# FORMULATION DEVELOPMENT AND TESTING OF THE ESSENTIAL OIL BLEND ON STRESS WITH SLEEP PROBLEMS

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# การพัฒนาสูตรน้ำมันหอมระเหยผสมและการทดสอบฤทธิ์ในผู้ที่มีปัญหาเครียดและนอนไม่หลับ

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# FORMULATION DEVELOPMENT AND TESTING OF THE ESSENTIAL OIL BLEND ON STRESS WITH SLEEP PROBLEMS

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A Dissertation Submitted in Partial Fulfillment of the Requirements

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BY

KANCHANAPA SATHIRACHAWAN

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Inhalation of many essential oils has been used as the contemporary or alternative treatment for stress and sleep problems. However, there are no scientific data supporting this treatment. The purpose of this study were to i) formulate the essential oil blend for decreasing the stress and sleep problem in the volunteers and ii) to evaluate the acute and chronic effect of essential oil blend on physiological response in volunteers with stress and sleep problems by investigating sympathetic activity, HPA axis and immune system. Then, this blend was used to investigate the acute and chronic effect of selected blend on stress with sleep situation as well as physiological response by assessing, vital signs and biomarkers including salivary α-Amylase, salivary cortisol and salivary immunoglobulin A (IgA). The result showed that the volunteers were satisfied with the B2 blend of essential oils containing limonene, linalyl acetate, menthol and linalool. Acute inhalation of B2 blend did not change blood pressure, body temperature and heart rate. After acute exposure to B2 blend inhalation, there are no change in salivary α-amylase and salivary cortisol level compared to pre-inhalation. For the chronic treatment with balm containing B2 blend, there was a significant reduction in stress score, blood pressure, alpha amylase and salivary cortisol ($p<0.05$) sleep score While IgA level was elevated following long-term treatment using balm containing B2 blend. In conclusion, long-term treatment of essential oil blend reduced stress by reducing the hypothalamic-pituitary-adrenal axis (HPA) and sympathetic activity but increased immunity, suggesting the benefit on improving sleep quality.

Keyword : essential oil blend, cortisol, alpha amylase, stress, insomnia

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# CHAPTER 1
# INTRODUCTIONS

## Background

Inhalation of many essential oils has been used as the contemporary or alternative treatment for stress and sleep problems. The use of essential oils and their components in aromatherapy has been recognized for a significant period, with their importance steadily increasing. Recent years have seen numerous scientific studies exploring the effects and mechanisms of action of these compounds on the central nervous system. Key areas of focus include their influence on stress, pain, anxiety, learning, memory, attention, arousal, relaxation, sedation, and sleep. Additionally, their impact on mood, behavior, and perception, as well as their applications in managing stress and sleep disorders, has been extensively examined. (1) However, there are no scientific data supporting this treatment with essential oil blend

## Objectives of the Study

To formulate the essential oil blend for decrease the stress and sleep problem on the volunteers.

To evaluate the effect of essential oil blend on stress and sleep problems in volunteer the by measuring biomarkers (sympathetic and HPA axis) vital sign Immunoglobulin A (IgA) and electroencephalogram (EEG).

## Significance of the Study

To investigates the activity of the hypothalamic-pituitary-adrenal (HPA) axis , one of the main neuroendocrine stress systems, with sleep problem in order to formulated the essential oil blend effect for stress with sleep problems and to identify the relation between the mechanism of essential oil on stress and sleep problems.

**Scope of the Study**

The aim of this study was to evaluate the most favorite formula of essential oil including bergamot oil, peppermint oil, eucalyptus oil, chamomile oil, ylang ylang oil, lavender oil, marjoram oil, vetiver oil and cedarwood oil in sleep balm by using inhalation technique. Then, the selected blend was investigated by a gas chromatography-mass spectrometry (GC-Ms). Formulation of essential oil blend were evaluated of effectiveness investigating stress and sleep measuring in many aspects such as vital sign, sympathetic and HPA axis, Immunoglobulin A (IgA) and electroencephalogram (EEG).

**Definition of terms**: essential oil blend; sleep problem; stress; brain wave; cortisol; alpha amylase, IgA

Conceptual framework

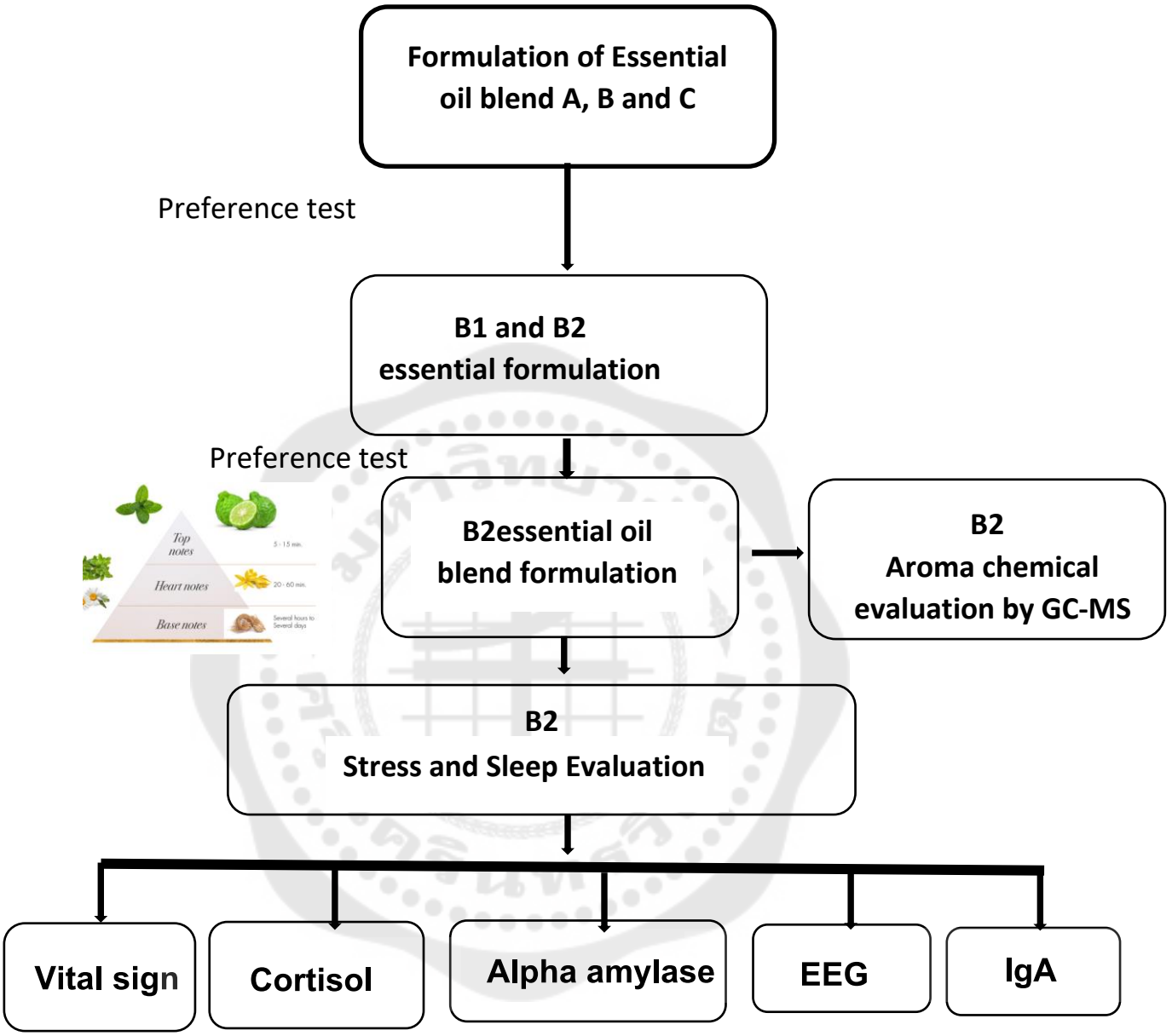


Figure 1 Conceptual framework

# CHAPTER 2
# LITERATURE REVIEW

## 1. Aromatherapy

Aromatherapy, an alternative therapeutic approach, involves the use of essential oils to alleviate symptoms related to discomfort and illness. The term "aromatherapy" was introduced in the 1930s by French chemist René-Maurice Gattefossé, following his observation that lavender essential oil (LEO) effectively treated a burn on his hand. Today, aromatherapy is widely utilized to address various conditions, including stress, sleep disturbances, and anxiety. However, the scientific evidence underpinning its efficacy remains incomplete and requires further investigation. (1)

Essential oils (EOs) have long been utilized in traditional medicine for managing various health conditions due to their diverse biological activities. Given the adverse effects often associated with conventional allopathic treatments for central nervous system (CNS) disorders, the pursuit of safer therapeutic alternatives holds considerable importance.(2)

Essential oils (EOs) have been utilized since ancient times as therapeutic agents, valued for their pharmacological and psychological benefits, and regarded as physical, mental, and spiritual healing aids. These complex mixtures of volatile compounds, produced as secondary metabolites by plants, are extracted using methods such as steam distillation, solvent extraction, and more advanced techniques like supercritical fluid extraction and ultrasound-assisted extraction. Research on humans and animals has demonstrated that EOs can exert various central nervous system (CNS) effects, including anxiolytic, neuroprotective, antidepressant, anticonvulsant, analgesic, and sedative properties. Consequently, EOs serve as safe and non-toxic adjunct therapies for managing CNS-related disorders, such as insomnia, depression, and Alzheimer's disease, when used at appropriate concentrations, as evidenced by studies over the past decade.(2)

Essential oils (EOs) are concentrated volatile compounds produced as secondary metabolites in aromatic plants, known for their distinctive aromas. Commonly used in traditional medicine and aromatherapy, certain EOs have been shown to reduce anxiety-related behaviors in both humans and animals. Their biological effects may stem from the combined action of all their constituents or primarily from the major components present in higher concentrations.(3) Interest in holistic approaches that integrate traditional and complementary therapies for health promotion has grown recently. Aromatherapy, a complementary therapy, utilizes essential oils extracted from plants, which are applied through various methods to elicit effects based on their chemical properties and modes of application.(4)

The chemical profiles of many essential oils suggest sedative and hypnotic properties; however, systematic research on these effects remains limited. Unlike traditional psychotropic drugs, aromatherapy appears to lack significant side effects, making it a promising area for further clinical and scientific investigation. The growing interest in the clinical use of essential oils highlights the need for more detailed studies on their pharmacological effects, particularly when inhaled.(5)

**1.1 Linalool**

Linalool, a monoterpene commonly found in essential oils from aromatic plants such as Lavandula angustifolia, Melissa officinalis, Rosmarinus officinalis, and Cymbopogon citratus, is widely used in the production of fragrances for shampoos, soaps, and detergents. When inhaled, linalool exhibits sedative effects, including hypothermia, reduced locomotion, and prolonged pentobarbital-induced sleep in mice.(3)

**1.2 Limonene**

Limonene, a key aromatic compound in essential oils from oranges, grapefruits, and lemons, is widely used in aromatherapy for its calming and sedative properties. These oils have been traditionally employed to help manage nervous disorders, heart issues, colic, asthma, and depression.(6)

## 2. Stress

The human stress response has developed to preserve homeostasis in response to real or perceived stress. (7) The hypothalamic–pituitary–adrenal (HPA) axis is a crucial regulatory pathway for maintaining homeostasis during stress. Cortisol, the end product of this pathway, is secreted in a pulsatile pattern, with its amplitude following a circadian rhythm. During acute stress, cortisol levels rise, driven by a surge in adrenocorticotropic hormone (ACTH). However, in chronic stress, while ACTH levels return to baseline, cortisol remains elevated due to increased adrenal sensitivity and decreased cortisol metabolism. Initially, elevated cortisol promotes survival in response to acute stress, but prolonged exposure can become maladaptive, contributing to various health issues, such as metabolic syndrome, obesity, cancer, mental health disorders, cardiovascular disease, and greater susceptibility to infections.(7)

In response to stress, the body initiates coordinated processes through the sympatho–adrenomedullary (SAM) and hypothalamic–pituitary–adrenal (HPA) axes to restore homeostasis, protect life, and promote evolutionary success. These responses were initially described in relation to physical injury, exertion, and perceived psychological threats.(7)

## 3. Sleep

Sleep is essential for human health, with insufficient sleep impairing concentration, judgment, daily functioning, and increasing irritability. Studies show that inadequate sleep disrupts homeostasis, neural functions, and mood. Even moderate sleep deprivation can negatively impact life quality and health, similar to the effects of complete sleep loss.(8)

Sleep disorders are linked to increased sympathetic activity, which raises blood pressure and heart rate, thereby increasing the risk of cardiovascular issues in patients in coronary care units. (9)

