Journal of Applied Pharmaceutical Science Vol. 7 (01), pp. 165-170, January, 2017 Available online at http://www.japsonline.com DOI: 10.7324/JAPS.2017.70123 ISSN 2231-3354 CC) BY-NC-5A

Chemical Composition and Effects of Blended Essential Oil on Humans

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ARTICLE INFO	ABSTRACT
Article history:	The blended essential oil was composed of eucalyptus, rosemary, patchouli, and pine oils. The blended oil was

Received on: 01/12/2016 Revised on: 18/12/2016 Accepted on: 30/12/2016 Available online: 31/01/2017

Key words: Aromatherapy, Mental stimulation, Eucalyptus, Rosemary, Pine, Patchouli. The blended essential oil was composed of eucalyptus, rosemary, patchouli, and pine oils. The blended oil was analyzed by GC-MS. The effects of blended oil on autonomic parameters and emotional changes in humans following inhalation were an investigation in this study. Thirty healthy volunteers participated in this study. Autonomic parameters, i.e. systolic blood pressure, diastolic blood pressure, mean arterial pressure and pulse rate were recorded. Additionally,emotional changes were assessed by means of visual analog scale. A quasi-experimental with one group pretest-post-test design was used to compare the differences in ANS parameters and emotional changes. Data was analyzed using paired t-test. The blended oil mainly contained 1,8-cineole (33.8%), camphor (14.2%), and α -pinene (12.2%). The blended oil showed significant increases in systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and pulse rate which indicated an increase of autonomic arousal. In addition, inhalation of the blended oil led to activation at the behavioral level, i.e. subjects feel more alert, more vigorous, and more cheerful than before the administration of the oil. This finding points towards an increase of arousal in terms of self-evaluation. Thus, the effects of blended oil by inhalation may be characterized by the concept of stimulating/activating effects.

INTRODUCTION

Stress affects numerous body systems such as cardiovascular, respiratory, digestive, and immune systems (Cooksley, 1996; Damain and Damain, 1995). When stress occurs, some people often take medicines such as psychotropic drugs. If they use the medicines improperly, it will become addictive and harmful to the body.

Therefore the current trend of using natural products has gained in the field of alternative medicine. Essential oil, one of the natural products, is used to treat or alleviate the symptoms of both physical and mental disorders. The use of essential oils in psycho-aromatherapy is very popular. Some studies have shown that essential oils are used for mental strain or dullness and lethargy or exhaustion such as eucalyptus, rosemary, cajuput, pine, bergamot, patchouli, and marjoram oils

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(Albert and Steven, 1996; Lis-Balchin, 2006; Pujiarti et al., 2012). It is interesting that the main chemical components of these essential oils are 1,8-cineole, limonene, camphor, and α -pinene which had been reported a stimulating effect (Heuberger et al., 2000; Kovar et al., 1987; Lis-Balchin, 2006; Steinmetz et al., 1987). Aromatherapy blending preparations may use alone or blend two, three or more oils together to match consumer's satisfaction. The blends usually smell better than a single oil. Blends of neroli and lavender showed an effective for postpartum mothers to improve physical and mental status (Imura et al., 2006). Blends of frankincense, bergamot, and lavender showed a positive effect on depression and pain on hospice patients with terminal cancer (Chang, 2008). Aromatherapy blending of rose, lavender, and sage oils was effective in decreasing the severity of menstrual cramps (Han et al., 2006). Rosemary oil exhibited stimulating effect, such as help to strengthen the brain, improve memory, fortify the heart, and energize mind (Atsumiand Tonosaki,2007; Diego et al., 1998; Hongratanaworakit, 2009; Lis-Balchin, 2006; Moss et al., 2003, Sayorwan et al., 2013).

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Rutledge and Jones (2007) recommend a combination of organically derived essential oils (eucalyptus, lemon, orange, camphor, rosemary, and peppermint) for fibromyalgia. Aromatherapists know that the refreshing effects of pine oil which communicate bracing, strengthening, and stabilizing psychological effects (Damain and Damain, 1995). Patchouli oil show stimulating effect on human physiological responses (Pujiarti *et al.*, 2012).

