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THE HARMONIZING EFFECTS OF CITRONELLA OIL ON MOOD STATES AND BRAIN ACTIVITIES

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ABSTRACT: Citronella oil is a famous odor and widely used in mosquito repellent application. However, the study of the effects of citronella oil on nervous system is rather limited. In this study we investigated the effects of inhaled citronella oil on emotional states and physiological parameters of the nervous system. Twenty healthy volunteers participated in this experiment. All subjects underwent autonomic nervous system recordings. These recordings included:, body temperature, heart rate, respiratory rate, and blood pressure; mood states were also evaluated as was electroencephalography (EEG) recording in pre-, during, and post-citronella inhalation The results were compared with control conditions. These assessments were measured before and after using a paired *t*- test statistical procedure. Our results indicated that citronella oil might be characterized onto the concept of "harmonization". Citronella significantly decreased blood pressure, heart rate, and respiratory rate after inhalation. Subjectively, participants reported feeling in a better mood and fresher. Moreover, the power of alpha and beta brain activities was increased. These results were then confirmed the stimulating effects harmonized together with relaxing effect of citronella oil.

Keywords: *Cymbopogon nardus* Rendle, EEG, alpha power, mood state, autonomic nervous system

INTRODUCTION

Aromatherapy originated in Europe approximately 5,000 years ago. The practice entailed the use of essential oils derived from plant extracts to promote physiological and psychological healing [1]. Traditionally, the essential oils are massaged into the body, added to bath water, or diffused into the air. This application is based on the belief that certain odors promote therapeutic effects ranging from healing minor skin irritations to altering mood states [1, 2]**.** In Thailand, data available from the Office of Agricultural Economics report a growing interest in supporting and exporting herbal plants. In particular kalmegh (Fah-Talay-Jorn) which is processed into capsule form for the alleviation of sore throats, aloe vera or medicinal aloe for wound and burn healing, plai for its relaxing therapeutic properties, as well as citronella oil as an insect repellent [3]. Therefore, nowadays there are more citronella plantations and an increased production of its oil commercially. This is illustrated by the fact that the Thai market for citronella oil production increased significantly from 51.0% in 2007 to 56.8% in 2008 [3].

Citronellal is cultivated in the tropical areas of Asia, America and South Africa. The citronella grass *Cymbopogon nardus* Rendle, a Poaceae is regarded as a medicinal plant, but is also widely used as culinary additive and for perfumery [1]. The benefits of citronella oil are its antiseptic properties. The oil is also used as a diuretic, antipyretic, to induce gastric relaxation in the treatment of irritable bowel syndrome [4, 5]. The use of citronellal essential oil has increased over fifty years for its use as an insect repellent, in particular as a repellent against mosquitoes, biting flies and fleas. It is found in many familiar insect repellent products, such as candles, lotions, gels, sprays, and towelette wipes [6]. Citronella oil has been registered for its repellent use in the United States since 1948. The United States Environmental Protection Agency considers oil of citronella as a biopesticide with a non-toxic mode of action [7]. Most research on citronella oil has focused on its effectiveness as a mosquito repellent. For example, Ansari found that after applying citronella lotion for 30 minutes the number mosquito bites were reduced by 75% [8] whereas Jaruwichiratana and colleagues also reported that a 14% citronella cream was effective against the Culex mosquito under field conditions for up to one hour which could prevent up to 90% of mosquito attacks [9]. From the above research findings and a meta-analysis by Kongkaew and her colleagues [10] reviewed the effectiveness of citronella preparations in preventing mosquito bites

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in laboratory experimental studies. This review indicated that volunteer olfactory times of different oil-containing insect repellent products varied. The sprayed form of the product was spent on olfactory time lasting at least 20 minutes whereas the cream products lasted 60 minutes. In addition, mean protection time of these products was around 82.28 minutes. The review also suggested that after inhaling the aroma, there were effects influencing on the nervous system and mood states. In the nervous system, arousal effects can be broadly divided into two major forms including cortical arousal effect which demonstrated by alteration of brain wave activity and autonomic arousal effect [11, 12]. For example, massaging rosemary in healthy volunteers could increase blood pressure and breathing rate resulting in more attentiveness, alertness and a cheerful mood state [13], whereas there was a significant decrease in the power of alpha wave over the bilateral mid-frontal regions [14]. These findings suggest that rosemary was capable of modulating the brain functions. By contrast, an inhalation of lavender oil caused a reduction of blood pressure, heart rate and respiratory rate [15]. These effects were consistent with Motomura [16] who demonstrated that lavender can change brain activity with an increase of theta 1 (3.5-5.5 Hz) and a decrease of beta 1 (13.5-20 Hz) waves.