Sleep epidemiology is an expanding field that examines the link between sleep disturbances and issues such as accidents, human errors, health problems, and the prevalence of sleep disorders. The health effects of sleep disturbances are both

immediate and long-term. Short-term effects include poor well-being, daytime sleepiness, fatigue, and impaired performance, impacting safety. Long-term effects include hypertension, inflammation, obesity, and glucose intolerance, which can lead to chronic diseases and early death. Sleep disturbances are also strongly associated with cognitive dysfunction, emotional regulation issues, social problems, and substance abuse. A recent USA Sleep Poll revealed that 64% of respondents experienced frequent sleep problems, but only 15% were officially diagnosed.(10)

Sleep disturbances are a significant public health issue, impacting physical, mental, and emotional well-being. While sleep is crucial for recovery from various physiological conditions, chronic sleep deprivation is common and negatively affects mood, cognitive function, performance, and homeostasis. Many individuals experience ongoing sleep deprivation and disorders. (1)

Common treatments involve the use of medications that promote or extend sleep;(1) Inhaling essential oils may offer a safe alternative to pharmaceutical treatments for mild to moderate sleep disturbances.(10) However, while drugs may not enhance sleep quality and can lead to side effects like addiction or resistance, essential oils have minimal side effects and show promising potential as therapeutic agents for treating sleep disorders(1) Inhaling essential oils may serve as a safe alternative to pharmaceutical treatments for mild to moderate sleep disturbances.(10)

**4. Essential oil for sleep and stress treatment**

Aromatherapy essential oils, including lavender (Lavandula officinalis), marjoram (Origanum majorana), ylang-ylang (Cananga odorata), and neroli (Citrus aurantium), are used to support hypertensive patients. Lavender helps balance the nervous system and alleviate insomnia, while marjoram activates the parasympathetic nervous system to relax the sympathetic system. Ylang-ylang addresses cardiac palpitations and hypertension, and neroli is effective for insomnia and depression. The primary chemical components of these oils are linalyl acetate, terpinen-4-ol, benzyl acetate, and limonene, respectively(4)

Inhalation is a rapid and effective aromatherapy method that triggers a central nervous system response within seconds. It works by absorbing volatile molecules through the nasal mucosa, which then enter the circulatory system via the lungs. The olfactory pathway not only conveys the sense of smell but also regulates memory, emotions, visceral functions, and brain activities such as alertness and sleep. Neurotransmitters involved in olfactory transmission, along with the Orexin system in the hypothalamus, activate brain regions responsible for producing neurotransmitters like GABA and serotonin, which are linked to insomnia and other disorders. Inhalation is a non-invasive method that minimizes toxicity and side effects, making it an effective approach for managing insomnia and related psychiatric conditions.(5)

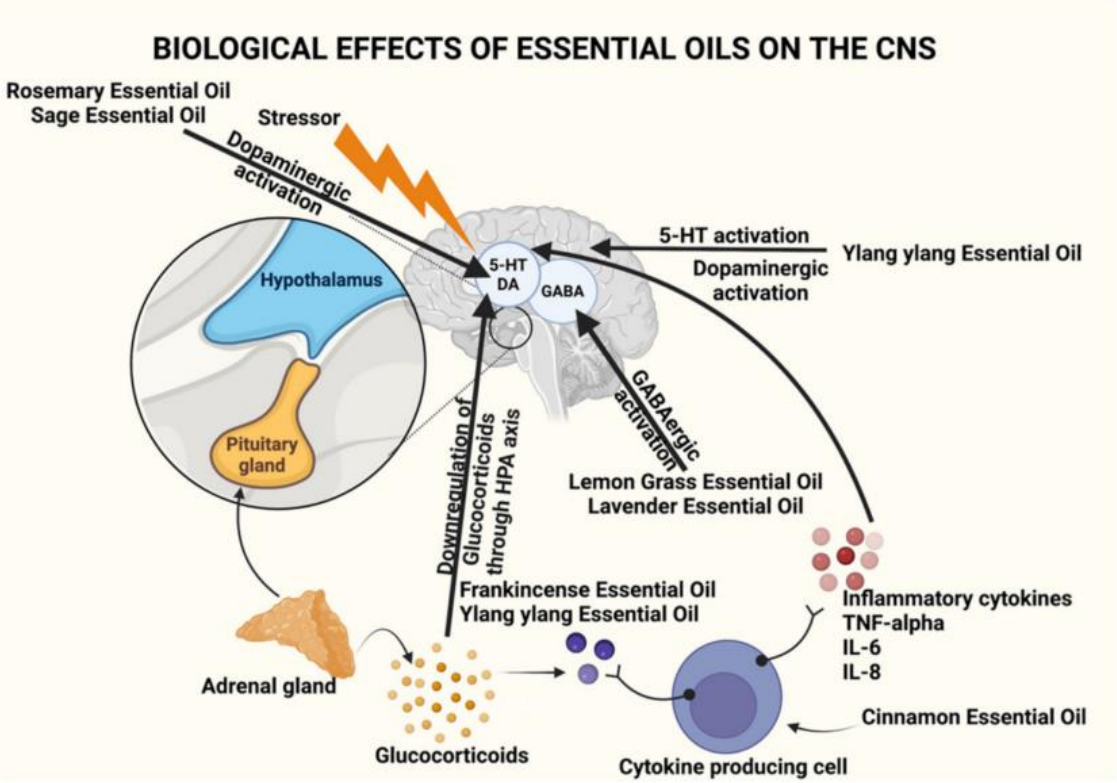


Figure 2 Biological effects of essential oils on the CNS through activation of various components of the brain [2]

Inhalation of essential oils (EOs) stimulates olfactory nerves, which are responsible for the sense of smell. Pain perception involves various brain regions, including the somatosensory cortex, ACC, PFC, insular cortex, amygdala, thalamus, cerebellum, and PAG. The analgesic effects of certain EOs may result from their action on these brain areas. Their antinociceptive, anxiolytic, and anticonvulsant effects are believed to arise from modulation of the GABAergic system, sodium (Na+) ion channels, and transient receptor potential (TRP) channels.(2)

**4.1 Effect of aroma chemical on stress and sleep problem**

Table 1 Biological effects of essential oils on the CNS through activation of various components of the brain.

| Plant/Source Essential Oil | Biological Activity | Purpose of Use | Reference |
|---|---|---|---|
| Bergamot oil<br>(Citrus bergamia)<br>Active Constituents<br><br>Monoterpene<br>limonene (25–53%) and high quantities of oxygenated compounds, such as linalool (2–20%), linalyl acetate (15–40%), γ-terpinene, and β-pinene | Reduction of nociceptive responses | Analgesic effect<br>Anxiolytic, stimulant or sedative and Antidepressive | (11), (12),(13), (14) |
| Lavender oil<br>(Lavandula Angustifolia)<br><br>Active Constituents (>20%)<br>Linalyl acetate (7.4–44.2%), linalool 11.4–46.7%) | GABAergic system interaction<br>Antagonist of NK-1 receptor inhibiting release of substance P, reduces peripheral and central nerve excitability<br>inhibition of voltage-gated | Anxiolytic, stress relief, mood enhancement, analgesic, and pain relief | (2, 15) |

| | | | |
|---|---|---|---|
| | calcium channels, reduction of 5-HT1A receptor activity, and increased parasympathetic tone | | |
| Peppermint oil (Mentha Piperita)<br><br>Active Constituents Menthol (40.7%), iso-menthone (23.4%) | Binds to the nicotinic/GABAA receptor and inhibits acetylcholinesterase | CNS stimulation, antioxidant, and memory retention Memory booster, modulated performance on cognitive tasks, and decreased mental fatigue | (2, 16, 17) |
| Eucalyptus oil (Eucalyptus Globulus)<br><br>Active Constituents 1,8-cineole (49.07–83.59%), α-pinene (1.27–26.35%) | Acetylcholinesterase inhibition | Anti-inflammatory, improves memory, and improves symptoms of Alzheimer's disease | (2) |
| Sweet Marjoram oil (Origanum majorana)<br><br>Active Constituents Monoterpenes α -terpinene α -terpinene terpinolene β -myrcene Monoterpenols | Analgesic, Antispasmodic | Anxiety/stress, Insomnia | (18), (19),(20) |

| | | | |
|---|---|---|---|
| terpinen-4-ol linalool α -terpineol<br>Esters<br>sabinene hydrate and linalyl acetate | | | |
| Vetiver oil (Vetiveria zizanioides).<br><br>Active Constituents<br>β-vetivenene and β-vetispirene.<br>The main compound was sesquiterpene | gamma aminobutyric acid (GABA) potentiation | pharmacological effects, e.g. antioxidant, antiinflammation, antifungal, anti-parasite, antibacterial, hepatoprotective, antidepressant, antianxiety, antihyperglycemia | (21) |
| Ylang ylang oil (Cananga Odorata)<br>Active Constituents<br>β-Caryophyllene (26.8%) | Activation of ANS and has effects on the 5-HT and DAergic system Direct binding onto CB2R receptor | Mood adjustment, relaxation, and antidepressant activity | (2) |

Various plant extracts have been studied for their effects, with lavender and bergamot essential oils (EOs) being the most commonly used for relaxation, either alone or in combination, showing pharmacodynamic interactions. Positive effects are linked to specific constituents in these oils, and the combined use of EOs with diverse molecular compounds can enhance therapeutic outcomes.(22)

4.2 Absorption of EO Molecules through Inhalation (Mechanisms behind the Effect of **EOs on Brain)**

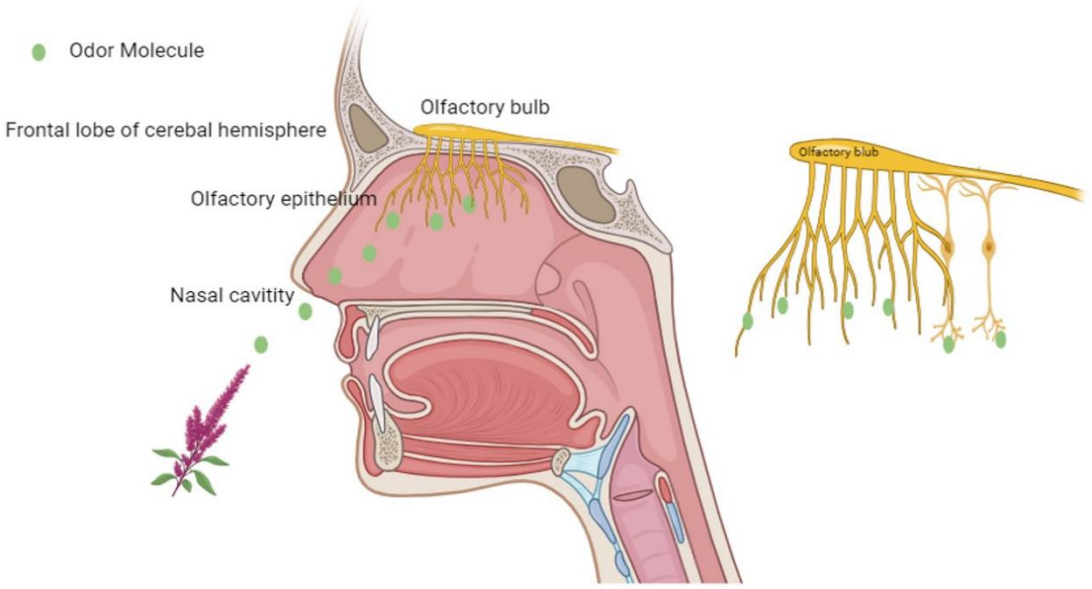


Figure 3. Inhaled essential oils (EOs) are delivered to the brain through the respiratory and olfactory systems: (a) EO molecules pass through the nasal cavity, reaching either the olfactory or respiratory system; (b) EO molecules follow a pathway within the olfactory system

Essential oils (EOs) consist of volatile compounds that, when inhaled, are delivered to the brain through the olfactory and respiratory systems. The olfactory system begins in the nasal cavity and connects to the olfactory bulb, which transmits odorant signals to brain regions such as the hypothalamus and hippocampus. Small EO molecules can directly reach the central nervous system (CNS) via sensory neuron axons, influencing emotional responses. Meanwhile, the respiratory system facilitates gas exchange, with vapor molecules diffusing through the respiratory epithelium, reaching the alveoli, and entering the bloodstream to be transported to the brain.(22)

**4.2.1 Transmission of Olfactory Odorant Signal (Through Activation of Nasal Olfactory Chemoreceptors)**

In summary, odor identification and processing involve olfactory sensory neurons (OSNs) that express specific odorant receptors. When essential oils are inhaled, odor molecules bind to these receptors, sending signals to the olfactory bulb, which processes and transmits them to the olfactory cortex. The olfactory cortex, including areas like the piriform cortex and olfactory tubercle, projects this information to the limbic system, facilitating emotional processing and connecting scent perception with memory and mood regulation (Figure 3).

