

## การศึกษาแบบสลับไขว้กลุ่มของการดมน้ำมันผักแขยงต่อระบบประสาทอัตโนมัติ และเวลาปฏิกิริยาในอาสาสมัครสุขภาพดี

### A Randomized Crossover Study on the Effects of Inhaled Rice Paddy Herb Oil (*Limnophila aromatica* (Lam.) Merr.) on Autonomic Nervous System and Reaction Time on Healthy Volunteers

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#### บทคัดย่อ

ผักแขยงเป็นผักที่มีกลิ่นที่ใช้ในอาหารไทยและระบบการแพทย์พื้นบ้านในแถบเอเชีย ศึกษาผลของการดมน้ำมันระเหยจากส่วนเหนือดินของผักแขยงที่มีต่อระบบประสาทอัตโนมัติและเวลาปฏิกิริยาในอาสาสมัครสุขภาพดี จำนวน 24 คน อายุ 18 ถึง 25 ปี โดยการวิจัยเชิงทดลองแบบสลับไขว้กลุ่ม ให้อาสาสมัครดมน้ำมันอัลมอนด์และน้ำมันผักแขยง (ความเข้มข้นร้อยละ 8 โดยปริมาตร ในน้ำมันอัลมอนด์) บันทึกระบบประสาทอัตโนมัติด้วยเครื่องวัดสัญญาณชีพรุ่น BIOM7000 ศึกษาเวลาปฏิกิริยาด้วยโปรแกรม Deary-Liewald บนจอคอมพิวเตอร์ เปรียบเทียบข้อมูลด้วยสถิติ paired t-test ที่ระดับนัยสำคัญทางสถิติ .05 วิเคราะห์เวลาปฏิกิริยาแบบง่ายด้วยค่าเฉลี่ยเวลาการตอบ และแบบตัวเลือกว่าด้วยจำนวนข้อถูกและค่าเฉลี่ยเวลาการตอบข้อถูก ผลการศึกษาพบว่า น้ำมันผักแขยงสามารถลดความดันโลหิตช่วงบน ความดันโลหิตช่วงล่าง และอัตราการเต้นของหัวใจ อย่างมีนัยสำคัญทางสถิติ ผลการศึกษาเวลาปฏิกิริยาพบว่า การดมน้ำมันผักแขยงสามารถลดเวลาปฏิกิริยาแบบง่ายได้อย่างมีนัยสำคัญทางสถิติ โดยสูรบน้ำมันผักแขยงมีผลต่อความดันโลหิตช่วงบนและช่วงล่าง และอัตราการเต้นหัวใจ และมีศักยภาพในการเพิ่มความสามารถในการตอบสนองต่อเวลาปฏิกิริยาในอาสาสมัครสุขภาพดี

**คำสำคัญ:** ผักแขยง ระบบประสาทอัตโนมัติ เวลาปฏิกิริยา

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วันที่รับ (received) 2 ก.พ. 2563 วันที่แก้ไขเสร็จ (revised) 16 เม.ย. 2563 วันที่ตอบรับ (accepted) 2 พ.ค. 2563

## Abstract

Rice paddy herb, a characteristic odor vegetable is commonly used in Thai dishes and Asian indigenous system of medicine. This study aimed to evaluate the effects of rice paddy herb aerial part essential oil inhalation on autonomic nervous system and reaction time tasks among healthy volunteers using a randomized crossover design. Twenty-four healthy participants aged between 18 and 25 years were received sweet almond oil and rice paddy herb oil (8% v/v in sweet almond oil) by inhalation. ANS activities were recorded using BIOM7000 Patient Monitor. The simple and choice reaction tasks were performed on computer screen by the Deary-Liewald reaction time program. Data were analyzed using paired t-test with a significance level of .05. Simple reaction times to responses as well as choice reaction correct items and times were recorded. The results showed that the rice paddy herb oil statistically significantly decreased the systolic blood pressure, diastolic blood pressure and heart rate. For reaction time tasks, the rice paddy herb oil statistically significantly decreased the responded times in simple reaction time test. In summary, the rice paddy herb oil affected systolic and diastolic blood pressure as well as heart rate and it showed potential on the reaction response improvement among healthy participants.