Accordingly, in the light of these findings it was felt that citronella oil was worthy of investigation in the light of the physiological and mood state changes after the inhalation or other aromatic oils.

In a previous study, the effects of citronella essential oil on autonomic nervous system activities and emotional activities were rather limited. The sedative effect of citronella was confirmed in experimental animals by Jager [17]. His research found that under standardized experimental conditions the motility of female mice was reduced from 100% for untreated animals to 50.18% by citronella. In addition, a citronella spray collar significantly reduced barking in a sample of thirty dogs [18]. In humans, Saeki and Shiohara demonstrated vital sign changes after inhaling citronella. The R - R interval on the electrocardiogram was increased, a reduction of blood pressure and simultaneously with calm and relaxed emotions [19]. In our present study, we measured ANS parameters including heart rate, blood pressure, breathing rate and skin temperature, as well as CNS detection, first time examining human brain wave by EEG spectral power analysis during citronella oil inhalation. The purpose of this study is to determine the effects of citronella oil in three dimensions encompassing: the central nervous system (brain wave), the autonomic nervous system and the subjective reports of mood states.

METHODS

Subjects

A total of 20 healthy subjects aged between 18 to 29 years (mean 21.40 ±2.76 years) with a body mass index of 18-25 kg/m^2 (mean BMI 20.68 ± 1.89) [20] were enrolled in this study. As a number of studies have indicated that there are different brain activities in the left-handed and right-handed subjects during olfactory tasks. Accordingly, only right handed participants were tested. Handedness was tested using Edinburgh Handedness Inventory scale [21]. The subjects were then screened for a normal sense of smell by the n-butyl alcohol test [22]. Personal health status was also recorded; including weight, height and blood pressure. Subjects with symptoms of upper respiratory tract infection, neurological or psychiatric disorders, hypertension, cardiovascular diseases or a history of smoking were excluded from this study [23]. In addition, female who were menstruating on the day of testing were also excluded [24]. In addition to the exclusion criteria outlined above all participants were given routine information for subjects preparing for a EEG recording, such as they were not allowed to apply any sprays, antiperspirants or perfumes to their hair twelve hours prior to testing. All subjects were advised not to be fatigued or drowsy at the start of the experiment.

The present study was approved by the Ethical Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University. The study was approved and given the Permissions no. COA NO.009/2011. Informed consents explaining all aspects of the study were given to participants and were handed out for the subjects to read and sign. The subjects were told that they had the rights to withdraw at any time.

Essential oil administration

The citronella oil was obtained from the Thai China Flavors and Fragrances Company. The composition was analyzed by gas chromatography/mass spectrometry (GC/MS) equipped with Finnigan DSQ MS detector, Thermo Finnigan model Trace GC Ultra. Identification of the oil's constituents was achieved by matching their mass spectra and retention times, indicated in NIST05 MS library; the percentage compositions also were computed from GC peak area. The result revealed that citronella oil consisted of three main kinds on α–citronellal 33.22%, geraniol 21.12% and citronellol 13.07%. One milliliter of sweet almond oil, the base oil, or 10% v/v citronella oil, diluted in base oil, was delivered using an oxygen pump system through plastic tube via respiratory masks in inhalation set for adults that permits selective airflow (2 liter/min). According to previous studies,

it has been found that the pleasantness of the oil smell could induce an autonomic variability [25, 26]. Therefore, the subjects were asked to inhale base oil and citronella oil and then rated the level of pleasantness on a 5-point Likert scale before starting the experiment. The subjects who rated the pleasantness of the oil within 2-4 point were allowed to proceed in the experiment.