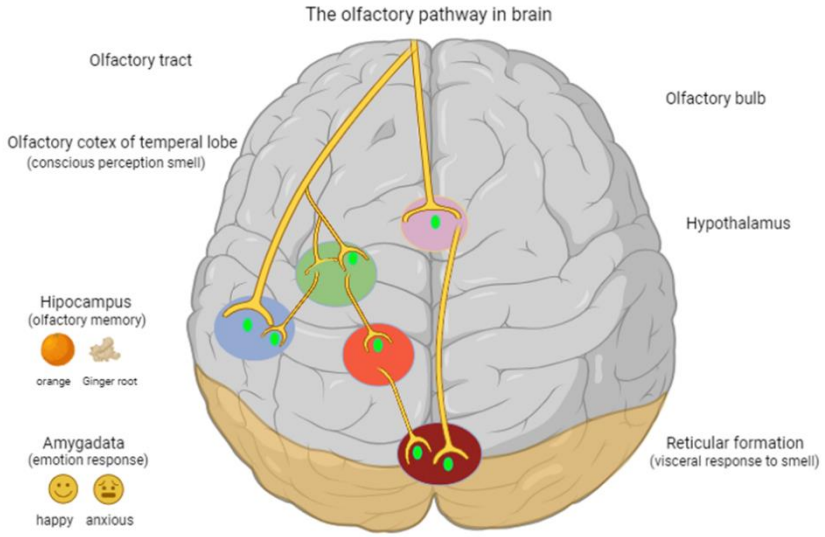


Figure 4 Inhaled odor molecules activate specific olfactory sensory neuron (OSN) receptors.

**4.2.2 Chemical Transport of Molecules (Direct Penetration of EO Molecules via Neuronal Pathway)**

Essential oils (EOs) can influence mood through both intra- and extracellular transport. After inhalation, small volatile compounds pass through the olfactory system and reach the brain. In intracellular transport, the compounds bind to receptors on target neurons, triggering internalization and receptor-mediated endocytosis. The molecules are then transported to the olfactory cortex and interact with the

hippocampus and amygdala via anterograde transport, involving the olfactory and trigeminal nerves. Extracellular transport occurs as molecules move through the paracellular cleft to the lamina propria and are then transported along axons to the brain. These molecules cross the blood-brain barrier (BBB) and cerebrospinal fluid barrier, reaching various brain regions. EO molecules can interact with neurotransmitter receptors, such as TRP, GABA, 5-HT, and DA, inducing anxiolytic or antidepressant effects.(22)

**5. Stress and sleep evaluation**

**5.1 Saliva cortisol**

Salivary cortisol concentrations have been shown to directly correlate with serum unbound cortisol levels in both men and women. Measuring cortisol in saliva offers several advantages over traditional serum measurements, including a non-invasive, stress-free collection process and the ability to assess biologically active, unbound cortisol. As a result, salivary cortisol measurements have gained increasing attention in recent research.(23)

**5.2 Salivary alpha amylase**

Salivary alpha-amylase (sAA) is a key enzyme in saliva that breaks down starches, aiding in energy extraction from starch. Its concentration in saliva is linked to starch intake and the number of salivary amylase gene copies in humans. sAA is released by acinar cells, which are innervated by both sympathetic and parasympathetic branches of the autonomic nervous system. While primarily a digestive enzyme, sAA is also useful for assessing sympathetic activity. Research has shown a connection between changes in sAA levels and physical or psychological stress. Traditionally, salivary cortisol, reflecting hypothalamic-pituitary-adrenal activity, is used to monitor short-term stress. Studies measuring both cortisol and sAA levels have found varying correlations: some show both increase under stress, while others suggest they function independently, indicating different stress response mechanisms or individual coping styles.(24)

Free salivary cortisol is a well-established non-invasive marker for hypothalamic-pituitary-adrenal (HPA) axis activity, but a similar marker for sympatho-adrenal medullary (SAM) system activity is not yet available. Salivary alpha-amylase (sAA) has been proposed as a potential marker for SAM activity, as its levels increase in response to both physiological and psychological stress. While salivary cortisol is widely used in stress research, direct measurement of adrenaline and noradrenaline in saliva does not reliably reflect SAM activity. The study found a significant association between sAA levels and plasma noradrenaline, suggesting that sAA concentrations may predict plasma catecholamine levels during stressful conditions.(25)

**5.3 Blood Pressure**

Blood pressure measurement often focuses on assessing the degree of nocturnal BP reduction, highlighting the growing interest in sleep quality, disorders, and duration. Typically, blood pressure decreases at night due to increased parasympathetic activity and reduced sympathetic activity during sleep. Consequently, sleep disorders, sleep duration, and quality can impact hypertension. (4)

**5.4 Electroencephalography (EEG)**

Electroencephalography (EEG) measures local field potentials resulting from the synchronization and desynchronization of neuronal activity in the brain. EEG patterns, known as electropharmacograms, are used to characterize drug effects on specific brain regions. This technique is commonly employed to assess sleep-wake cycles and EEG power activity, making it a valuable tool for studying the impact of essential oils on brain function.(26) EEG recordings provide insights into brain wave patterns by analyzing their amplitude and frequency. Brain waves are classified into four primary categories based on their frequency ranges: alpha waves (8–13 Hz), beta waves (15–30 Hz), theta waves (4–7 Hz), and delta waves (0–4 Hz). Alpha waves are predominantly observed during states of mental relaxation, while beta waves are typically associated with periods of focused cognitive activity or heightened mental tension in response to external stimuli.(27)

# CHAPTER 3
# MATERIALS AND METHODS

## 1.Materials

Essential oil

Centrifuge (Satorious Stedim, Germany)

Blood pressure monitors

ELISA kit (R&D Sytems, USA)

BioTek® Synergy™ HT (Multi-Detection Microplate Reader, USA)

Salivary cortisol enzyme immunoassay assay kit (96-well plate) (SALIMATRICS, 101 Innovation Blvd., State Collage, PA 16803 UAS )

Salivary alpha amylase assay kit (96-well plate) (SALIMATRICS, 101 Innovation Blvd., State Collage, PA 16803 UAS)

Brain Wave EEG instrument

Gas chromatography Mass spectrometer GCMS (Sci Spec co.,Ltd)

## 2. Methods

Essential oil blends selection and formulation

In this study, a blend of essential oils was formulated to address stress and sleep issues in volunteers. The selected oils included Bergamot (Citrus bergamia), Peppermint (Mentha piperita), Eucalyptus (Eucalyptus globulus), Chamomile (Chamomilla recutita), Ylang-ylang (Cananga odorata), Lavender (Lavandula angustifolia), Marjoram (Origanum majorana), Vetiver (Chrysopogon zizanioides), and Cedarwood (Cedrus atlantica). Three different formulations were created, each with distinct scent profiles: floral, unisex, and aromatic.

### 2.1 Gas chromatography mass spectrometer by headspace technique analysis test

Essential oil samples were selected based on a preference test to assess the aromatic chemical composition in the blended oils. The essential oils were obtained through hydrodistillation. For some herbs, 1g of dried, crushed plant material was mixed

with 20 ml of ethanol and 20 ml of distilled water, then stored at room temperature for 2 days. A 0.6 ml portion of this mixture was combined with 0.6 ml distilled water and 0.2 ml solvent A (ethyl acetate: hexane: methylene chloride, 5:1:1, v/v/v). The mixture was agitated for 2 minutes, and 4 µl of the supernatant was injected into the chromatograph.

For analysis, a Hewlett Packard GC 5890 coupled with an MS engine 5989B in EI mode was used for compound identification. The GC was equipped with an Rtx-5MS capillary column (30m x 0.25mm, 0.25µm film). The temperature program ranged from 50°C (2 minutes) to 250°C (10 minutes) at a rate of 8°C/min, with a helium flow rate of 1 ml/min. A Thermo Finnigan GC-MS with similar conditions was also used. The GC/MS interface and ion source were maintained at 200-250°C, with electron energy set to 70eV and electron emission at 100µA.

**2.2 Sleep lotion formulation preparation**

Sleep lotion formulation as shown in Table 1

Table 2 Formulation of sleep lotion for stress and sleep problems.

| No. | Ingredients | Part | % w/w | Function |
|---|---|---|---|---|
| 1 | Water | A | 75-85 | Solvent |
| 2 | Glycerin | | 0.5-2 | Humectant |
| 3 | Fractionated Coconut oil | B | 3-5 | Emollient |
| 4 | Argan oil organic | | 0.5-2 | Emollient |
| 5 | Sweet almond oil | | 0.5-2 | Emollient |
| 6 | Jojoba oil | | 0.5-2 | Emollient |
| 7 | Phenoxyethanol | C | 0.7-1 | Preservative |
| 8 | Essential oil blend formulation | | 3-6 | Active ingredients |
| 9 | Sodium Polyacryloyidimethyl Taurate and Hydrogenate Polydecene and Trideceth-10 | | 0.5-3 | Emulsifier |
| | | | 100 | |

Sleep balm product preparation

Sleep balm in each sample prepared by 2g of essential oil blend, and LEO dissolved in 100 g. base sleep balm in amber glass bottle.

**2.4 Preference test**

The research study scent satisfaction in essential oil blend tests the preference by sniff essential oil formulation direct on the scent paper strip. The number of participants were 30 persons in Srinakharinwirot University (SWU) students, aged between 18-22 years old. Volunteers receive the smelling paper strip in 3 formulation and then test by smell. After that, the questionnaire classified by hedonics scaling test and strength test were evaluated the scent satisfaction.

In this study, preference scale was used for satisfaction measuring. It was statement to respondents rates their level of satisfaction. The statement may be positive or negative. Usually a 5 point scale of satisfaction like the following is used (Albert et al.,2008) which shown in Table 2.

| Level | Score | Meaning |
|---|---|---|
| 5 | 4.21-5.00 | Excellent |
| 4 | 3.41-4.20 | Good |
| 3 | 2.61-3.40 | Moderate |
| 2 | 1.81-2.60 | Low |
| 1 | 1.00-1.80 | Very low |

**3. Study population**

**3.1 Sample size calculation**

$$\frac{N}{1 + Ne^2}$$

n = Sample

N = Population

e = i.e. error (0.05)

$$\frac{60}{1 + 60(0.05)^2}$$

n = 52 people

**4. Clinical criteria**

Inclusion criteria, the volunteers must have two or more of the following risk factors and voluntarily participate in the project and sign the consent form. And they are able to follow instruction of procedures required for the study and must be able to come for follow-up examinations in the 4weeks

The participants were 60 male and female employees aged between 25-50 years, recruited from Mae Fah Luang University Hospital and Tel-Dan Co., Ltd and also had the stress and sleep problems between mild to moderate stages. The questionnaires were used to evaluate the sleep quality and stress condition.

The participants were divided into 3 groups. The experimental group (n=20/group) was selected to inhale an essential oil blend (1 from 3 formulations) which was the most favorite, whereas the placebo and positive control groups were inhaled base oil and lavender oil, respectively. Participants were received sleep balm massage on forehead for 15 -20 minutes before sleep, every night for 4 weeks

Exclusion criteria included participants with sleep disorders (Pittsburgh Sleep Quality Index score of 5 or higher), severe stress, anosmia, or a history of allergy to herbal remedies. Individuals using hypnotics or sedatives, suffering from depression, or unable to follow the study procedures were also excluded. Volunteers who had used antidepressants within 12 weeks prior to the study, those who smoked or had alcohol dependence, and those who were pregnant, lactating, or planning pregnancy during the study period were not eligible. Additionally, individuals with a history of adverse reactions to essential oil-containing products or those who chose to withdraw from the study were excluded.

**5. Salivary cortisol sample collection**

Saliva collection for acute condition was performed for all participants in MFU hospital at two-time (before and after essential oil inhalation) points on a single day. Saliva collection for chronic condition was collected two time per day (morning and afternoon) for 3 days before and after treatments.

The group of participants brush their teeth without toothpaste or rinse mouth with drinking water to removed food residue, then wait for 10 minutes before collecting saliva sample. To collect saliva sample, tilt head forward and drool through a funnel into a 15 ml tube for no more than 30 minutes. The tube containing the saliva sample will be placed in a container and store in the refrigerator at 4°C.The container containing the saliva samples will be collected be the researcher on the following day. All of sample will be stored in at -20 °C freezer until analyzed.

Saliva was collected using the passive drooling method from each participant to investigate diurnal variations in hormonal secretion influenced by the circadian rhythm. This method is a quick, non-invasive, and reliable approach for measuring biologically active, unbound plasma cortisol levels in adults. The passive drool technique is regarded as the gold standard for saliva collection in biological testing, as it facilitates the storage of samples for subsequent analysis.