**Keywords:** *Limnophila aromatica*, autonomic nervous system, reaction time

## Introduction

The odors of essential oils are therapeutically effective on humans due to the sense of smell or olfactory system. The aroma acts via the limbic system, particularly the amygdala and hippocampus and in turn may cause both physiological and psychological effect<sup>1</sup>. Measurement of the odor effects on physiology can be divided into two forms of arousal, the autonomic arousal (blood pressure, heart rate, respiratory rate and skin temperature)<sup>2</sup> and the cortical arousal (reaction time tasks)<sup>3</sup>. Citronella oil significantly decreased blood pressure and heart rate and respiratory rate<sup>4</sup> whereas Cinnamomum porrectum oil<sup>5</sup> and jasmine oil<sup>6</sup> caused a considerably significant increase in heart rate and respiratory rate as well as systolic and diastolic blood pressure. Reaction time task, a method basically used to measure psychological effect, 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<sup>7</sup>.

Rice paddy herb (*Limnophila aromatica* (Lam.) Merr., Scrophulariaceae family) is commonly used in Thai dishes due to its characteristic odor<sup>8</sup>. It is an annual or perennial native to Southeast Asia. Rice paddy herb is used in Asian 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<sup>9</sup>. The effects of rice paddy herb essential oil on brain activities have never been studied. The effects of rice paddy herb essential oil on brain activities have never been studied. This study was to evaluate the effects of rice paddy herb essential oil inhalation on autonomic nervous system and reaction time tasks. The sweet almond oil was used as essential oil diluent. It is a natural fixed oil from the seed of *Prunus dulcis* (Mill.) D.A. Webb that has been widely used as a non-olfactory stimulating carrier oil<sup>10</sup>. An experimental research was performed within participants using randomized crossover design.

## Research Objective

The research objective was to evaluate the effects of rice paddy herb essential oil inhalation on autonomic nervous system (blood pressure, heart rate, respiratory rate, skin temperature) and reaction time tasks among healthy volunteers.

## Research hypothesis

Research hypothesis was that rice paddy herb essential oil could affect autonomic nervous system (blood pressure, heart rate, respiratory rate, skin temperature) and reaction time tasks among healthy volunteers.

## Methodology

### Participants

A total number of 24 healthy volunteers were recruited from Kanchanabhisek Institute of Medical and Public Health Technology. The sample size in this study was calculated from the previous study<sup>6</sup>. This study used technical sample size calculation by computer G\*Power 3.1. Type II error was at 0.05. Power of test was at 80% and effect size was at 1.2. 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. Regarding a randomized crossover design, each participant was randomly assigned to receive firstly rice paddy herb oil or sweet almond oil by choosing the drawing lots.

### Inclusion and exclusion criteria

The male and female healthy participants aged between 18 and 25 years old who had a normal range of body mass index (BMI) i.e. between 18.5 and 22.9 kg/m<sup>2</sup> based on WHO and Asian criteria values<sup>11</sup> were recruited. Inclusion criteria were right-handed participants evaluated by the Edinburgh Handedness Inventory scale<sup>12</sup> and a normal sense of smell screened

using the n-butyl alcohol test<sup>13</sup>. The personal health status of all the participants which included weight, height and blood pressure was also recorded. All the participants were nonsmokers, without any symptoms of upper respiratory infection, hypertension or cardiovascular disease. They should not have current or past history related to neurological illness, epilepsy and loss of consciousness longer than 30 minutes. They should take neither central nervous system medication nor sedative drugs. Each participant was asked to inhale the rice paddy herb oil diluted with the sweet almond oil to fill out the pleasantness form "Odor familiarity five-point Likert scale". Only the potential participants who indicated the oil pleasantness within the range of 2-4 were included as the research participants. The participants who were allergic to essential oils or had a headache were excluded from the experiment.

### Study design

A within-participant design by assigning each participant to receive both rice paddy herb oil and sweet almond oil in a random order was used. A randomized two-group crossover design (rice paddy herb oil at first and sweet almond oil at first) was performed. The washout period was 7 days. The experimental study was conducted at Kanchanabhisek Institute of Medical and Public Health Technology. It was performed during July 2018 to April 2019.

