# Effect of Orange Essential Oil Inhalation on Mood and Memory in Female Humans

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## Abstract

Orange essential oil has previously been shown to have anti-anxiety activity in animal and human studies. However, the mood and cognitive enhancing effects have not been investigated. Therefore, the present study aimed to investigate the effect of orange essential oil on working memory and emotion in women. Eighty healthy females which age 18-25 years old were entered into a double-blinded, randomized, placebo-controlled, crossover designed study. Subjects were assigned to inhale placebo or orange essential oil for five minutes, and then their working memory and cognitive function were evaluated using Cognitive Drug Research (CDR) Computer Assessment Battery Test. The mood assessment was also evaluated using Bond-Lader Visual Analogue Scales. The results showed that orange essential oil enhanced spatial working memory, including: 1) the continuing of attention as indicated by increasing digit vigilance accuracy ($t$ = -2.275, $P$ = 0.027) and choice reaction time accuracy (T2) ($t$ = -2.129, $P$ = 0.038); 2) the quality of memory as indicated by the elevation of the percentages of word recognition accuracy ($t$ = -2.312, $P$ = 0.026), picture recognition accuracy ($t$ = -8.286, $P$ = 0.000), and spatial working memory accuracy ($t$ = -2.110, $P$ = 0.041); 3) the speed of memory as indicated by the reduction of reaction time of word recognition ($t$ = 3.558, $P$ = 0.001), picture recognition ($t$ = 5.049, $P$ = 0.000), and spatial working memory ($t$ = 2.694, $P$ = 0.010). The orange essential oil also improved alertness ($t$ = -8.431, $P$ = 0.000) and calmness ($t$ = -3.912, $P$ = 0.000). In conclusion, we suggest that orange essential oil has a potential as a cognitive enhancer and could also be used for relaxation.

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***Keywords:*** *Orange essential oil, essential oil inhalation, mood, memory*

## Introduction

Aromatherapy is one of the alternative medical methods that use essential oils for relieving symptoms, relaxing, and resting the body.[1] Odor molecules of essential oils are transmitted to the brain by olfactory sensory neurons in the nasal cavity. The neuronal signals are sent along the olfactory bulb to the prefrontal cortex, orbitofrontal cortex, and limbic system, particularly the amygdala-hippocampal formation. These are critical for expression of emotion and emotional memory.[2] In recent years, aromatherapy has focused on emotional stress and cognitive performance in both animal and human models. Several lines of evidence have suggested that inhalation of lavender essential oil exerts anxiolytic-like activity in mice via the serotonergic system.[3] Rose essential oil possessed anxiolytic-like activity, as tested using the elevated plus-maze test in adult male rats.[4] Previous studies investigated human models using inhalation of rosemary, lavender, roman chamomile, peppermint, and ylang-ylang essential oils to modulate cognitive performance and mood.[5-7] A recent study found that inhalation of lavender and rosemary aroma oils decreased cortisol levels (stress marker). However, only lavender aroma reduced stress and mood index.[8] The mentioned essential oils provide beneficial effects on mood and cognition, but they are expensive. Searching for other inexpensive essential oils which could provide the same beneficial effects is still necessary. The orange essential oil, commonly known as orange sweet, *Citrus sinensis*, has shown anti-anxiety in animal and human studies.[9,10] However, the cognitive enhancing effect and mood modulation of orange essential oil is rarely reported. Thus, the present work examined the effect of orange essential oil on mood and working memory in female humans.

## Materials and Methods

### Subjects

Eighty healthy Thai females, aged 18-25 years old (mean age 20.40 ± 1.16 years), were recruited to participate in this study (approved by the Ethical Committee of Mae Fah Luang University (REH-56035). Before the testing, each subject was asked to give a written consent and also screened for physical health by a physician in order to assure good health. The subjects were then randomly allocated to either a placebo (inactive control oil) or orange essential oil-treated group. The individuals were excluded if they showed respiratory tract infection, sinusitis, cold,

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neuropsychological disease, or alcohol addiction. The subjects who were taking prescribed or non-prescribed drugs or nutraceutical compounds which influence the nervous system were also excluded. All subjects were asked not to do the test during their menstrual period because mood and smell ability of woman might be changed during their periods. Therefore, if the subjects were having a period, they were asked to wait until their period had passed before performing the test.