**5.1 Salivary Sample preparation**

Saliva sample was extracted by centrifuge for 10 minutes at 1200 rpm at 4 °C with mutifuge S-R (Heraeus from kendro laboratory product Germany) collected total supernatant of saliva sample was transfered into 1.5 ml microtube stored at -20 °C freezer until analyzed with ELISA test kit.

**5.2 Instrument Parameters**

**5.2.1 Salivary cortisol enzyme immunoassay assay**

Salivary cortisol levels were measured using a quantitative enzyme-linked immunosorbent assay (ELISA) (SALIMATRICS, USA) following the manufacturer's instructions. Reagents were incubated at room temperature for 1.5 hours prior to use. Standard dilutions (3.0, 1.0, 0.33, 0.11, 0.037, 0.012 µg/dL) were prepared, with a reagent diluent used as a blank. Saliva samples and standards were added to the wells,

and 25 μL of each was pipetted into the appropriate wells. The enzyme conjugate was diluted (1:1600) and added to each well, followed by a 5-minute incubation with a plate rotator at 500 rpm. After washing the plates, TMB substrate solution was added and incubated for 25 minutes in the dark. A stop solution was added, and absorbance was measured at 450 nm using a BioTek® Synergy™ HT Microplate Reader. Results were quantified from a standard curve and expressed as μg/dL. All procedures were carried out at room temperature.

**5.2.2 Salivary α-amylase enzyme immunoassay assay**

α-amylase product in saliva was measured by Salivary α-amylase quantitative enzyme-linked immune absorbent essay (ELISA) following manufacturer's instruction from (SALIMATRICS, USA). Briefly, All reagents were incubated at room temperature of 1.5 hours before use Subsequently, Standard dilution was prepared concentration of Ctrl-H and Ctrl-L Unit/ml. Heat the α-Amylase Substrate to 37ºC in for 20 minutes, Saliva samples are to be diluted with the α-Amylase Diluent provided. Prepare a final dilution is 1:200. And then add 8 μL of controls and/or diluted saliva samples to individual wells. Subsequently, add 320 μL of the preheated (37ºC) α-Amylase Substrate to each well simultaneously using a multichannel pipette. Discard pipette tips to avoid reagent contamination. And then reading kinetic at 37ºC in plate reader immediately. Place plate in reader and start reader and mix (500-600 rpm) at 37ºC. Transfer plate again and read the OD at exactly 3 minutes. Save 3-minute OD readings. Calculations Subtract the one-minute reading from the three-minute reading and multiply by the conversion factor. The conversion factor takes the 1:200 sample dilution into account for the controls and pre-diluted samples.

# CHAPTER 4
# RESULTS

**4.1 Preference test of essential oil blend for stress with sleep problems.**

The result of preference test showed that formulation B was the most satisfied compared with other formulations. The volunteers suggested to reduce intensity and increase the balance of overall of scent suitable for using prior to sleep and relaxation.

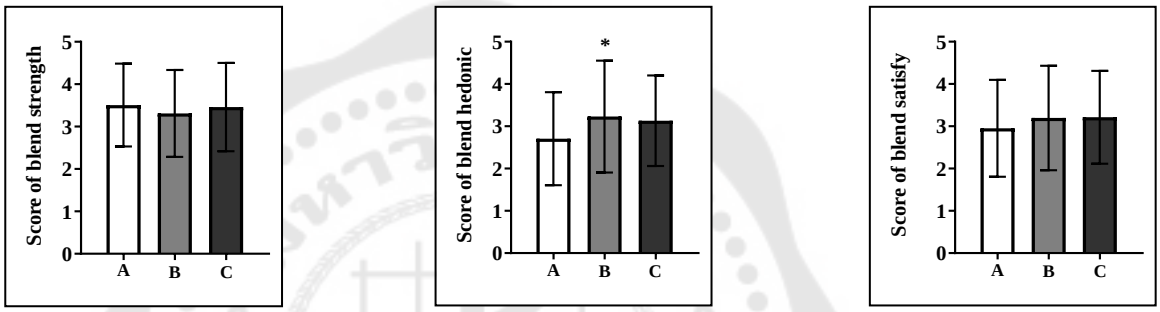


Figure 5 Preference test of essential oil blend for stress with sleep problem in volunteers. There are 3 formulations (A, B, C) of essential oil blend. Data are expressed as means± SEM (n=20/group) *$p$<0.05 compared to A

Table 3 The development of essential oil blend formulation from the preference test

| List of essential oil blend stress with sleep formulation | % B1 | % B2 |
|---|---|---|
| Bergamot oil | 49.02 | 58.14 |
| Peppermint oil | 24.51 | 17.44 |
| Marjoram oil | 9.80 | 11.63 |
| Ylang ylang oil | 9.80 | 5.81 |
| Chamomile oil | 4.90 | 5.81 |
| Vetiver oil | 1.96 | 1.16 |
| รวม | 100.00 | 100.00 |

The volunteer's opinion of formulation development B1 and B2 was shown in figure 2. These was no significance between B1 and B2 however B2 was selected due to its content including higher bergamot oil to give freshness and unisex scent profile and reduce the floral middle note. The result of blending effect to B2 has a light citrus and freshness character compared with B1. Therefore, B2 formulation were used in the study investigating the effect of essential oil blend on stress with sleep problem.

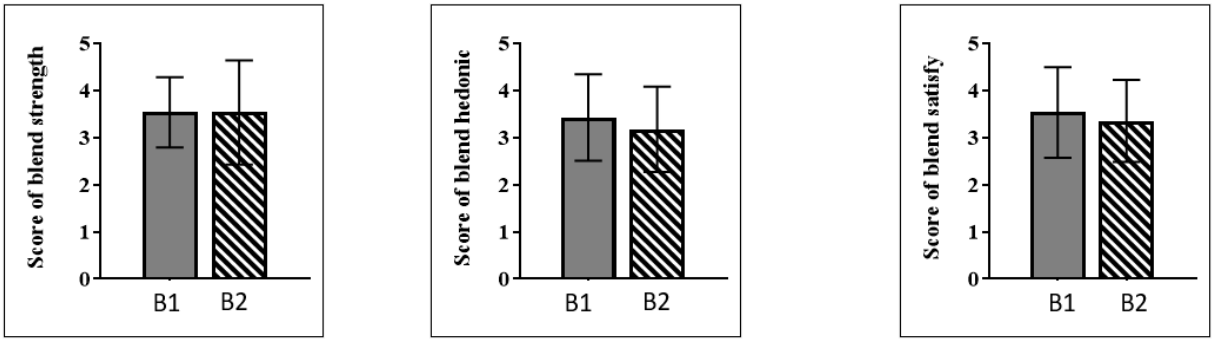


Figure 6 Preference test of essential oil blend development for stress with sleep problem in

volunteers. There are 2 formulations B1 and B2 using the same essential oil but different ratio. Data are expressed as means $\pm$ SEM (n=20/group)

**4.2 Qualitative and quantitative characterization of essential oil blends from Gas chromatography–mass spectrometry (GC–MS)**

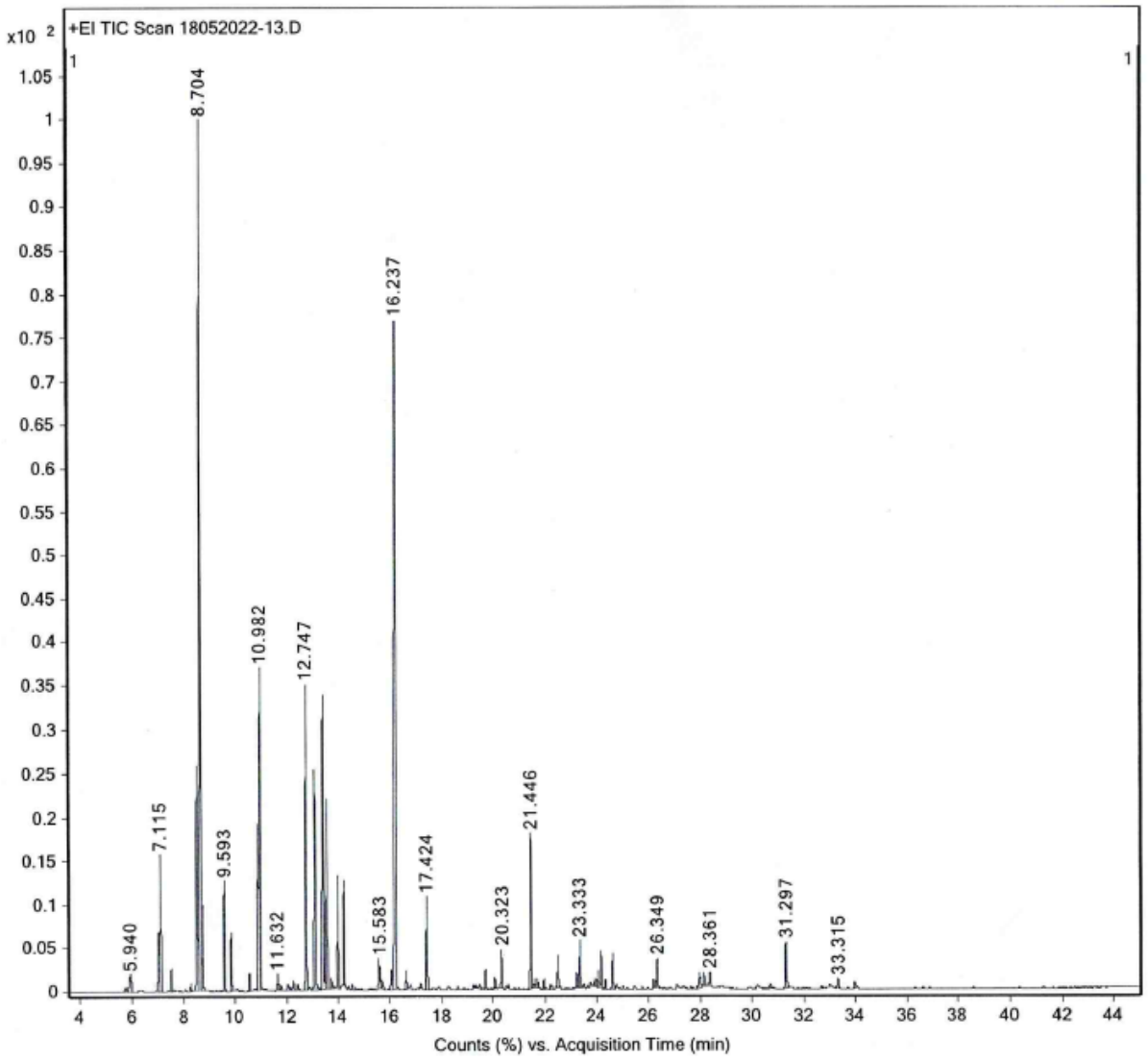


Figure 7 Gas chromatography mass spectrometer
by headspace technique analysis test

Aroma chemical containing in blend essential oil selected from preference test was shown in the % area peak. Limonene, menthol and linalool were the main components of aroma chemical showed in the formulation B2.

Table 4 Percentage compositions of essential oil blend B2 formulation

| no. | RT | Component name | % area | % in the formulation |
|---|---|---|---|---|
| 1 | 8.7 | limonene | 20.22 | 26.56 |
| 2 | 13.42 | menthol | 7.18 | 9.43 |
| 3 | 10.98 | linalool | 6.34 | 8.33 |
| 4 | 12.74 | isomenthone | 5.67 | 7.45 |
| 5 | 13.08 | benzyl acetate | 4.73 | 6.21 |
| 6 | 8.53 | p-*cymene* | 4.64 | 6.10 |
| 7 | 10.88 | trans-sabinene hydrate | 3.4 | 4.47 |
| 8 | 13.55 | terpine-4-ol | 3.38 | 4.44 |
| 9 | 21.44 | trans-caryophyllene | 2.77 | 3.64 |
| 10 | 7.11 | β-pinene | 2.42 | 3.18 |
| 11 | 13.97 | α-terpineol | 1.85 | 2.43 |
| 12 | 14.22 | methyl chavicol | 1.66 | 2.18 |
| 13 | 9.59 | g - terpinene | 1.62 | 2.13 |
| 14 | 16.23 | linalyl acetate | 1.52 | 2.00 |
| 15 | 17.42 | menthyl acetate | 1.52 | 2.00 |
| 16 | 31.29 | benzyl benzoate | 0.94 | 1.23 |
| 17 | 23.33 | germacrene D | 0.9 | 1.18 |
| 18 | 8.75 | 1,8-cineole | 0.88 | 1.16 |
| 19 | 20.32 | nerol acetate | 0.65 | 0.85 |
| 20 | 24.15 | α-farnesol | 0.62 | 0.81 |
| 21 | 24.61 | δ-cadinene | 0.61 | 0.80 |
| 22 | 26.34 | caryophyllene oxide | 0.56 | 0.74 |
| 23 | 5.94 | α-pinene | 0.54 | 0.71 |
| 24 | 22.49 | α-humulene | 0.54 | 0.71 |
| 25 | 16.63 | geranial | 0.36 | 0.47 |

| no. | RT | Component name | % area | % in the formulation |
|---|---|---|---|---|
| 26 | 28.37 | α-candinol | 0.26 | 0.34 |
| 27 | 33.31 | franesol acetate | 0.2 | 0.26 |
| 28 | 33.96 | benzyl salicylate | 0.14 | 0.18 |
| | | **Total** | | **100.00** |

The analysis of essential oil blend B2 was performed by Expert Centre of Innovative Herbal Product, Thailand Institute of Scientific and Technological Research by Gas Chromatography-Mass spectrometry (GCMS) method. The samples were analyzed on headspace gas chromatography mass spectrometry instrumentation described with instrument of Agilent Technologies 7890 B, Triple Quad Mass Selective Detector 7000 D, capillary. The column was set at HP-5Ms (30m x 0.25 mm, film thickness 0.25 μm). The column temperature was set at 50°C-230 °C,4°C/min, injector mode pulse split 20:1,230 °C, Detector MSD,EI 70eV, scan mode, 40-400amu, The carrier gas was helium 10.0 psi, flow 1.2 ml/min, average velocity 40 cm/sec.

