐ เปน ☐ไมเปน ความดันโลหิตที่วัดได........................
- ทานมีโรคประจำตัวอื่น คือ.............. และ/หรือ เคยเขารับการผาตัด..................................
- ทานจำเปนตองใชยารักษาโรคประจำตัว คือ

ชนิด	ขนาด	ปริมาณ
....	....	....
....	....	....

2. ที่คิดว่าสุขภาพร่างกายของท่านตอนนี้เป็นอย่างไร

☐ เจ็บปวย ☐ ปกติตามเคย☐แข็งแรงดี ☐ แข็งแรงดีมาก

3. ทานเคยแพสิ่งตอไปนี้ หรือไม

☐ สารเคมี____________________☐อาหาร___________☐น้ำหอม ☐เกสรดอกไม้

☐อื่นๆ โปรดระบุ____

4. ทานเคยประสบอุบัติเหตุรายแรง หรือไม

☐เคยที่อวัยวะ____________________________เมื่อ_______________________________☐ ไม่เคย

5. เวลานอนตามปกติ____ชั่วโมง

6. ทานมีปญหาเรื่องนอนหลับในชวง 1 เดือนที่ผานมา หรือไม

☐ เปน ☐ ไมเปน ☐ ไมทราบ ☐ ไม่แน่ใจ

7. ทานมีปญหาการไดยิน หรือไม

☐ มี ☐ ไม่มี

8. ทานมีปญหาในการดมกลิ่น หรือไม

☐ มี ☐ ไม่มี

9. ทานไดรับการฝงเครื่องกระตุนหัวใจ

☐ มี ☐ ไม่มี

10. ทานคิดวาสุขภาพจิตของทานเปนอยางไร ☐ เจ็บปวย ☐ ไมดี ☐ ดี

11. ทานสูบบุหรี่หรือไม ☐ ไม่เคยและ☐ สูบ☐ เคยสูบแตหยุดสูบแลว

12. ทานดื่มสุรา เครื่องดื่มที่มีแอลกอฮอลหรือไม ☐ ไม่เคยเลย☐ บอยครั้ง ☐ บางครั้ง

- น้ำอัดลม ☐ ใช่ประมาณวันละ______________________________☐ไมใช ☐ บางครั้ง

-ชา-กาแฟ ☐ ใช่ประมาณวันละ______________________________☐ไมใช ☐ บางครั้ง

APPENDIX C

The Edinburgh Handedness Inventory test

Date.................................

แบบทดสอบถนัดมือขวา

ให้ท่านอ่านกิจกรรมในแต่ละข้อแล้วให้คะแนนตามความเป็นจริงมากที่สุดโปรดตอบทุกข้อตามมือข้างที่ถนัดหากไม่เคยมีประสบการณ์ในกิจกรรมนั้นๆให้เว้นช่องว่างไว้

วิธีการให้คะแนน

+ ในช่องมือข้างที่ถนัดขณะทำกิจกรรมนั้นซึ่งมืออีกข้างพอที่จะทำได้บ้าง

++ ในช่องมือที่ถนัดข้างเดียวโดยที่มืออีกข้างที่ไม่สามารถทำกิจกรรมนั้นได้เลย

+/+ ในทั้ง 2 ช่องถ้าสามารถทำกิจกรรมในแต่ละข้อนั้นได้ดีทั้ง 2 มือเท่าๆกัน

กิจกรรม	ข้างขวา	ข้างซ้าย
1.เขียนหนังสือ		
2. วาดรูป		
3. โยนหรือปาของ		
4. ใช้กรรไกร		
5. ถือแปรงสีฟัน		
6. ถือมีดหั่นของ		
7. ถือช้อน		
8. กวาดพื้น		
9. ถือก้านไม้ขีดไฟ		
10. มือข้างที่ถือฝาขณะเปิดฝากล่องหรือขวด		
คะแนนรวม		

.... ผู้ประเมิน

การคิดคะแนน ผลรวมของช่องข้างขวา – ช่องข้างซ้าย×100
―――――――――――――――
ผลรวมทั้งหมด

เกณฑ์ ได้คะแนนต่ำกว่า -40 แสดงว่าถนัดมือซ้าย

ได้คะแนนระหว่าง -40- +40 แสดงว่าถนัดทั้งสองข้าง

ได้คะแนนมากกว่า +40 แสดงว่าถนัดข้างขวา

APPENDIX D

Score sheet for odor test (Butanol Threshold)

Score sheet for odor test (Butanol Threshold)

Date..............................

Score sheet for odor test (butanol threshold)

Step	Concentration	1	2	3	4	5
11	$2.25x10^{-5}$	B	W	B	B	W
10	$6.77x10^{-5}$	B	B	W	W	B
9 (Start)	$2.03x10^{-4}$	W	B	W	B	B
8	$6.09x10^{-4}$	W	B	W	B	B
7	$1.82x10^{-3}$	W	W	B	B	B
6	$5.48x10^{-3}$	B	W	B	B	B
5	0.0164 %	B	B	B	B	W
4	0.049 %	W	B	B	B	W
3	0.148 %	W	B	B	B	B
2	0.44 %	W	B	B	B	B
1	1.33 %	B	W	B	B	W
0	4 %	B	W	B	B	W
score						

B = smell buthanol W= smell water

Key: ✓correct ✕ incorrect

APPENDIX E

Odor Familiarity

Odor Familiarity

Date..................................