Autonomic Nervous System (ANS) and mood measurement

Simultaneously, mood state and ANS parameters (e.g. blood pressure, heart rate, skin temperature and respiratory rate) were recording using the life scope 8 bedside monitor (Nihon Kohden, Japan) for ANS parameters, while mood state was rated by the Geneva Emotion and Odor Scale (GEOS) [27]. This particular scale is consisted of a 100 millimeters, monopolar visual analog scale following by 5 factors: pleasant (good), unpleasant (bad, uncomfortable, disgusted, frustrated and/or stressful), sensual (romantic), relaxed (serene, drowsy), and refreshing (energetic).

Procedure

To reduce circadian variation, all experiments were conducted in the morning (8-12 am) and done in the silent room with an ambient temperature of 24±1 ºC and 40-50% humidity. Prior to the experiment, the subjects would be inquired whether they had any olfactory problems – none did. After they sat comfortably in the adjustable armchair, the ANS electrodes were then attached to suitable positions. The researcher monitored ANS parameters (e.g., heart rate, skin temperature and respiratory rate) every 1 minute; systolic and diastolic blood pressure every 5 minutes. Three sections of this examination, including the first part was served as a base line trial (resting period), taking approximately 10 minutes. Later the second and third trials, they took 20 minutes each. Sweet almond oil was administered in the second trial, whereas 10% v/v citronella oil diluted in sweet almond oil was applied to the third trial. The researcher required the subject to subjectively rate their mood state on a scale at the end of the first trial, the procedure was also repeated in the second and third trials.

EEG recording

A set of 31 electrodes with 1 additional ground electrode were placed onto the subject's head according to the international 10-20 system at FP1, FP2,FZ, F3, F4, F7, F8, FT7, FC3, FCZ, FC4, FT8, T3, T4, T5, T6, TP7, TP8, C3, CP3, C4, CZ, CPZ, CP4, P3, P4, PZ, O1, O2 and OZ. Two mastoids were used as a recording reference (average of both mastoids, Al + A2/2). The electro-oculogram (EOG) was measured by placing 4 electrodes in two external acanthi (HEOL and HEOR), left supraorbital (VEOU) and infraorbital (VEOL) regions. Electro-Cap is made of an elastic spandex-type fabric with recessed silver/silver chloride (Ag/AgCl) electrodes attached to the fabric. Electrode impedances were adjusted to below 5 kOhms. Acquire Neuroscan version 4.3 (Neurosoft, INC) used as recording system. An online filter was set to band pass; with low frequency of 70 Hz and high frequency of DC. A/D rate was 500 Hz and the gain was set at 19. Notch filter was open at 50 Hz [28, 29]. The relative power spectrum of the respective frequency bands derived by Fast Fourier Transformation (FFT) was expressed as follows: Delta (0-3.99 Hz), Theta (4-7.99 Hz), Alpha1 or low frequency alpha (8-10.99 Hz), Alpha2 or high frequency alpha (11-12.99 Hz) and Beta (13-29.99 Hz) wave ranges. The studied areas were divided into the left anterior (Fp1, F3, F7), right anterior (Fp2, F4, F8), right posterior (P4, T6, O2), left posterior (P3, T5, O1), and middle (Fcz, Cz, Cpz) [30].

EEG experimental conditions were identical to those of ANS experiment. The procedure was divided into 4 sessions of 7-min each. Baseline EEG recording was done in both eyes-close and eyes-open sessions. The participants were then exposed to undiluted sweet almond oil and lastly 10% v/v citronella oil diluted in sweet almond oil.

DATA AND STATISTIC ANALYSIS

The SPSS statistical package 16 was used for data analysis on the effects of citronella on physiological and mood states in two steps (before and after) treatments. A paired *t*-test was carried out on the data concerning blood pressure, heart rate, skin temperature, and respiratory rate as well as power of brain wave and rating of mood state.

RESULS

Autonomic Nervous System parameters

The mean and standard derivation (SD) values of the ANS parameters in the experiment are shown in Table 1. The data on various ANS parameters were compared during resting, sweet almond oil and citronella oil inhalation Our results showed significantly decreased heart rate (p-value <0.05) during the sweet almond oil treatment compared with those of resting. When subjects inhaled citronella, the blood pressure, heart rate and respiratory rate were significantly decreased compared with sweet almond oil inhalation. The skin temperature, on the contrary, was not significantly changed.