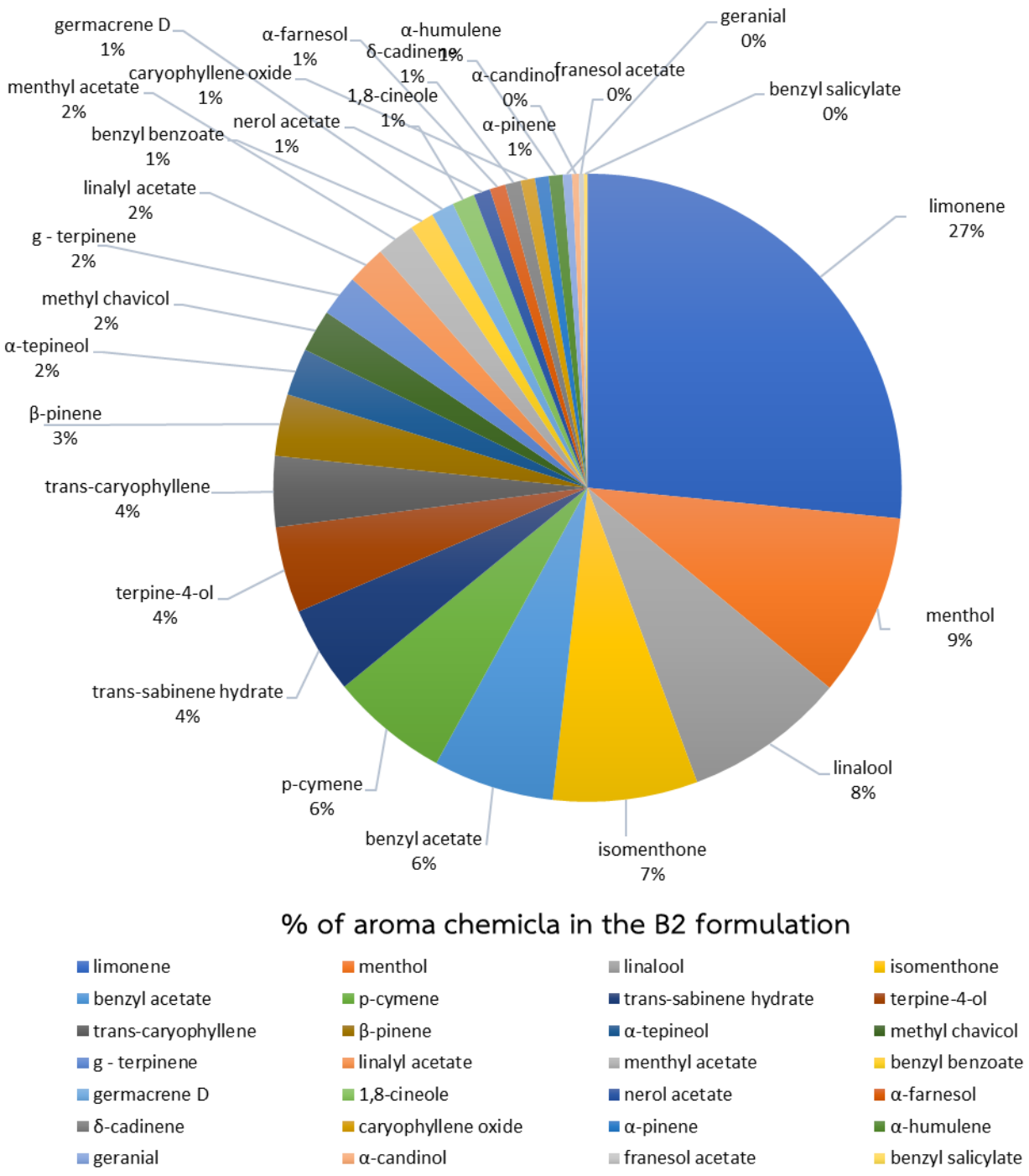


Figure 8 The ratio of aroma chemical in essential oil blend formulation B2

Limonene, a common terpene found in citrus fruits, is assumed to reduce stress and mood disorders. Dopamine and γ-aminobutyric acid (GABA) have been reported to play an important role in modulating anxiety in different parts of the brain. Limonene treatment increased dopamine levels in the striatum of mice. Depolarization-induced GABA release was enhanced by limonene pre-treatment in PC-12 cells.(28) Limonene, a principal component at 26.56%, is known for its potential to reduce stress and improve mood disorders (6, 28, 29). Dopamine and γ- aminobutyric acid (GABA) are reported to play important roles in these properties of limonene. (28)

Interestingly, a blended essential oil is composed of aromatic chemicals that produce mood-enhancing effects due to the synergistic interaction of their properties. For example linalyl acetate, l-menthol enhances dopamine-mediated neurotransmission (30), decreased the level of cortisol in the blood (31) and showed less anxiety. Moreover, Linalool acts on the central nervous system (CNS) primarily as an antidepressant agent. It has harmonizing effects that improve mood and reduce symptoms of stress, anxiety, and depression. (32)

The essential oil (EO) blend is more effective than its individual constituents, meaning the bioactivity of single molecules does not fully represent the effects of the whole oil.(33) Interactions between individual chemicals in essential oils can produce various effects. These include additive effects, where the combined activity of individual molecules equals that of the mixture; antagonistic effects, where the activity of the combined chemicals is notably lower than an additive outcome; and synergistic effects, where the combined activity is significantly greater than the sum of the individual effects(34). Such interactions highlight how the whole essential oil often has a more potent or nuanced effect than its individual components alone.(35)

**4.3 The effects of using a balm containing blended essential oils on changes in stress levels, as assessed by stress questionnaire evaluation**

The study on the effects of using a balm containing blended essential oils on stress, as measured by the questionnaire developed by the Department of Mental Health's stress evaluation tool, revealed the following:

Participants were divided into three groups. Group 1 (control group) using a balm without essential oils, and Group 2 using a lavender essential oil balm. Both groups did not show significant changes in stress levels after using the product. In contrast, Group 3 (experimental group), using a blended essential oil balm specifically formulated for stress and insomnia reduction, exhibited a significant decrease in self-reported stress levels after applying for four weeks ($p < 0.01$). Furthermore, participants in Group 3 reported good feelings of after inhaling the essential oil blend.

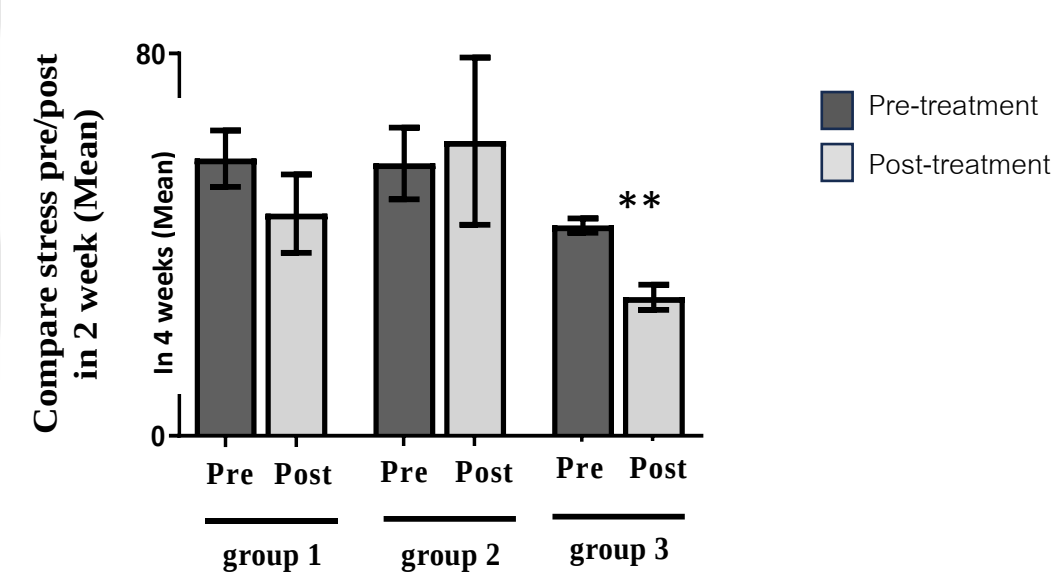


Figure 9 The effect of essential oil blend in sleep balm products on self-evaluation of after using for 4 weeks. The data was compared between pre and post inhalation including 3 groups, group 1= base sleep balm, group 2= lavender essential oil sleep balm and group 3 =essential oil blend for stress with sleep problem **p<0.01 was significant compared to pre-treatment. Data was express as mean±SEM.

**4.4 The effects of balm containing blended essential oils on changes in sleep.**

The effects of using a balm containing blended essential oils on sleep quality were assessed by using the Pittsburgh Sleep Quality Index (PSQI).

Participants were divided into three groups. Group 1 (control group) used a balm without essential oils, Group 2 used a lavender essential oil balm, and Group 3 (experimental group) used a blended essential oil balm designed to alleviate stress and insomnia. After four weeks of use, there was no statistically significant changes in sleep quality observed in all groups

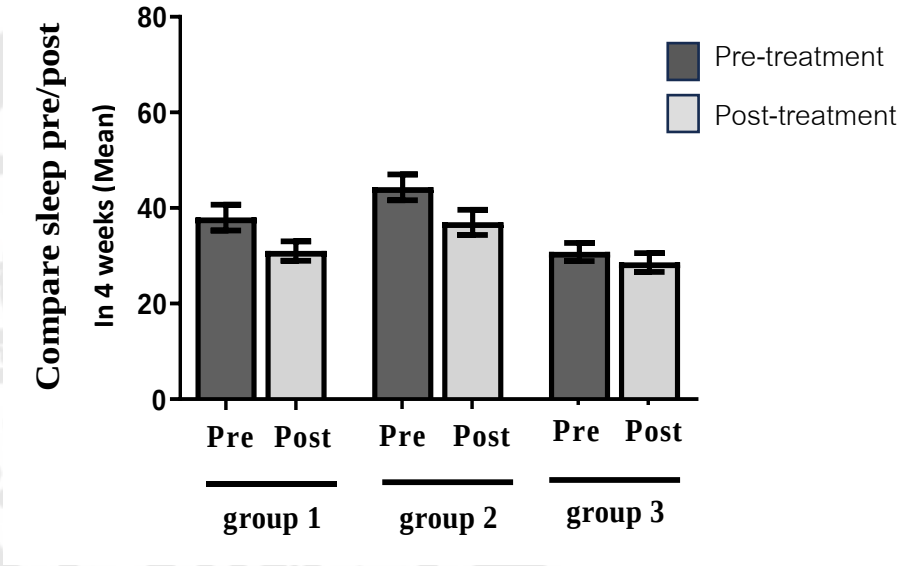


Figure 10 The of essential oil blend in sleep balm products on sleep quality after 4 weeks treatment. The data was compared between 4-week treatment with pre and post inhalation including 3 groups, group 1= base sleep balm, group 2= lavender essential oil sleep balm and group 3 =essential oil blend for stress with sleep problem. Data express as mean±SEM (n=20/group).

After four weeks of using balm containing essential oil blend, participants in group 3 significantly reduced self-reported stress levels, as measured by the Department of Mental Health's stress assessment tool, with statistical significance ($p < 0.01$). However, no significant changes in sleep quality were observed.

### 4.5 Vital sign

The main objective of this study was to investigate the change in vital sign before and after using aromatherapy. There was a no significant difference in systolic blood pressure, diastolic blood pressure, mean arterial blood pressure, heart rate and temperature between before and after inhaling essential oil blend.