**The preparation of orange essential and base oils**

The orange essential oil used in this study was from Botanicessence, Bangkok, Thailand. The essential oil was extracted from orange peel by a cold pressing method. Fresh orange peel was washed and then dried by heating in an oven at 50 °C for two hours. The peel was then removed from the oven and the oil was extracted by pressing.

Coconut oil was used as the base oil or placebo because it does not have the same scent as the orange essential oil. Before testing, either orange essential oil or coconut oil was placed in amber glass bottles. Therefore, the subjects would not know whether it was orange or coconut essential oil.

**Mood and cognitive testing**

This study was double blinded and placebo controlled. A random list of numbers was computer-generated. Either 50 µl of essential oil or placebo oil containing sterilized cotton was placed in a 10-ml amber glass bottle. After the subjects were randomly assigned to coconut oil (placebo) or treated group, each was assigned to inhale placebo or orange essential oil for five minutes in a quiet experimental room, 3x3 $m^2$. Their working memory and cognitive function were evaluated using a Cognitive Drug Research (CDR) Computerized Assessment Battery Test. The mood assessment was evaluated using Bond-Lader Visual Analogue Scale. All subjects were screened for baseline intellectual ability by using cumulative Grade Point Average (GPA) and CDR Computerized Assessment Battery Test as a pretest baseline score in order to avoid confounding errors induced by intellectual differences.

**The Bond-Lader Visual Analogue Mood Scale**

The Bond-Lader Visual Analogue Mood Scale is a questionnaire of 16-bipolar-10-cm-analogue scales for measuring subjective experience of mood state. The test consists of three factor scores: self-rated alertness (items 1, 3-6, 9, 11-12, 15), calmness (2, 10), and contentment (7-8, 10-14, 16).[11]

**CDR Computerized Assessment Battery Test**

The CDR Computerized Assessment Battery Test was modified from the Cognitive Drug Research Computerized Assessment Battery Test. It has been used for cognitive assessments including attention, working memory, executive function, and motor skills.[12,13] The CDR system is a computer-based cognitive testing tool composed of six sets of test and was assigned to each subject at the same difficulty level to avoid learning effect on the test. The test was presented on high-resolution monitors. The selected test took approximately 20 minutes to complete. The tests were administered in the following order:

***Word presentation:*** Fifteen words were presented sequentially on the monitor for one second each with an inter-stimulus interval of one second. The subjects were required to memorize these words.

***Delayed word recognition:*** Thirty words, which consisted of 15 words presented previously and distracting words, were presented one at a time on the monitor in a randomized order. For each word, the subjects indicated whether or not it was included in the 15-word list by pressing the "yes" or "no" button within a second. The delayed word recognition test was performed 30 minutes after the subjects had been presented with the list of words.

***Picture presentation:*** Twenty pictures were presented sequentially on the monitor for three seconds each, with an inter-stimulus interval of 1 second. The subjects were required to memorize these pictures.

***Delayed picture recognition:*** Twenty pictures, which consisted of the previously presented pictures and distracting pictures, were randomly presented one at a time on the screen. For each picture, the subjects indicated whether or not it was a picture in the 20-picture list by pressing the "yes" or "no" button within a second. The delayed picture recognition test was performed 30 minutes after the subjects had been presented with the pictures.

***Simple reaction time:*** The word "yes" was presented on the monitor. The subjects pressed the "yes" response button as quickly as possible if the word "yes" was presented on the monitor. The reaction time it took the subjects to respond was recorded.

***Digit vigilance task:*** The monitor screen was divided into two parts, left and right. A target number was randomly selected and constantly displayed to the right of the monitor screen. A series of numbers were presented on the left of the screen. The subjects pressed the "yes" button as quickly as possible if the number on the left screen matched the target number on the right screen.

***Choice reaction time (T1):*** Either the word "no" or "yes" was displayed on the monitor and the subject was instructed to press the matched button as quickly as possible. The time it took for the subjects to react was recorded as the reaction time.