Essential oils may be used alone or blend two, three or more oils together to match an individual's physical and emotional needs. Good blends usually combine all three notes. Top notes (such as eucalyptus, pine, and cajuput) are highly volatile and give the initial scent. Middle notes (such as neroli, rose, rosemary, and sweet marjoram) round out the blend and last a little longer. Base notes (such as patchouli, vetiver, and sandalwood) are long-lasting and acts as a fixative. Generally, the blends comprise of top note 20-40%, middle note 50-70%, and base note 10% (Hudson, 1999; Wildwood, 2000). In this study, we selected the essential oils that contained 1,8-cineole, limonene, camphor, and α -pinene which had been reported a stimulating effect (Heuberger et al., 2000; Kovar et al., 1987; Lis-Balchin, 2006; Steinmetz et al., 1987). The eucalyptus (Eucalyptus globulus Labill.) and pine (Pinuspalustris Mill.) oils were used as a top note. The rosemary (Rosmarinus officinalis L.) oil was used as a middle note. The patchouli (Pogostemoncablin (Blanco) Benth.) oil was used as a base note.

There are a variety of approaches to evaluate the physiological and psychological effects of essential oil such as measuring changes in autonomic parameters, e.g. breathing rate, blood pressure, pulse rate, skin temperature and skin conductance, changes in brain wave activities, e.g. electroencephalogram, contingent negative variation, changes in mood, cognitive performances and emotion (Bensafi et al., 2000; Diego et al., 1998; Heuberger et al., 2000; Heuberger et al., 2006; Hongratanaworakit et al., 2005; Hongratanaworakit et al., 2007; Hongratanaworakit, 2009; Hongratanaworakit, 2010; Inoue et al., 2003; Moss et al., 2003; Rutledge and Jones, 2007). However, up to now, no experiments about the effects of blended oil on human autonomic parameters and on emotional changes after inhalation have been carried out. Therefore, the main objective of this study was: (a) to investigate the chemical composition of blended oil; (b) to evaluate the effects of blended oil on autonomic parameters, as well as on emotional changes in humans following inhalation.

MATERIALS AND METHODS

Subjects

Thirty healthy subjects (10 males and 20 females) aged between 21 and 23 years (mean age 22.27 ± 2.67 years) took part in the experiments. They were fully briefed, given written informed consent to all aspects of the study (Faculty of Pharmacy, Srinakharinwirot University Ethics Committees) and were free to withdraw at any time. Subjects did not show any symptoms of upper respiratory infections and did not have a history of cardiovascular disease. Forty-eight hours prior to testing subjects were asked to abstain from food, beverages, and toiletries containing the essential oil, as well as from any stimulants (for example, caffeine and nicotine).

Essential oil preparation and administration

The essential oils were obtained from Thai-China Flavours and Fragrances Industry Co., Ltd, Thailand. The blended oil was prepared in a volume ratio and composed of eucalyptus: rosemary: patchouli: pine oils (2:6:1:1). The blended oil was analyzed by GC/MS. Blended oil was diluted to 10% v/v concentration in swe *et al*mond oil.

Gas chromatography/mass spectrometry (GC/MS) analysis

Chemical analysis of single/pure essential oils and blended oil was achieved by gas chromatography/mass spectrometry. GC/MS was carried out on a Trace GC Ultra/DSQ Quadruple spectrometer/Finnigan gas chromatograph fitted with a fused-silica BPX5 capillary column (30 m, 0.25 mm, 0.25 µm film thickness). The carrier gas was helium at 1.0 mL/min. Injector temperature was 180°C. A split ratio of 1:100 was used. Transfer line temperature was 240°C. Column temperature was 60°C for 1 min, then programmed from 60 - 240°C at 3°C/min. The MS were obtained from using an ionization energy of 70 eV and ion source temperature of 200 °C. The individual components were identified: (a) by comparing the Kovats gas chromatographic retention indices of the peaks on the HP-5MS column with the Adams Essential Oil Mass Spectra library; (b) by comparison of their MS with those stored in the MS database (NIST05 library) and with those reported in the literature (Adams, 2004; Lakusic et. al., 2013).