### Ethical considerations

The research proposal was approved by the Ethical Review Committee from the Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University for research which involved human research participants (COA No. 154/2018). The informed consent was obtained from each participant after the full explanation of the study.

### Rice paddy herb oil and sweet almond oil administration

Rice paddy herb oil and sweet almond oil were used in this study. *Limnophila aromatic.* was collected from The Northeast of Thailand and was authenticated by Associate Professor Dr. Nijisiri Ruangrunsi. The voucher specimen was deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. The essential oil was obtained from the fresh aerial parts of *L. aromatica* by hydrodistillation. Sweet almond oil purchased from Thai China Flavors and Fragrances industry (Thailand) was used as the essential oil diluent. The concentration of rice paddy herb oil was 8 % v/v in sweet almond oil.

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).

### Instruments

BIOLIGHT M7000 Multi-Parameter Patient Monitor – BIOM7000 was employed to monitor and record ANS parameters of the participants simultaneously in real time. CPU and monitor for the Deary-Liewald reaction time program (2011)<sup>7</sup> classified into simple reaction time and choice reaction time were used to monitor and record reaction time tasks completed by the participants.

### Outcome parameters

There were four ANS parameters including blood pressure, heart rate, respiratory rate and skin temperature. This study employed the Deary-Liewald reaction time program (2011) classified into simple reaction time and choice reaction time.

### Procedures

The participants were asked not to use hair spray, antiperspirants and any perfumes, 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. The procedure instructions were explained to the participants in Thai. The experiment was divided into 2 sessions: the first one for ANS parameters in which each participant had 2 appointment dates (the essential oil or the sweet almond oil) and the other one for reaction time task in which each participant had 2 appointment dates (the essential oil or the sweet almond oil) with 7-day washout period interval.

### So, each participant came for 4 appointment dates.

Each participant was asked to sit in a quiet room with the temperature at  $24 \pm 1^\circ\text{C}$ , relative humidity at 50-65% in a comfortable chair. Each participant was asked to inhale only one oil (either sweet almond oil or rice paddy herb oil diluted in sweet almond oil)/appointment/day. Each appointment date was separated by 7-day washout period. For ANS parameters, the first and second appointment dates consisted of 2 processes. In these processes, when participant felt comfortable for 10 minutes as rest, ANS activity was recorded for the first time. The intervention (the essential oil or the sweet almond oil) was applied while ANS activity was recorded for 10 minutes for the second time. For the reaction time, each participant was asked to sit in the same condition. The intervention (the essential oil or the sweet almond oil) was applied for 8 minutes. Then, each participant was instructed to click button on mouse whenever target picture appeared randomly on computer screen by responding to a single stimulus rapidly for single reaction time task and choosing the correct box rapidly from 4 boxes of stimulus for choice reaction time task.

## Statistical analysis

SPSS version 22 was used for data analysis. Shapiro-Wilk test was performed to verify normal distribution of the data. The comparison between and within sweet almond oil and rice paddy herb oil was performed by paired t-test (parametric). A value of  $p < .05$  was considered statistically significant. Values were presented as mean and standard deviation.

## Results

### Demographic Data

The participants in this study were 5 males and 19 females aged  $19.67 \pm 0.76$  years and BMI were  $20.03 \pm 2.32 \text{ kg/m}^2$ .

### Autonomic nervous system parameters

The effects on ANS were displayed in Table 1. Each participant inhaled the sweet almond oil (SO) and the rice paddy herb essential oil diluted in sweet almond oil (RO). 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 ( $p < .05$ ). For RO inhalation, systolic blood pressure, diastolic blood pressure and heart rate statistically significantly decreased ( $p < .05$ ). Skin temperature statistically significantly increased ( $p < .05$ ). The comparison of ANS changes between RO and SO inhalation showed that the rice paddy herb essential oil statistically significantly decreased the systolic blood pressure, diastolic blood pressure and heart rate but had no effects on respiratory rate and skin temperature.

### Reaction Time

The effects on reaction time were demonstrated in Table 2. 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 ( $p < .05$ ). For the choice reaction time, the rice paddy herb essential oil inhalation showed no effects on correct answers as well as the response times.