***Choice reaction time (T2):*** Either a triangle or a square was displayed on the monitor. The subjects were instructed to press the "red button" when they saw a triangle or the "blue button" when they saw a square as quickly as possible.

***Spatial working memory:*** Nine windows, illuminated with four yellow lights, were displayed on the monitor. The subjects were required to memorize the position of the illuminated windows. The subjects

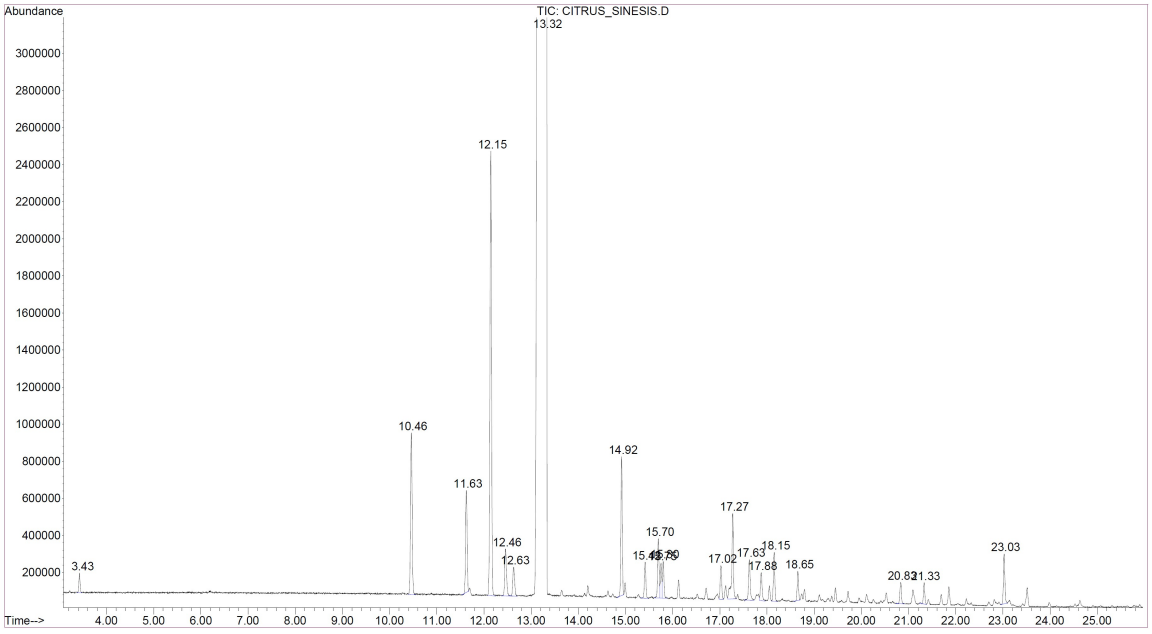


**Figure 1** Chromatogram of orange essential oil analyzed by GC-MS. The experiment was performed with an HD column with the flow rate of 1 ml/min. Oven temperature was set at 40-240 °C. Hexane was used as the solvent. Injector and detector were set at 250 and 230 °C, respectively. Mass spectrometer was set to determine molecular weights of 20-300.

determined if the position of the illuminated window was the same with the one previously shown. The subjects were required to press the "yes" or "no" response button as quickly as possible.

***Numeric working memory:*** Five numbers were displayed sequentially on the monitor for the subjects to memorize. Then, a series of 30 numbers were presented. The subjects decided whether or not the number was in the five-number list. The subjects were required to press the "yes" or "no" response button as quickly as possible.

**Analysis of the orange essential oil component**

Gas chromatography-mass spectrometry (GC-MS) was used for the analysis of volatile organic compounds of orange essential oil. The GC-MS analysis was provided by the Scientific and Technological Instruments Center of Mae Fah Luang University. An HD column was used with a flow rate of 1 ml/min. The oven temperature was set at 40-240 °C. Hexane was used as the solvent. Injector and detector were set at 250 and 230 °C, respectively. Mass spectrometer was set to determine molecular weights of 20-300. The components of orange essential oil determined by GC-MS are shown in Table 1. The certificate of analyses of orange essential oil was provided by Botanicessence and is shown in Table 2.