Mood state response

The mean and SD values of mood state response are shown in Table 2. After a citronella inhalation, subjects felt that they had significant increases in pleasant emotions; good, fresh, relaxed and calm feelings (p-value <0.05). No significant change was observed in the case of other mood states (p-value > 0.05, data not shown).

Table 1 Mean and SD values of ANS parameter change during resting, sweet almond oil and citronella inhalation.

Parameters (n=20)	Rest Mean ± SD	Sweet almond oil Mean ± SD	Citronella Mean ± SD	p-value rest and SO	p-value SO and CI
Systolic Blood Pressure	105.42 ± 7.61	105.07 ± 7.89	103.36 ± 7.60	0.764	0.001*
Diastolic Blood Pressure	64.17 ± 8.88	63.62 ± 7.86	62.48 ± 7.70	0.757	0.021*
Heart Rate	70.84 ± 13.74	69.04 ± 11.98	66.61 ± 12.01	0.006*	0.000*
Skin Temperature	31.19 ± 2.37	31.58 ± 2.17	31.47 ± 2.40	0.118	0.312
Respiratory rate	16.29 ± 2.85	15.58 ± 3.26	14.76 ± 3.07	0.067	0.003*

* Significant difference, p-value < 0.05
SO = Sweet almond oil, CI = Citronella oil

Table 2 Mean and SD values of emotional state changes during resting, sweet almond oil and citronella oil inhalation.

Emotion	Rest		SO		CI		p-value	p-value
(n=20)	Mean	SD	Mean	SD	Mean	SD	rest and SO	SO and CI
Good	54.75	16.66	57.40	17.92	68.90	20.97	0.516	0.004*
Fresh	48.30	22.46	47.35	17.10	54.25	14.67	0.850	0.040*
Relaxed	52.20	23.80	50.85	22.38	71.15	16.24	0.804	0.002*
Calm	46.45	26.97	52.85	24.95	65.85	17.50	0.229	0.048*

* Significant difference, p-value < 0.05
SO = Sweet almond oil, CI = Citronella oil

Table 3 Mean brain waves' power during eyes closed, sweet almond oil and citronella oil inhalation.

Brain area	EC	SO	CI	p-value EC and SO	p-value SO and CI
Alpha1 Power (8-10.99 Hz) (μV^2)					
left anterior	8.31	7.71	8.93	0.140	0.093
right anterior	9.10	8.44	10.19	0.177	0.016*
Center	12.66	11.62	14.17	0.202	0.012*
left posterior	9.42	9.26	12.57	0.762	0.002*
right posterior	10.76	10.68	14.52	0.921	0.003*
Alpha2 Power (11-12.99 Hz) (μV^2)					
left anterior	2.59	2.51	2.79	0.462	0.279
right anterior	2.68	2.59	2.97	0.446	0.142
Center	3.95	3.74	4.52	0.328	0.029*
left posterior	4.54	4.70	5.52	0.457	0.006*
right posterior	6.79	7.16	7.97	0.227	0.153
Beta Power (13-30Hz) (μV^2)					
left anterior	0.28	0.28	0.31	0.890	0.032*
right anterior	0.29	0.30	0.33	0.930	0.093
Center	0.37	0.36	0.43	0.878	0.003*
left posterior	0.32	0.32	0.39	0.944	0.000*
right posterior	0.35	0.36	0.42	0.860	0.000*

* Significant difference, p-value < 0.05
EC = Eyes closed session, SO = Sweet almond oil, CI = Citronella oil

EEG data

The EEG power was calculated for each frequency band among resting, sweet almond oil and citronella oil inhalation. Our results revealed power changing of each brain wave frequency during three experimental sessions (Table 3) and expressed by topographic maps in Figure 1. There were noticeable changes of band power in alpha1 waves that significantly increased during the citronella inhalation in all brains areas (p-value <0.05), except for left anterior (p-value =0.093). Conversely, the power of alpha2 waves were significantly increased in central (p-value =0.029) and left posterior (p-value =0.006) brain areas. Furthermore, beta wave power was also increased in all brain areas during citronella oil inhalation, except for right anterior brain region. However, no significant change was observed in the case of theta wave power (p-value > 0.05, data not shown). In Figure 1, the topographic map shows after inhaling citronella compared with resting and sweet almond oil inhalation. The alpha1 wave power increased obviously in bilateral temporal and central areas whereas the power of alpha2 wave increased mainly in posterior brain area. In addition, an increase of beta wave power was observed in anterior and posterior parts of the brain.