## Discussion

The rice paddy herb essential oil (8% v/v in sweet almond oil) was administered to the healthy participants through inhalation. The sweet almond oil used as diluent was also inhaled for comparison. Twenty-four healthy volunteers (aged  $19.67 \pm 0.76$  years) participated in this study. Allocation to inhale SO first or RO first was randomized and the washout period was 7 days.

The effects of rice paddy herb essential oil on ANS function of the brain i.e. blood pressure, heart rate, skin temperature and respiratory rate were revealed. The systolic, diastolic blood pressure and heart rate after the rice paddy herb oil inhalation statistically significantly decreased. The essential oil consists of various chemical compounds which can distinctively interact with the brain receptors<sup>14</sup>. 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<sup>15</sup>. 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. Previous study on the effects of essential oil inhalation on the ANS parameters has reported

**Table 1** ANS parameter changes between sweet almond oil and rice paddy herb oil inhalation

Parameter	Sweet almond oil (SO)			Rice paddy herb oil (RO)			P-value between SO and RO change
	n = 24 Mean (SD)			n = 24 Mean (SD)			
	Rest	Intervention	P-value	Rest	Intervention	P-value	
Systolic blood pressure (mmHg)	103.0 1 (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 (°C)	31.73 (1.30)	32.28 (1.19)	.006*	31.57 (1.09)	32.73 (2.03)	.003*	.133

\*Significant difference, p-value < .05

**Table 2** The simple reaction time and choice reaction time between sweet almond oil and rice paddy herb 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 (item)	38.75 (1.51)	39.04 (1)	.338
Correct mean (s)	469.08 (49.13)	459.00 (56.37)	.562

\* Significant difference, p-value < .05, s = seconds



similar results. A previous study conducted on the harmonizing effect of Ylang-Ylang oil inhalation on humans reported that Ylang-Ylang oil inhalation could decrease heart rate, diastolic blood pressure and systolic blood pressure significantly<sup>16</sup>. However, the respiratory rate was not affected by both sweet almond oil and rice paddy herb essential oil. In addition, the increase in skin temperature was influenced by sweet almond oil inhalation but not by rice paddy herb essential oil inhalation. So, it should be concerned for the use of sweet almond oil as diluent of the essential oil for skin temperature outcome.

The effects of rice paddy herb essential oil on reaction time tasks were demonstrated. The participants were asked to respond and give correct answers rapidly in both simple and choice reaction time tasks 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<sup>7</sup>. The rice paddy herb 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. A recent previous study conducted on the effects of peppermint oil inhalation on ANS and spatial abilities employed reaction time tasks reported that the peppermint oil inhalation could significantly decrease the test times of the tasks. One of the inclusion criteria in this study was the participant whose pleasantness scale of the rice paddy herb essential oil (8% v/v in sweet almond oil) ranged 2-4 via "Odor familiarity five-point Likert scale". Chamine and Oken<sup>17</sup> evaluated the effects of lavender aroma, which is commonly used for stress reduction and concluded that aroma hedonics (pleasantness and intensity) played a role in the beneficial effect on working memory and physiologic function. Accordingly, the effective property of the rice

paddy herb essential oil was confined to this odor's familiarity.

## Conclusion

The rice paddy herb oil could induce beneficial effects on the ANS parameters by decreasing diastolic blood pressure, systolic blood pressure and heart rate as well as less reaction time spent on completing tasks. The findings of the rice paddy herb oil inhalation provide new insights and implications to aromatherapy.

## Applications for this study

This crossover study design can be applied for any essential oils that are diluted in any carrier oils. ANS measurement and reaction time tasks can provide useful information for the effects of the essential oils or the carrier oils on psychophysiological brain functions. However, rice paddy herb oil should be used with cautions for those who have certain health problems such as low blood pressure and low pulse rate. From this study, rice paddy herb oil could be used as aromatherapy for alternative medicine<sup>18</sup> because of its positive effects among healthy volunteers.

## Acknowledgements

The researchers would like to express gratitude to Graduate School, Chulalongkorn University for the scholarship from the 90<sup>th</sup> Anniversary of Chulalongkorn University (Ratchadaphiseksomphot Endowment Fund); the Faculty of Medicine, Mahasarakham University.

The researchers would like to thank Kanchanabhishek Institute of Medical and Public Health Technology for assistance and technical support and College of Public Health Science, Chulalongkorn University for the research equipment.

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