Acquisition of autonomic parameters and emotional changes

Autonomic parameters, i.e. systolic blood pressure (SBP), diastolic blood pressure (DBP), MAP (mean arterial blood pressure), and pulse rate (PR), were recorded simultaneously. The autonomic parameters were measured in the dominant arm by sphygmomanometer using an automated system (Digital Electronic Model DS-155E, Japan). Visual analog scales (VAS) were used to assess subjective emotional conditions. They consisted of 100 mm lines for 4 items: relaxation, vigor, mood and alertness. Each subject was asked to mark his or her feeling for each item between the two possible extremes: relaxed (on the left) and tense (on the right) for the item 'relaxation', vigorous (on the left) and feeble (on the right) for the item 'vigor', cheerful (on the left) and bad tempered (on the right) for the item 'mood', alert (on the left) and tired (on the right) for the item 'alertness' (Heuberger et al., 2006; Hongratanaworakit et al., 2005; Hongratanaworakit et al., 2007; Hongratanaworakit, 2009; Hongratanaworakit, 2010; Sayorwan et al., 2013).

Experimental protocol

The experimental protocol was modified from Diego *et al.* (1998). The pilot study to test the validity of the modified protocol was done in thirty healthy volunteers which were a different group from the experimental group. Lavender oil

(considered a relaxing oil) and rosemary oil (considered a stimulating oil) were selected in the pilot study. In addition, the experimental protocol has been previously used by our group (Taorang et al., 2009). A quasi-experimental with one group pretest-post-test design was used to compare the differences in ANS parameters and emotional changes. Experiments were performed between 08.00 and 12.00 AM to decrease circadian variation of ANS parameters. All experiments were conducted in a bright and quiet room. Ambient temperature was 25+1°C. Upon arrival, the subjects were interviewed about their personal data, e.g. sex, age, height, weight. In addition, they were asked about the rating of emotional responses. After completion of the interview and the rating scales, autonomic parameters were measured. Blended oil was presented in 50 mL amber glass vial which the subjects held about 3 inches from their nose and inhaled continually for a period of six minutes. After completion of the trial, autonomic parameters were repeatedly measured at the end of the trial. The subjects were asked to rate the VAS.

Statistical analyses

All data are presented as the mean \pm standard error mean. The SPSS version 11.5 was used for statistical analysis. The effects of blended oil, lavender oil, and rosemary oil on ANS parameters and emotional responses were determined by comparing the mean values between before and after inhalation using paired t-test. A p-value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

In the present investigation blended oil was administered by sniff to healthy subjects. Autonomic parameters, i.e. SBP, DBP, MAP, and PR, were recorded as indicators of the arousal level of the autonomic nervous system (ANS). In addition, subjects had to rate their emotional condition in terms of alertness, vigor, and mood in order to assess subjective behavioral arousal. A quasiexperimental with one group pretest-post-test design was used to compare the differences in ANS parameters and emotional changes.

The pilot study to test the validity of protocol was done in thirty healthy volunteers which were a different group from the experimental group. Lavender oil (considered a relaxing oil) was selected in the pilot study. The mean and SEM of autonomic and emotional parameters of the lavender oil were presented in Table 1. Pre-post t-tests revealed the following significant autonomic changes. SBP, DBP, and MAP of subjects significantly decreased after sniffing the lavender oil (P<0.05 for all). No significant effects of the lavender on PR was found (P>0.05). In addition, subjects felt more relaxed and less alert than before the administration of the lavender oil (P<0.05 for all). Inhalation of lavender oil decreased the level of arousal of the ANS, i.e. decreases of SBP, DBP, and MAP. Moreover, inhalation of lavender oil led to deactivation at the behavioral level, i.e. subjects feel more relaxed and less alert than before the administration of the oil. This is in accordance to a study done by Moss *et al.* (2003) and Sayorwan *et al.* (2012). Thus, the effects of lavender oil by inhalation may be characterized by the concept of relaxing effects.

 Table 1: Mean and SEM of autonomic and emotional parameters for the lavender oil.

Parameters	PRE	POST	∆ Pre - Post	t	p value
SBP	111.7 <u>+</u> 1.9	107.8 <u>+</u> 1.6	+3.9	+4.92	0.000*
DBP	74.4 <u>+</u> 1.0	71.9 <u>+</u> 1.1	+2.5	+3.14	0.004*
MAP	86.9 <u>+</u> 1.2	83.8 <u>+</u> 1.1	+3.1	+5.65	0.000*
PR	73.4 <u>+</u> 2.1	72.2 <u>+</u> 1.9	+1.2	+1.00	0.324
RL	46.1 <u>+</u> 3.4	33.7 <u>+</u> 3.1	+12.4	+3.59	0.001*
AL	45.6 <u>+</u> 3.6	54.9 <u>+</u> 3.5	-9.3	-2.88	0.007*

SBP: systolic blood pressure DBP: diastolic blood pressure, MAP: mean arterial blood pressure, PR: pulse rate, RL: relaxation, AL: alertness, SEM: standard error mean. * Significant differences (p<0.05).