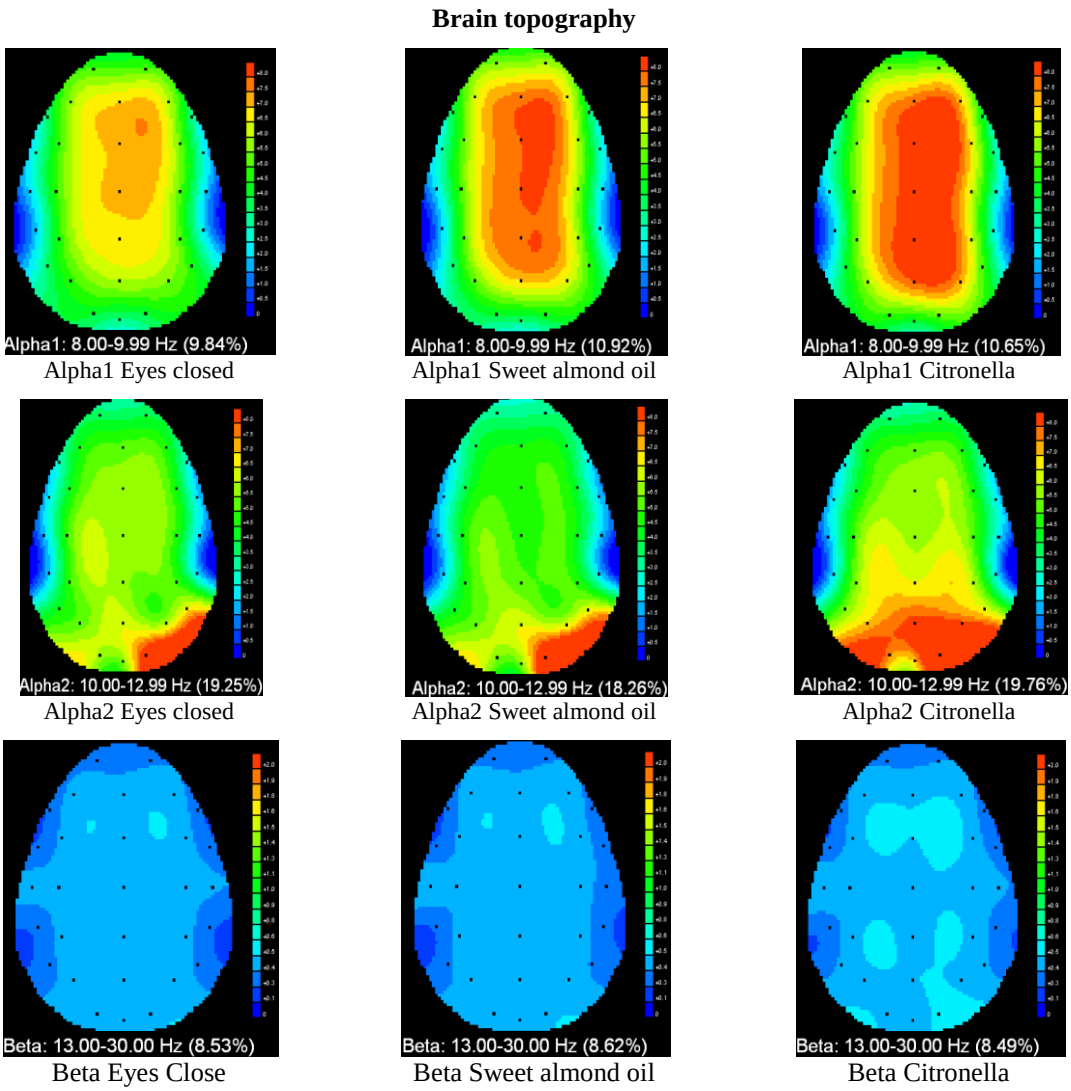

Figure 1 Brain Topographical map of the distribution of alpha and beta wave activities. The red areas indicate a higher power of brain wave in each frequency