Have you ever had these symptoms after inhalation? (Answer more than one item)

☐ Headaches / Dizziness .... ☐ Nausea / Vomiting ....

☐ Runny nose.. ☐ Allergy ....

☐ Respiratory difficulty............................. ☐ No symptoms.........................

How do you feel the smell of the following essential oils?

Score/Odor	Very much 5	Like 4	Moderately 3	Don't like 2	Hate 1

APPENDIX F

Case record Autonomic Nervous System

Case record Autonomic Nervous System

Date...............................

Activity	No	Times	Blood pressure		Pulse	Temp	RR	Note
			Systolic	Diastolic				
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							
	1							
	2							
	3							
	4							
	5							
	6							
	7							
	8							
	9							
	10							

APPENDIX G

Case record electroencephalography

Date...............................

Case record electroencephalography

Gender ....

Age ....

DOB ....

Handed ....

EEG operator: ....

No.	Procedure	Duration	Time	EEG recorded file	Sequence file	Bad channel/Remark
1	Apply EEG Cap	30 min				
2	EEG baseline (Eye open)	5min				
3	EEG baseline (Eye close)	5 min				
4	Sweet Almond /Essential oil (Eye close)	8 min				

Note ....

....

....

APPENDIX H

Certificate of Approval

AF 02-12

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University
Jamjuree 1 Building, 2nd Floor, Phyathai Rd., Patumwan district, Bangkok 10330, Thailand,
Tel/Fax: 0-2218-3202 E-mail: cccu@chula.ac.th

COA No. 154/2018

Certificate of Approval

Study Title No. 087.1/61 : EFFECTS OF INHALED ESSENTIAL OILS ON CENTRAL NERVOUS SYSTEM, AUTONOMIC NERVOUS SYSTEM, EMOTIONAL STATES AND REACTION TIME

Principal Investigator : MISS PAKAMON THANATUSKITTI

Place of Proposed Study/Institution : College of Public Health Sciences, Chulalongkorn University

The Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University, Thailand, has approved constituted in accordance with the International Conference on Harmonization – Good Clinical Practice (ICH-GCP).

Signature: Prida Tasanapradit
(Associate Professor Prida Tasanapradit, M.D.)
Chairman

Signature: Nuntaree Chaichanawongsaroj
(Assistant Professor Nuntaree Chaichanawongsaroj, Ph.D.)
Secretary

Date of Approval : 2 July 2018   Approval Expire date : 1 July 2019

The approval documents including

1) Research proposal
2) Patient/Participant Information Sheet and Informed Consent Form
3) Researcher
4) Questionnaire
5) Advertising leaflet

Protocol No. 087.1/61
Date of Approval -2 JUL 2018
Approval Expire Date -1 JUL 2019

The approved investigator must comply with the following conditions:

1. *The research/project activities must end on the approval expired date of the Research Ethics Review Committee for Research Involving Human Research Participants, Health Sciences Group, Chulalongkorn University (RECCU). In case the research/project is unable to complete within that date, the project extension can be applied one month prior to the RECCU approval expired date.*
2. *Strictly conduct the research/project activities as written in the proposal.*
3. *Using only the documents that bearing the RECCU's seal of approval with the subjects/volunteers (including subject information sheet, consent form, invitation letter for project/research participation (if available).*
4. *Report to the RECCU for any serious adverse events within 5 working days*
5. *Report to the RECCU for any change of the research/project activities prior to conduct the activities.*
6. *Final report (AF 03-12) and abstract is required for a one year (or less) research/project and report within 30 days after the completion of the research/project. For thesis, abstract is required and report within 30 days after the completion of the research/project.*
7. *Annual progress report is needed for a two- year (or more) research/project and submit the progress report before the expire date of certificate. After the completion of the research/project processes as No. 6.*

VITA

NAME	Pakamon Thanatuskitti
DATE OF BIRTH	04 July 1982
PLACE OF BIRTH	Suphanburi, Thailand

INSTITUTIONS ATTENDED

1) Bachelor's degree (2004) in Science (Applied Thai Traditional Medicine) faculty of Medicine, Mahasarakham University

2) Master's degree (2009) in Science (Pharmaceutical Chemistry and Natural Products) Faculty of Pharmaceutical Sciences, Khon-Kaen University

PUBLICATION

Pakamon Thanatuskitti "The Effects of Inhaled Rice paddy herb oil (*Limnophila aromatica* (Lam.) Merr.) on Autonomic Nervous System and Reaction Time on Healthy Volunteers: A Randomized Crossover", Journal of The Royal Thai Army Nurses, Vol 21, No 2 (2020): May-August

REFERENCES

VITA