**Statistical analysis**

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS). The comparison was made between the placebo and orange essential oil inhalation utilizing paired sample *t* tests. Statistical significance was regarded at $P < 0.05$.

# Results

**Determination of effective orange essential oil constituents by GC-MS**

Figure 1 showed the chromatogram of orange essential oil. The analysis of the oil constituents from Figure 1 was provided in Table 2. The main components of orange essential oil were limonene (95.21% v/v), beta-myrcene (1.5% v/v), alpha-pinene (0.54% v/v), and linalool (0.4% v/v). The other compounds were found in lesser amounts in comparison to the amount of the main components.

**Effect of orange essential oil inhalation on mood**

Table 3 showed the effects of treatment on the alertness, calmness, and contentment. The subjects who inhaled orange essential oil showed a significant increase in alertness ($t$ = -8.431, $P$ = 0.000) and calmness compared to placebo ($t$ = -3.912, $P$ = 0.000). However, contentment was not significantly different compared to the placebo.

**Effect of orange essential oil inhalation on working memory**

Prior to the investigation of orange essential oil inhalation effects on working memory, baseline data for both inhaling conditions (placebo or orange essential oil) of each individual task score were subjected to *t* test. No significant changes in any parameters were observed. The pretest baseline scores and posttest baseline scores for each condition are presented in Table 4. Participants who inhaled orange essential oil showed a significant increase in percent accuracy of delayed word recognition ($t$ = -2.312, $P$ = 0.026), delayed picture recognition ($t$ = -8.286, $P$ = 0.000), digit vigilance ($t$ = -2.275, $P$ = 0.027), choice reaction time (T2) ($t$ = -2.129, $P$ =

**Table 1** The components of orange essential oil analyzed from the chromatogram in Figure 1.

| Peak No. | Retention time (min) | Identified constituents | Area (percent) |
|---|---|---|---|
| 1 | 3.43 | Heptane | 0.05 |
| 2 | 10.46 | Alpha-pinene | 0.54 |
| 3 | 10.63 | Sabinene | 0.33 |
| 4 | 12.15 | Beta-myrcene | 1.50 |
| 5 | 12.46 | Octanal | 0.18 |
| 6 | 12.63 | Delta 3-carene | 0.11 |
| 7 | 13.32 | Limonene | 95.21 |
| 8 | 14.92 | Linalool | 0.40 |
| 9 | 15.42 | Tranns-p-mentha | 0.12 |
| 10 | 15.70 | Cis limunene oxide | 0.18 |
| 11 | 15.75 | Carveol | 0.10 |
| 12 | 15.80 | Trans limonene oxide | 0.11 |
| 13 | 17.02 | Alpha-terpineol | 0.11 |
| 14 | 17.27 | Decamal | 0.32 |
| 15 | 17.62 | Trans carveol | 0.14 |
| 16 | 17.88 | 2-cyclohexan-1-ol | 0.10 |
| 17 | 18.15 | 2-cyclohexan-1-one | 0.15 |
| 18 | 18.65 | E-citral | 0.08 |
| 19 | 20.83 | Alpha-copaene | 0.07 |
| 20 | 21.33 | Dodecanal | 0.06 |
| 21 | 23.03 | Valencene | 0.16 |

**Table 2** The certificate of analyses of orange essential oil provided by Botanicessence, Bangkok, Thailand.

| Product Name : | Orange Sweet | RESULT |
|---|---|---|
| Botanical Name : | *Citrus sinensis* | |
| Description : | Yellow to pale orange | COMPLIES |
| Odor : | Distinctive aroma from pressed peel | COMPLIES |
| Specific Gravity : (20 ºC) | 0.8450-0.8550 | 0.8456 |
| Refractive Index : (20 ºC) | 1.4700-1.4750 | 1.4729 |
| Optical Rotation : | +94º to +100º | +97.4º |
| Pesticide Results : | Less than 5 ppm total | COMPLIES |
| Shelf Life : | 24 Months | |