4.5.1 Acute vital sign

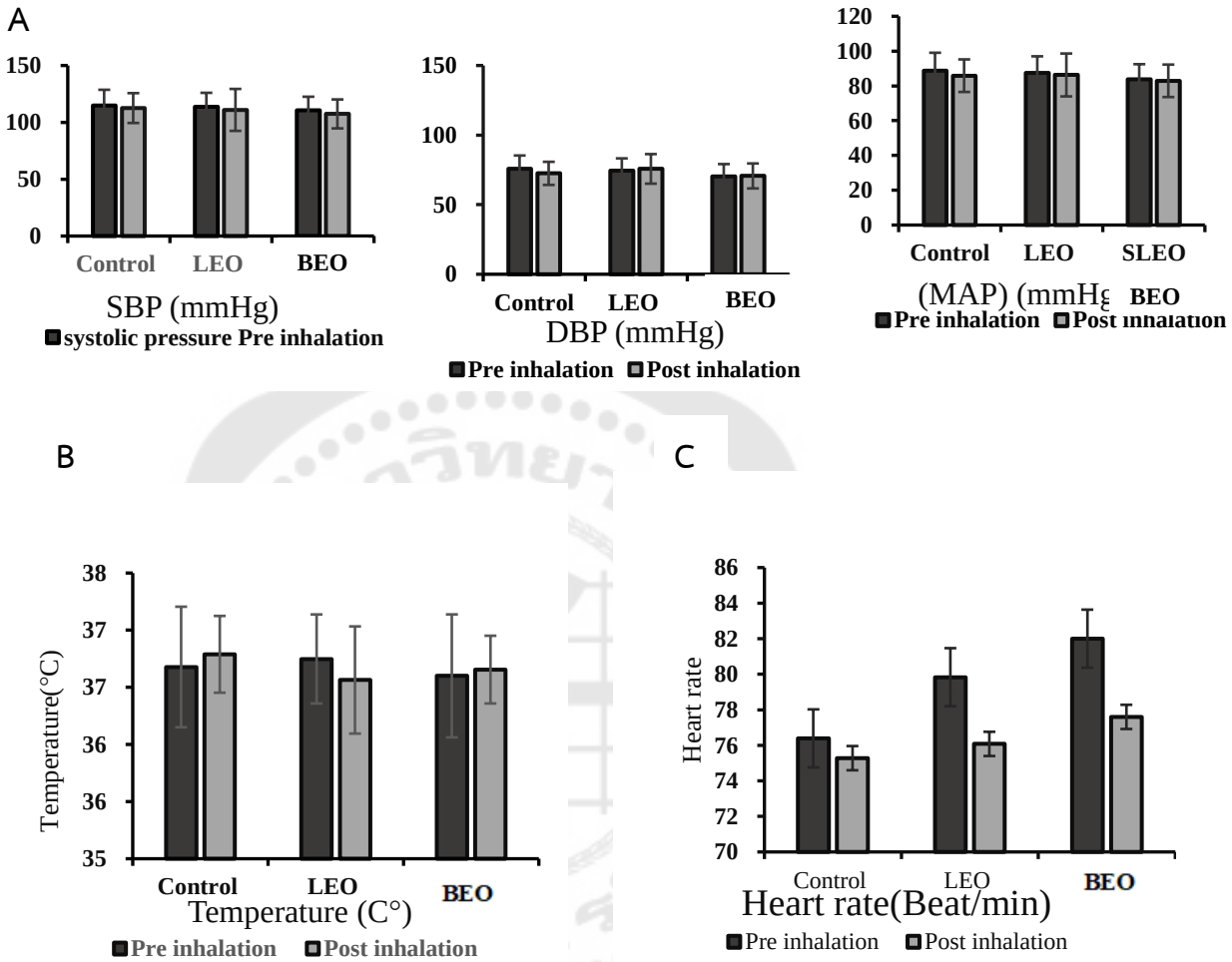


Figure 11 Short -term effect of essential oil blend inhalation on vital sign in volunteers including A: systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) B: temperature and C: heart rate compared between before and after inhalation. Data and express as mean±SEM (n=20/group).

There was no significantly different in all group of vital signs. However, Heart rate in BEO tend to be reduce after inhalation for short -term treatment.

4.5.2 Chronic vital sign

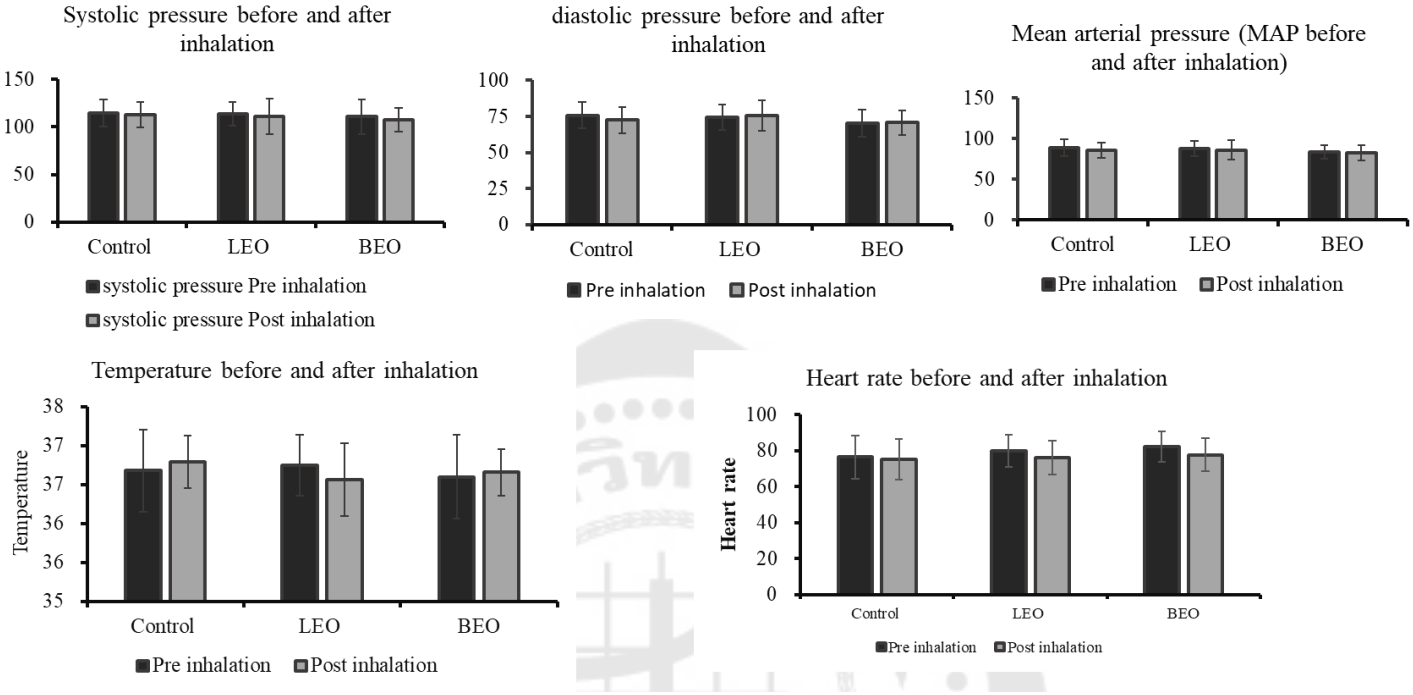


Figure 12 Long-term effect of essential oil blend inhalation on vital sign in volunteers including A: systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) B: temperature and C: heart rate compared between before and after inhalation. Data and express as mean±SEM (n=20/group).

There was no significantly different in all group of vital signs for long -term treatment.

4.6 Salivary Cortisol

4.6.1 The Acute effect of essential oil blend on saliva cortisol

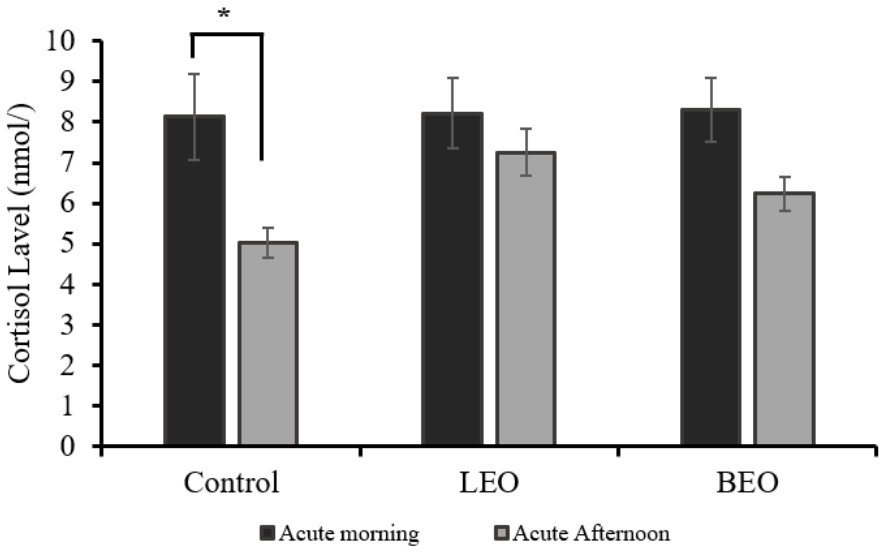


Figure 13 Acute effects of essential oil blends inhalation on salivary cortisol in volunteers. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and BEO (Blend essential oil) inhalation of essential oil blend formula B2. Data and express as mean±SEM (n=20/group) * $p<0.05$ was significant compared to pre-treatment.

There was significantly difference in control group ($p < 0.05$), before and after the short-term treatment.

4.6.2 The chronic effect of essential oil blend on saliva cortisol in the morning

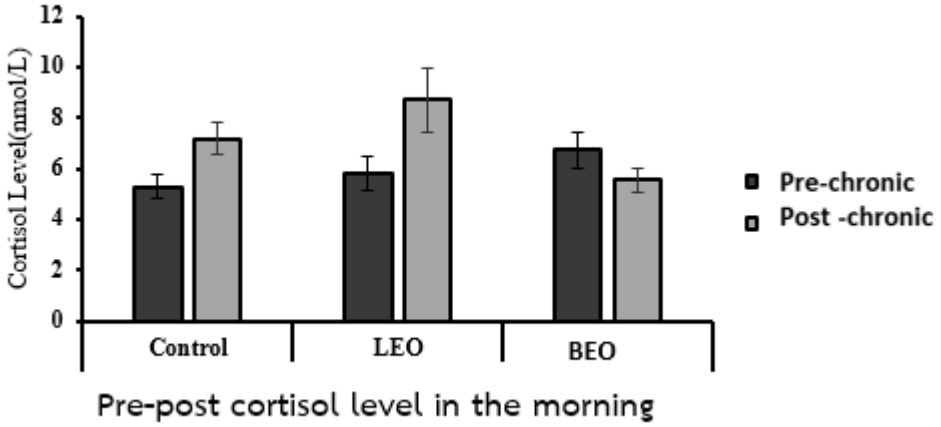


Figure 14 Chronic effects of essential oil blends inhalation on salivary cortisol in volunteers in the morning. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and BEO (Blend essential oil) inhalation of essential oil blend formula B2. Data and express as mean±SEM (n=20/group)

The salivary cortisol level before and after long-term inhalation of essential oil blend were investigated. Both group of control and LEO group tended to increase cortisol level but BEO was decrease of cortisol level but all group no significant different.

There was no significant difference between before and after inhaling in all group. In summary, long term of using essential oil blend could not effectively to reduce cortisol level in the morning on volunteers.

4.6.3 The chronic effect of essential oil blend on saliva cortisol in the afternoon

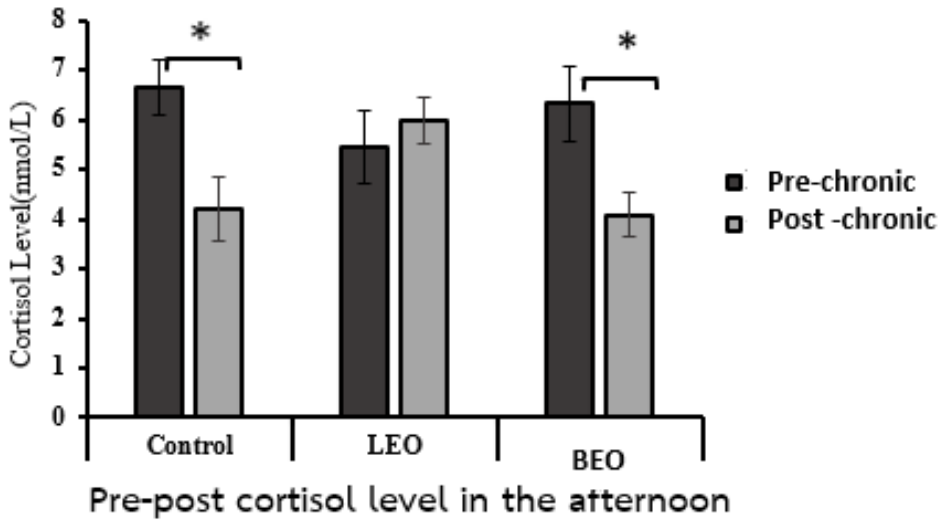


Figure 15 Chronic effects of essential oil blends inhalation on salivary cortisol in volunteers in the afternoon. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and BEO (Blend essential oil) inhalation of essential oil blend formula B2. Data and express as mean±SEM (n=20/group). * $p<0.05$ was significant compared to pre-treatment.