In addition, rosemary oil (considered a stimulating oil) was also selected in the pilot study. The mean and SEM of autonomic and emotional parameters for the rosemary oil were presented in Table 2. Pre-post t-tests revealed the following significant autonomic and emotional changes. SBP and PR of subjects significantly increased after sniffing the rosemary oil (P<0.05 for all). No significant effects of the rosemary on DBP and MAP were found (P>0.05 for all). Moreover, subjects felt more alert than before the administration of the rosemary oil (P<0.05). No significant effects of the rosemary oil on relaxation was found (P>0.05). Inhalation of rosemary oil increased the level of arousal of the ANS, i.e. increases of SBP and PR. Moreover, inhalation of the rosemary oil led to activation at the behavioral level, i.e. subjects feel more alert than before the administration of the oil. Our findings are consistent with the study done by Moss et al. (2003) and Sayorwan et al. (2013). Thus, the effects of rosemary oil by inhalation may be characterized by the concept of stimulating/activating effects.

From the pilot study showed that the protocol was accurate and can be used to test the blended oil.

Table 2: Mean and SEM of autonomic and emotional parameters for the rosemary oil.

Parameters	PRE	POST	∆ Pre - Post	t	p value
SBP	111.4 <u>+</u> 1.8	112.9 <u>+</u> 1.9	-1.5	-2.20	0.028*
DBP	74.9 <u>+</u> 1.3	75.3 <u>+</u> 1.4	-0.4	-0.26	0.800
MAP	87.1 <u>+</u> 1.3	87.8 <u>+</u> 1.3	-0.7	-1.25	0.213
PR	73.6 <u>+</u> 2.0	76.7 <u>+</u> 2.0	-3.1	-3.21	0.001*
RL	46.2 <u>+</u> 2.7	40.1 <u>+</u> 3.1	+6.1	-2.25	0.024*
AL	44.4 <u>+</u> 3.6	36.6 <u>+</u> 3.5	+7.8	-2.00	0.045*

SBP: systolic blood pressure DBP: diastolic blood pressure, MAP: mean arterial blood pressure, PR: pulse rate, RL: relaxation, AL: alertness, SEM: standard error mean. * Significant differences (p<0.05).

The mean and SEM of autonomic and emotional parameters for the blended oil are presented in Table 3. Pre-post ttests revealed the following significant autonomic and emotional changes. SBP, DBP, MAP, and PR of subjects significantly increased after sniffing the blended oil (P<0.05 for all). In addition, subjects felt more alert, more vigorous, and more cheerful than before the administration of the blended oil (P<0.05 for all).

 Table 3: Mean and SEM of autonomic and emotional parameters for the blended oil.

Parameters	PRE	POST	∆ Pre - Post	t	p value
SBP	111.2 <u>+</u> 1.9	117.4 <u>+</u> 1.8	-6.2	-8.67	0.000*
DBP	73.1 <u>+</u> 1.5	75.6 <u>+</u> 1.2	-2.5	-3.11	0.004*
MAP	85.8 <u>+</u> 1.5	89.6 <u>+</u> 1.2	-3.8	-6.04	0.000*
PR	74.6 <u>+</u> 1.7	79.7 <u>+</u> 1.8	-5.1	-5.38	0.000*
AL	44.7 <u>+</u> 3.1	36.3 <u>+</u> 2.6	+8.7	+2.83	0.009*
\mathbf{V}	48.9 <u>+</u> 3.7	41.2 <u>+</u> 2.6	+7.7	+2.33	0.027*
Μ	35.9 <u>+</u> 3.1	29.4 <u>+</u> 2.2	+6.5	+2.63	0.014*
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SBP: systolic blood pressure DBP: diastolic blood pressure, MAP: mean arterial blood pressure, PR: pulse rate, AL: alertness, V: vigor, M: mood, SEM: standard error mean. * Significant differences (p<0.05).