DISCUSSION

In the present study, citronella oil was administered by inhalation to healthy subjects. Brain wave activity and ANS parameters, such as blood pressure, heart rate, respiratory rate and skin temperature, were recorded as indicators of the arousal level of nervous system. In addition, subjects had to subjectively rate their mood state in terms of good, bad, active, drowsy, fresh, relaxed, stressed, uncomfortable, romantic, frustrated, calm, and disgusted in order to assess subjective behavioral arousal.

The results of this study support previous studies indicating citronella balancing effect. The observed effects of citronella are not precisely characterized by concept like stimulant or relaxation since inhalation of citronella oil significantly decreased the level of ANS arousal shown by a reduction in blood pressure, heart rate and respiratory rate. This finding is consistent with those reported by Saeki and Shiohara [19]. The power of alpha1 (8-10.99 Hz), alpha2 (11-12.99 Hz) and beta (13-30 Hz) activities were significantly increased. Since several reports had demonstrated the effects of citronella on physiological responses. Our finding also suggests an apparent influence on main component. After analyzing by GCMS, there were main components citronella is citronellal, geranoil and citronellol which a monoterpene. Various studies have shown that monoterpines and their derivative compounds also exhibit several types of pharmacological properties, such as antinociception, antidepressant and sedative effects [31]. In citronellal, previous studies found mice treated with citronellal presented behavioral alterations such as decrease of spontaneous activity, ataxia and sedation. [32, 33]. Furthermore, Azarmi and colleagues found that the vascular effect of geraniol, other main components of citronella oil. Geraniol was able to reduce the contractile response to noradrenalin in vascular walls and heart leading to a further rate of aorta relaxation with lower blood pressure and lower

heart rate [34]. In comparison with other volatile oils comprising similar components as those of citronella, such as rose oil *(Rosa Damascena* Mill) having main components of geraniol and citronellol, such as after applying rose oil to the abdomens of forty healthy subjects a significant decrease of breathing rate, blood oxygen saturation and systolic blood pressure as well as more calm, more relaxed and less alert was observed than the subjects in the control group. After applying rose oil on 40 healthy participants' abdominal surface, the subjects from the study group revealed decreases of breathing rate, blood oxygen saturation and systolic blood pressure, and more calm mental state, the felt more relaxed and less alert than subjects in the control group [35]. Furthermore, Khyaudeen indicated that thirty subjects who inhaled rose oil for fifteen minutes had a significant reduction of blood pressure and feeling more relaxed. In EEG reports, rose oil significantly decreased beta wave power but increased alpha wave power [36]. These findings were similar to our results in terms of alpha wave power increment but were different to the beta wave power changes after citronella inhalation. The significant increase in power of beta wave may relate to high arousal levels. For example, the Sugnano study found the healthy participants had more brain beta wave activity and felt fresher after jasmine oil inhalation [37]. Thus, this study supported that citronella inhalation induced a significant increase in relaxation and fresh feelings in participants. Overall, the changes observed could be interpreted as reflecting the harmonious status of arousal and relaxation, the so-called "relaxed concentrate". Similar changes have been reported in other studies. For example, Hongrattaworakit tested the effects of Ylang-Ylang oil and its effect characterized by the concept of "harmonization". They found that after the oil inhalation induced a decrease in blood pressure and pulse rate, an increase in subjective emotions, including feelings of attentiveness and alertness [38]. Similarly, Morinush studied the combination effect of peppermint oil and eucalyptus oil resulted in similar findings [39]. In short, these results were possibly relevant in individually increasing the cognitive and mental relaxation effects as evidenced by the increase of alpha and beta brain powers.

CONCLUSION

In conclusion, our study explored the effects of inhaled citronella oil on the CNS and other psycho-physiological changes. Our findings support the influence of citronella oil on brain wave activity, autonomic nervous system response and mood states, which emphasizes the effects of citronella harmonization.

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