The salivary cortisol level before and after long-term inhalation of essential oil blend in the afternoon were investigated both group control group ($p < 0.05$), and BEO ($p < 0.05$) before and after the long-term treatment in the afternoon. However, there are no significant observed in LEO group but the significant difference was observed in LEO group. Therefore, the aromatherapy could effectively reduce afternoon cortisol level for long-term treatment in volunteers.

4.7 Salivary alpha amylase

4.7.1 The acute effect of essential oil blend on salivary alpha amylase

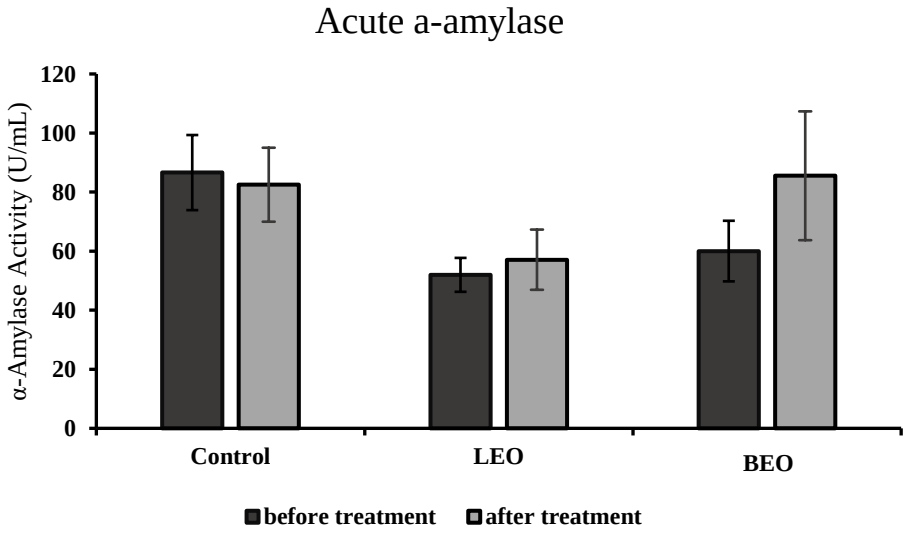


Figure 16 Acute effects of essential oil blends inhalation on salivary cortisol in volunteers. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and (Blend essential oil) inhalation of essential oil blend formula B2. Data and express as mean±SEM (n=20/group).

The three group of acute salivary alpha amylase level of the volunteers who have stress with sleep problem. The volunteers were divided into three groups, control group, positive control (LEO) and experimental group (BEO) respectively.

Following inhalation of sweet almond oil, lavender oil or essential oil blend, the alpha amylase level in all group did not change significantly.

4.7.2 The chronic effect of essential oil blend on salivary alpha amylase in the morning

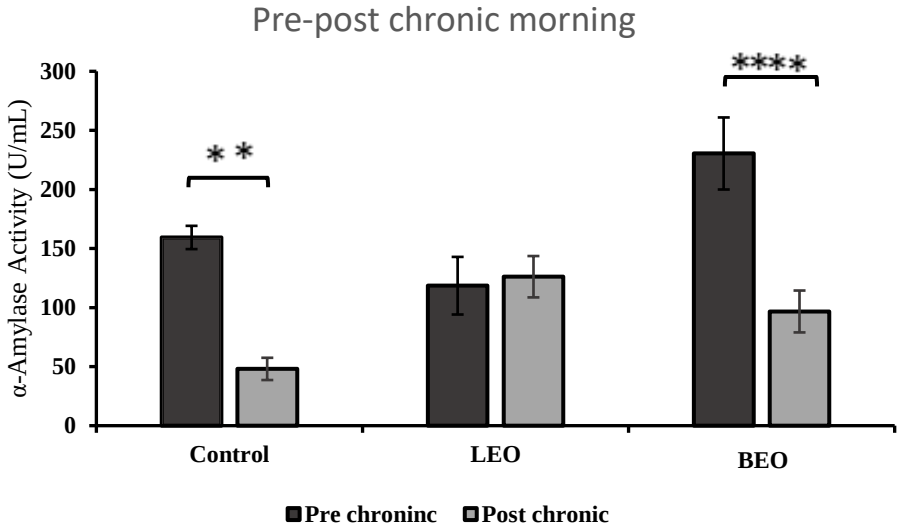


Figure 17 Chronic effects of essential oil blends inhalation on salivary cortisol in volunteers in the morning. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and (Blend essential oil) inhalation of essential oil blend formula B2. Data and express as mean±SEM (n=20/group). * $p$<0.01, * $p$<0.0001 were significant compared to pre-treatment.

The salivary alpha amylase level before and after long-term inhalation of essential oil blend in the morning were investigated both group control group ($p < 0.01$), and BEO ($p < 0.0001$) before and after the long-term treatment in the morning. However, there are no significant observed in LEO group show significant difference in LEO. Therefore, the aromatherapy could effectively reduce cortisol level after for long-term treatment in the morning.

4.7.3 The chronic effect of essential oil blend on salivary alpha amylase in the afternoon

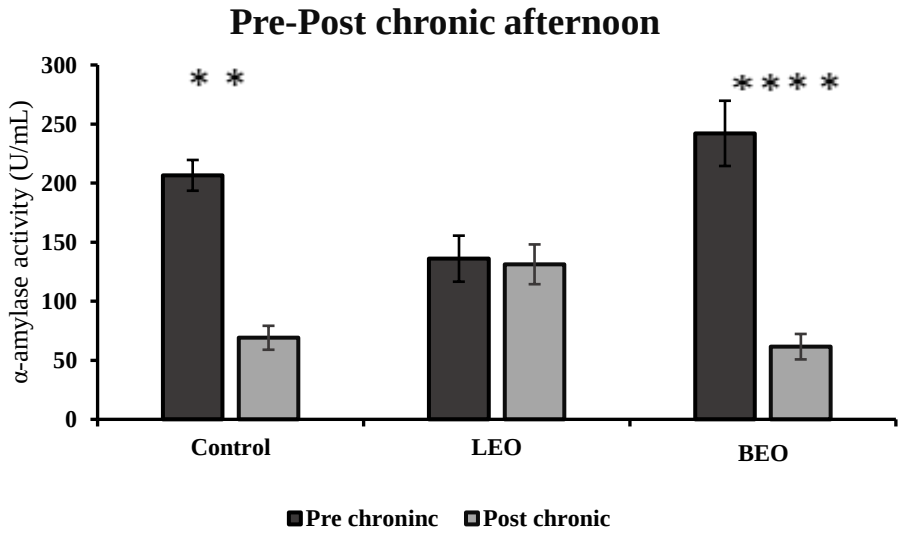


Figure 18 Chronic effects of essential oil blends inhalation on salivary cortisol in volunteers in the afternoon. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and (Blend essential oil) inhalation of essential oil blend formula B2 Error bars indicates standard deviation. Data express as mean±SEM (n=20/group). * $p$<0.01, * $p$<0.0001 were significant compared to pre-treatment.

There was a significant difference in control group ($p < 0.01$), and BEO ($p < 0.0001$) before and after the long-term treatment in the afternoon. Although, the results did not show significant difference in LEO. Therefore, the aromatherapy could effectively reduce alpha amylase level for long term in the afternoon in volunteers.

4.8 Saliva Immunoglobulin A (IgA)

4.8.1 The Chronic effect of essential oil blend on Saliva Immunoglobulin A in the morning

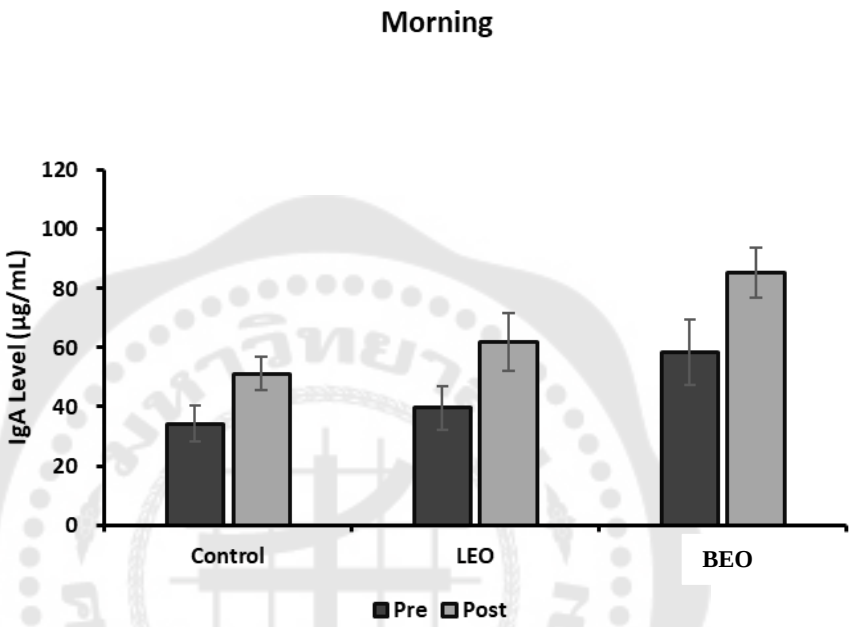


Figure 19 Chronic effects of essential oil blends inhalation on salivary Immunoglobulin A in volunteers in the morning. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and (Blend essential oil) inhalation of essential oil blend formula B2 Error bars indicates standard deviation. Data and express as mean±SEM (n=20/group)

The salivary Immunoglobulin A level before and after long term treatment inhalation of essential oil blend were investigated in all groups tends to increase but there was no significance.

**4.8.2 The Chronic effect of essential oil blend on Saliva Immunoglobulin A in the afternoon**

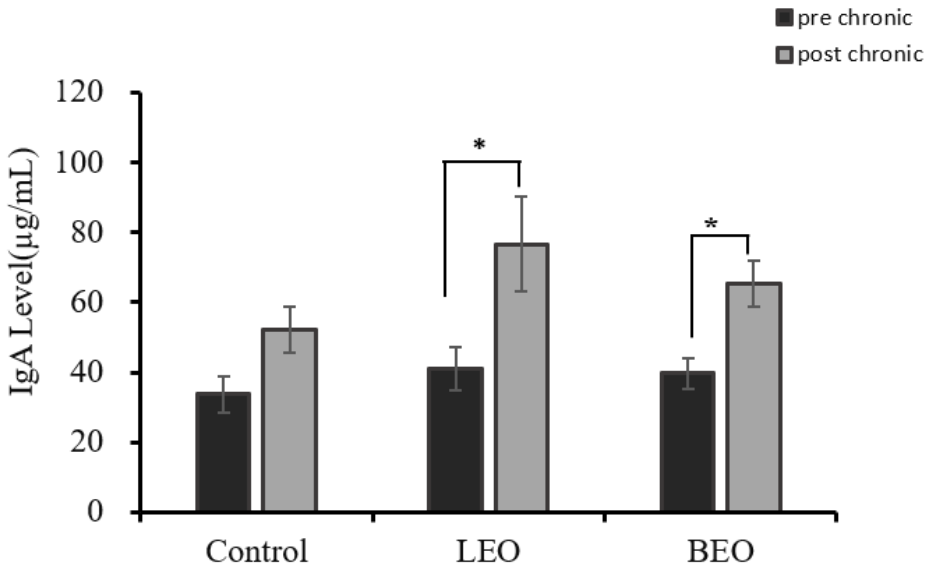


Figure 20 Chronic effects of essential oil blends inhalation on salivary Immunoglobulin A in volunteers in the afternoon. Control = inhalation of sweet almond oil, LEO= inhalation of lavender oil and (Blend essential oil) inhalation of essential oil blend formula B2 Error bars indicates standard deviation. Data and express as mean±SEM (n=20/group)

The salivary immunoglobulin A level before and after long-term inhalation of essential oil blend investigated in control group tended to increase but there was no significance.

Afternoon IgA of both LEO and BEO groups significantly increased ($p < 0.05$) when compared to before the long-term treatment. Therefore, the aromatherapy could effectively increase afternoon Immunoglobulin A following long-term treatment.