The chemical composition of the single/pure essential oils and blended oil was determined by gas chromatography-mass spectrometry (GC/MS), as shown in Table 4. The blended oil mainly contained 1,8-cineole (33.8%), camphor (14.2%), and  $\alpha$ -pinene (12.2%). Limonene (3.7%),  $\beta$ -pinene (3.4%), and p-cymene (3.5%) were the minor components found in the blend. The major component observed in the single/pure essential oils, eucalyptus and rosemary oils was 1,8-cineole (23.2-83.3%), whereas the major components of pine oil were  $\alpha$ -pinene (17.2%), terpinolene (15.1%), and  $\alpha$ -terpinene (14.0%).

 Table 4: Chemical compositions of blended essential oil preparations and single essential oils.

	%Area					
Compounds	KI*	Blended oil	Eucalyptus	Rosemary	Patchouli	Pine
Tricyclene	926	0.4	-	1.0	-	0.3
α-Pinene	939	12.2	6.8	19.2	-	17.2
Camphene	954	6.8	-	13.2	-	4.1
β-Pinene	979	3.4	1.1	6.8	-	0.1
Myrcene	988	0.7	0.4	1.1	-	0.4
α-Phellandrene	1002	-	-	0.1	-	0.4
δ-3-Carene	1011	-	-	-	-	0.5
1,4-Cineole	1014	1.8	-	-	-	-
α-Terpinene	1017	-	-	-	-	14.0
p-Cymene	1026	3.5	3.8	4.1	-	3.2
Limonene	1029	3.7	4.6	3.5	-	8.8
1,8-Cineole	1031	33.8	83.3	23.2	0.1	7.8
γ-Terpinene	1059	0.2	-	-	-	1.9
Terpinolene	1088	1.4	-	-	-	15.1
Linalool	1096	0.4	-	0.5	-	-
endo-Fenchol	1116	0.4	-	-	-	2.2
α-Campholenal	1126	-	-	0.5	-	-
1-Terpineol	1133	0.8	-	-	-	4.0
cis-β-Terpineol	1144	-	-	-	-	2.4
Camphor	1146	14.2	-	19.2	-	-
Camphene hydrate	1149	-	-	-	-	0.2
Isoborneol	1160	1.2	-	1.3	-	0.6
Borneol	1169	2.3	-	2.2	-	1.9
Terpinen-4-ol	1177	0.2	-	-	-	0.6
a-Terpineol	1188	2.7	-	0.5	-	11.0
γ-Terpineol	1199	0.9	-	-	-	3.0
Isobornyl acetate	1285	2.0	-	2.6	-	-
δ-Elemene	1338	-	-	-	0.1	-
α-Longipinene	1352				-	0.1

0 Detek oulene	1201	0.4			4.0	
p-Patchoulene	1301	0.4	-	-	4.9	-
β-Elemene	1390	0.1	-	-	0.9	-
Cycloseychellene	1407	-	-	-	0.6	-
Longifolene	1408	-	-	-	-	0.3
E-Caryophyllene	1419	0.6	-	0.6	2.2	-
α-Guaiene	1439	1.0	-	-	15.0	-
Seychellene	1446	0.8	-	-	8.4	-
α-Humulene	1454	-	-	0.1	-	-
α-Patchoulene	1456	0.6	-	-	6.4	-
Aciphyllene	1501	0.2	-	-	2.9	-
γ-Patchoulene	1502	0.3	-	-	4.1	-
α-Bulnesene	1509	1.3	-	-	20.8	-
Nootkatene	1518	-	-	-	0.2	-
7-epi-α-Selinene	1522	-	-	-	0.2	-
Norpatchoulenol	1555	-	-	-	0.4	-
Caryophyllene oxide	1583	0.2	-	0.3	-	-
Pogostol	1653	-	-	-	1.3	-
Patchouli alcohol	1658	1.7	-	-	31.6	-
Cyperotundone	1695	-	-	-	0.2	-

*KI = Kovats gas chromatographic retention indices of the peaks on the HP-5MS column

The blended oil caused a significant increase of blood pressure. Since blood pressure is determined by the activity of the sympathetic branch of the ANS, an increase of blood pressure shows an increase in sympathetic tone, i.e., an increase of autonomic arousal (Andreassi, 2000; Heuberger *et al.*, 2006; Hongratanaworakit *et al.*, 2005; Hongratanaworakit *et al.*, 2007; Hongratanaworakit, 2009; Hongratanaworakit, 2010; Hugdahl, 1995; Sayorwan *et al.*, 2013).