**4.9 Brain wave**

**4.9.1 Comparison of brain wave of pre- essential oil Inhalation during eye closed**

Table 5 Comparison of brain wave of pre- essential oil Inhalation during eye closed

| Type of Brainwave | Brainwave of pre- essential oil Inhalation during eye closed (μV) | | |
|---|---|---|---|
| | Control | LEO | BEO |
| Delta | 1.96 | 3.27 | 2.32 |
| Theta | 4.19 | 1.42 | 1.24 |
| Alpha | 1.57 | 7.52 | 1.19 |
| Beta | 7.91 | 1.34 | 3.24 |
| Gamma | 2.66 | 4.00 | 4.21 |

In control group, the main brain waves of pre- essential oil inhalation during eye closed were theta and beta wave. In LEO group, the main brain waves of pre-essential oil Inhalation during eye closed were delta, alpha and gamma wave. For BEO group, the main brain waves of pre- essential oil inhalation during eye closed included beta and gamma wave.

4.9.2 Comparison of brain wave of post- essential oil Inhalation during eye closed

Table 6 Comparison of brain wave of post- essential oil Inhalation during eye closed

| Type of Brainwave | Brain wave of post- essential oil Inhalation during eye closed (μV) | | |
|---|---|---|---|
| | Control | LEO | BEO |
| Delta | 1.36 | 3.57 | 1.24 |
| Theta | 4.11 | 6.95 | 8.14 |
| Alpha | 1.31 | 7.37 | 6.09 |
| Beta | 7.36 | 1.94 | 1.31 |
| Gamma | 2.21 | 5.12 | 2.43 |

In control group, the main brain wave of post- essential oil Inhalation during eye closed was beta wave. Interestingly, the main brain waves of post- essential oil inhalation during eye closed in both LEO and BEO groups were theta and alpha wave, suggesting the relaxation of aromatherapy.

**4.9.3 Comparison of brain wave of pre- essential oil Inhalation during eye opened**

Table 7 Comparison of brain wave of pre- essential oil Inhalation during eye opened

| Type of Brainwave | Brain wave of pre- essential oil Inhalation during eye opened (μV) | | |
|---|---|---|---|
| | Control | LEO | BEO |
| Delta | 1.65 | 1.89 | 2.92 |
| Theta | 4.26 | 4.52 | 1.03 |
| Alpha | 1.16 | 3.93 | 1.20 |
| Beta | 7.24 | 1.87 | 5.16 |
| Gamma | 2.46 | 3.02 | 4.78 |

In control group, the main brain waves of pre- essential oil inhalation during eye opened was beta wave. In LEO group, the main brain waves of pre- essential oil inhalation during eye opened were theta, alpha and gamma wave. For BEO group, the main brain waves of pre- essential oil inhalation during eye opened included beta and gamma waves.

**4.9.4 Comparison of brain wave of post- essential oil Inhalation during eye opened**

Table 8 Comparison of brain wave of post- essential oil Inhalation during eye opened

| Type of Brainwave | Brain wave of post- essential oil Inhalation during eye opened (μV) | | |
|---|---|---|---|
| | Control | LEO | BEO |
| Delta | 1.11 | 1.01 | 2.07 |
| Theta | 4.76 | 3.41 | 8.45 |
| Alpha | 1.22 | 1.42 | 9.01 |
| Beta | 3.63 | 8.52 | 3.37 |
| Gamma | 1.82 | 1.70 | 3.58 |

In control group, there are no notable brain waves of post- essential oil inhalation during eye opened. Surprisingly, in LEO group, the main brain waves of post-essential oil inhalation during eye opened was beta wave, suggesting the stimulation effect. Remarkably, the main brain waves of post- essential oil inhalation during eye opened in BEO group included theta and alpha waves, advocating the relaxation of essential blend.

# CHAPTER 5
# DISCUSSION

## 5.1 Satisfaction with the Essential Oil Blend by preference test

Volunteers expressed the highest satisfaction with the essential oil blend formula B, which predominantly consisted of Thai essential oils, including bergamot oil, peppermint oil, ylang-ylang oil, chamomile oil, marjoram oil, and vetiver oil. After refining the formula, it was found that volunteers preferred the B2 blend over B1. The B2 formula comprised approximately 50% bergamot oil, making it the most favored formulation.

## 5.2 Effects of Balm Containing Essential Oil Blend on Stress and Sleep Quality

The use of balm containing the B2 essential oil blend, which primarily consists of bergamot oil, demonstrated a statistically significant reduction in self-reported stress levels among participants, as assessed by the Department of Mental Health's stress evaluation scale, after of use. However, no significant changes in sleep quality were observed.

These findings suggest the potential of the B2 essential oil blend as a promising intervention for stress reduction. Given the known impact of stress on sleep quality, the continued use of the balm for up to four weeks may yield more pronounced effects on both stress and sleep. However, future studies should aim to control confounding factors, such as sources of stress, underlying causes of sleep problems, and environmental variables, to enhance the accuracy of the findings. Such efforts could contribute to further refinement of the balm formulation and the development of more precise usage guidelines.

These findings align with the study conducted by Sangkhamee et al. (2022)(36), which demonstrated that back massages using bergamot essential oil three times per week for four weeks significantly reduced stress levels, as assessed by a stress questionnaire. Additionally, the intervention lowered blood pressure, heart rate, respiratory rate, and the levels of cortisol and aldosterone. Bergamot essential oil, which contains key components such as limonene, linalyl acetate, and linalool(37), has also

been shown to possess anxiolytic properties. For example, inhalation of limonene was found to reduce anxiety in rats during the elevated plus maze test, producing anxiolytic effects comparable to diazepam. These findings suggest that prolonged use of balm containing a bergamot-based essential oil blend can effectively alleviate stress. Furthermore, it may have potential benefits in mitigating symptoms of insomnia, warranting further investigation.(38)

Implications and Future Directions

Suggest that the essential oil balm containing the B2 formula holds promise for reducing stress among individuals experiencing stress-related conditions. However, as stress has a considerable impact on sleep quality, future studies should focus on controlling stress-related factors and addressing the underlying causes of sleep problems. Additionally, external environmental factors that might influence experimental outcomes should also be controlled.

Further research is recommended to refine the essential oil balm product and optimize its usage methodology. Such efforts will contribute to the accurate and effective application of essential oil-based interventions for stress reduction and improved sleep quality.

### 5.3 Effects of Inhaling the B2 Essential Oil Blend on Vital sign

The inhalation of the B2 essential oil blend exhibited potential effects on vital signs, including reductions in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate. Although these changes indicated a favorable trend, were not statistically significant. Further investigation with a larger sample size or prolonged exposure may be warranted to clarify these effects.

The body releases many of hormones under stress condition. These hormones cause the heart to beat faster and the blood vessels to constrict. These actions increase blood pressure later. There's no proof that stress causes long-term high blood pressure. But reaction of stress can raise blood pressure(39). The inhalation of an essential oil had immediate and continuous effects on the home SBP, daytime BP, and the stress reduction. Essential oils may have relaxation effects. These actions increase blood

pressure later. There's no proof that stress causes long-term high blood pressure. But reaction of stress can raise blood pressure.(40) limonene is the main component of bergamot oil related with the previous research of inhalation of orange peel Aroma which is study on the effects of exotic oils containing limonene on blood pressure changes. The GC-MS characterization results proved that the distillate contained the compound d-limonene is able to provide a relaxing effect and has the ability to lower and raise blood pressure in respondents because the d-lemonene compound is able to inhibit neuroinflammation and nitrite levels in the hippocampus and relieve anxiety through DAergic and GABAergic nerve activity mediated by A2A receptors.(41) The inhalation of essential oils was assessed for its impact on vital signs, including blood pressure, heart rate, respiratory rate, and body temperature. The results indicated that exposure to the essential oil blend may influence these physiological parameters. The sympathetic nervous system is typically activated during stress, as evidenced by physiological responses such as increased blood pressure, elevated heart rate, rapid breathing, and higher body temperature. In this study, the inhalation of the B2 essential oil blend (primarily composed of bergamot oil) resulted in a trend toward reduced blood pressure, including systolic blood pressure, diastolic blood pressure, and mean arterial pressure, as well as a decrease in heart rate compared to pre-inhalation levels. These findings are consistent with the study by Ni et al. (11), which reported that patients who inhaled bergamot essential oil for 30 minutes before surgery experienced a reduction in anxiety, heart rate, and systolic blood pressure, although diastolic blood pressure remained unchanged. Thus, this study suggests that inhalation of a bergamot-based essential oil blend may help reduce sympathetic nervous system activity.

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**5.4 Effects of B2 Essential Oil Blend Inhalation on Salivary Alpha-Amylase**

Inhalation of the B2 essential oil blend showed no statistically significant effect on salivary alpha-amylase levels when compared to pre-inhalation measurements. Inhalation of the B2 essential oil blend or lavender oil demonstrated a trend toward reduced salivary alpha-amylase levels compared to pre-inhalation levels. However, these reductions were not statistically significant. In contrast, participants who inhaled sweet almond oil exhibited a trend toward increased salivary alpha-amylase levels. Further research is recommended to assess the potential long-term effects or variations under different conditions. The use of the essential oil blend was evaluated for its impact on salivary alpha-amylase levels, a biomarker commonly associated with sympathetic nervous system activity. The results indicated that inhalation of the essential oil blend

did not produce a statistically significant change in alpha-amylase levels when compared to baseline measurements. Further investigation is needed to explore the potential long-term effects or variations under different conditions.

Salivary alpha-amylase is a biomarker of stress, as the secretion of this enzyme reflects sympathetic nervous system activity. Cozma et al 2017(42) highlighted that both salivary alpha-amylase and cortisol levels can be utilized in diagnosing stress-related cardiovascular conditions. The present study found that volunteers who inhaled the B2 essential oil blend (primarily composed of bergamot oil) or lavender oil showed a tendency to have reduced salivary alpha-amylase levels compared to baseline measurements. This is consistent with a previous study, which reported that patients who inhaled bergamot essential oil before surgery experienced a reduction in anxiety and salivary alpha-amylase levels. Therefore, this study suggests that inhalation of a bergamot-based essential oil blend may help to reduce sympathetic activity.

In summary, this study suggests that inhalation of a bergamot-based essential oil blend may reduce stress levels, likely through the modulation of autonomic nervous system activity. Prolonged use of a balm containing this essential oil blend for four weeks may further alleviate stress and potentially provide relief for symptoms of insomnia.

**5.5 Effects of B2 Essential Oil Blend Inhalation on Salivary Cortisol**

Inhalation of the B2 essential oil blend showed no statistically significant effect on salivary cortisol levels when compared to pre-inhalation measurements. Therefore, the acute treatment of aromatherapy could not effectively reduce cortisol level in volunteers

In conclusion, the experimental group (BEO) tend to decrease the acute cortisol level more than that of positive control group (LEO). However, the control group was the most decreased cortisol level at the acute state, probably due to the preference of scent was difference, the control group were treated with water shown more positive result, compared with scented group. Therefore, the scent administration might be the effect of stress of volunteer during the inhalation.(43)

Inconclusion, it was noticeable that the experimental group (SLEO) decrease the acute cortisol level compared with positive control group (LEO). However, the control group was the most decreased cortisol level at the acute state, probably due to the preference of scent was difference. That while, the control group were treated with water shown more positive result, compared with scented group. Therefore, the scent administration might be effect of stress of volunteer during the inhalation.(43)

### 5.6 Effects of B2 Essential Oil Blend Inhalation on Salivary IgA

Chronic inhalation of either the BEO or LEO significantly increased salivary IgA levels. Similarly, the previous study showed that Inhalation of the B2 essential oil blend showed statistically significant effect on salivary salivary IgA levels in BEO and LEO group as a positive control when compared to pre-inhalation measurements. Related with the  evaluating the effect of aromatherapy on a stress marker in healthy subjects study suggested that the rate of change in the salivary s-IgA level and interval required until the salivary s-IgA level increases depend on the type of aroma oil.(44)

### Suggestion

This study suggests that inhalation of an essential oil blend, primarily composed of bergamot oil, has a potential to reduce stress levels, likely due to its effect on reducing the activity of autonomic nervous system. However, the number of participants in this study was relatively small. Increasing the sample size in future research could yield more reliable and generalizable findings. Additionally, the prolonged use of the bergamot-based essential oil blend for four weeks may not only further reduce stress but also improve the effectiveness of insomnia treatment.

Future studies should aim to control stress-related factors, underlying causes of sleep disturbances, and other environmental variables that may impact the results. Such efforts will help refine the development of essential oil-based products and optimize their application methods, ensuring more accurate and effective use.

The study suggests that the use of essential oil products for stress reduction shows promise for further development, particularly for individuals with sleep difficulties, as stress is known to impact sleep quality.

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