In addition, PR of subjects significantly increased after sniffing the blended oil. Since pulse rate is mainly controlled by the ANS, the increase of pulse rate after sniffing of blended oil is likely to show a decrease in vagal tone, i.e., an increase of ANS arousal. The change in the cardiovascular parameter, i.e., pulse rate, was consistent with an increase of peripheral autonomic nervous activity (Andreassi, 2000; Heuberger et al., 2006; Hongratanaworakit et al., 2005; Hongratanaworakit et al., 2007; Hongratanaworakit, 2009; Hongratanaworakit, 2010; Hugdahl, 1995; Sayorwan et al., 2013). Inhalation of blended oil increased the level of arousal of the ANS, i.e. increases insystolic and diastolic blood pressure, mean arterial blood pressure, and pulse rate. Moreover, inhalation of blended oil led to activation at the behavioral level, i.e. subjects feel more alert, more vigorous, and more cheerful than before the administration of the oil. This finding points towards an increase of arousal in terms of self-2000; evaluation(Andreassi, Heuberger et al., 2006; Hongratanaworakit et al., 2005; Hongratanaworakit et al., 2007; Hongratanaworakit, 2009; Hongratanaworakit, 2010; Hugdahl, 1995; Sayorwan et al., 2013). Thus, the effects of blended oil by inhalation may be characterized by the concept of stimulating/activating effects, which has also been described for rosemary oil (Hongratanaworakit, 2009; Sayorwan et al., 2013), kaffir lime oil (Hongratanaworakit et al., 2007), sandalwood oil(Heuberger et al., 2006), jasmine oil (Hongratanaworakit, 2010), and sweet orange oil (Hongratanaworakit et al., 2005). In addition, our findings clearly support previous studies indicating the stimulating effect of rosemary oil, patchouli oil, pine oil and the main chemical components in these oils (Albert and Steven, 1996; Atsumi and Tonosaki, 2007; Heuberger *et al.*, 2000; Kovar *et al.*, 1987; Lis-Balchin, 2006; Pujiarti *et al.*, 2012; Steinmetz *et al.*, 1987). Although our findings agree with other reports, it is important to assess further biochemical measures (e.g., noradrenaline), as these would further confirm the presence of stimulating/activating effects.

Blended oil mainly contained eucalyptus and rosemary oils. These essential oils contain abundant oxides and monoterpenes, and have the main action of stimulating the nervous system under sympathetic control, leading to increase in alertness and vigor (Lis-Balchin, 2006; Sanders et al., 2002). Steinmetz et al.(1987)described the effect of 1,8-cineole and camphor, two of the constituents of blended oil, on the central nervous system. Their results showed both compounds stimulated the activity of the central nervous system, respiration, and circulation. Moreover, 1,8-cineole, the main component of blended oil, increased respiration rate after administration of the substance (Heuberger et al., 2008). Furthermore, pepper oil, estragon oil and grapefruit oil showed a stimulating effect on the sympathetic activity. These essential oils consist of some component, i.e.  $\alpha$ -pinene and  $\beta$ pinene, which are possible that these components mediate the stimulating effect on the sympathetic activity (Hongratanaworakit, 2009; Lis-Balchin, 2006). Therefore, it is possible that these components such as 1,8-cineole, camphor or  $\alpha$ -pinene mediate the stimulating effect of the blended oil on the nervous system (Hongratanaworakit, 2009; Hugdahl, 1995).

#### CONCLUSIONS

In conclusion, our investigation demonstrates the stimulating effect of the blended oil. The blended oil showed significant increases of systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and pulse rate which indicated an increase of autonomic arousal. In addition, inhalation of the blended oil led to activation at the behavioral level, i.e. subjects feel more alert, more vigorous, and more cheerful than before the administration of the oil. This finding points towards an increase of arousal in terms of self-evaluation. Thus, the effects of blended oil by inhalation may be characterized by the concept of stimulating/activating effects

### ACKNOWLEDGEMENTS

The authors were grateful to the people who participated in the study.

**Financial support and sponsorship:** This work was supported by grants from Srinakharinwirot University (11/2014).

Conflict of Interests: There are no conflicts of interest.

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# How to cite this article:

Hongratanaworakit T, Srimuang P, Wichanpreechar A, Akarapattaranithi T, Taorang B, Rawdlaurh P., Chemical Composition and Effects of Blended Essential Oil on Humans. J App Pharm Sci, 2017; 7 (01): 165-170.