**Table 3** Effect of orange essential oil on mood assessing by Bond-Lader Visual Analogue Mood Scales

| Mood factor | Group | Pretest baseline score | Posttest baseline score | *t* test | *P* value |
|---|---|---|---|---|---|
| Alertness | Placebo | 5.80±1.58 | 5.98±1.62 | -0.590 | 0.558 |
| | Orange | 4.95±1.70 | 6.94±1.49*** | -8.431 | 0.000 |
| Calmness | Placebo | 6.35±1.74 | 6.82±1.92 | -1.302 | 0.199 |
| | Orange | 5.72±1.73 | 6.78±1.52*** | -3.912 | 0.000 |
| Content-ment | Placebo | 8.19±1.38 | 8.32±1.35 | -1.458 | 0.152 |
| | Orange | 7.65±1.55 | 7.65±1.56 | -0.109 | 0.914 |

Subjects were assessed for alertness, calmness and contentment by using paired sample *t* test. Data are presented as mean ± SD (n = 40 / group). *, **, *** *P* value < 0.05, 0.01, and 0.001, compared to the placebo-treated group, respectively.

0.038), and spatial working memory ($t$ = -2.110, $P$ = 0.041). In addition, participants with post-inhalation of orange essential oil showed a significant decrease in reaction time for delayed word recognition ($t$ = 3.558, $P$ = 0.001), delayed picture recognition ($t$ = 5.049, $P$ = 0.000), and spatial working memory ($t$ = 2.694, $P$ = 0.010). However, a significant difference was not observed in percent accuracy and reaction time of numeric working memory. The current data suggest that inhalation of orange essential oil could improve working memory by: 1) the continuing of attention, as indicated by the increased percent accuracy of digit vigilance and choice reaction (T2); 2) the speed of memory, as indicated by the decreased reaction time of delayed word recognition, delayed picture recognition, and spatial working memory; 3) quality of memory as indicated by the increase of percent accuracy of word recognition, delayed picture recognition, and spatial working memory.

## Discussion

The physiological activation of the olfactory system is via binding of inhaled odor molecules to olfactory receptors. These chemical signals are transduced into electrical signals and sent to the brain areas to be perceived as smell, particularly in the prefrontal cortex (PFC), orbitofrontal cortex, and limbic system such as the hippocampus and amygdala.[14] These parts of the brain regulate olfactory memory and emotional responses to olfactory stimuli.[14] The current evidence also indicates that the prefrontal cortex is a crucial site of integration between mood and cognition.[15] Thus, olfaction plays an important role on regulation of mood and memory involving with these brain areas.

Our data show the changes of alertness and calmness together with the improvement of working memory after orange essential oil inhalation. Therefore, the inhalation of orange essential oil might improve mood (alertness and calmness), which finally improves attention and working memory.

The olfactory sensations are the primary input to the amygdala. Olfactory signals are sent from basolateral amygdala to orbitofrontal cortex and hypothalamus via the medial dorsal thalamic nucleus.[16] The orbitofrontal cortex provides the perception of emotions while the hypothalamus provides the expression of emotions.[16] Our study reports that the major component in orange essential oil is limonene (95.21%) which probably is responsible for mood and memory effects shown in this study. Previous study has demonstrated that sweet orange, containing limonene 97.66%, possesses anxiolytic activity in rats evaluated in elevated plus maze and light-dark paradigms.[9] In addition, limonene epoxide, a synthesized limonene from plants, was found to decrease anxiety in mice observed in elevated plus maze test.[17] A study in healthy volunteers has reported that inhalation of sweet orange essential oil in the volunteers could reduce acute anxiety symptoms.[18] Tsunetsugu and colleagues demonstrated that inhalation of limonene enhanced relaxation and soothing by suppressing the sympathetic nervous system.[19] Moreover, increase in subjective alertness was observed after limonene inhalation.[20] Thus, inhalation of orange essential oil has the effect on emotion and mood. These findings are in good agreement with the increase in subjective

alertness and calmness observed in our study. Previous studies have demonstrated that anxiety induced an impairment of both verbal and spatial working memory capacities (e.g., spatial attention and executive function).[21] Neuroimaging study showed that there was a relationship between emotion and cognition mediated by amygdala in the case of emotion, and lateral prefrontal cortex in the domain of cognition.[22] Hence, the inhalation of orange essential oil might modulate mood and working memory.

Working memory, mediated by prefrontal cortex, is used for short-term storage and manipulation of executive process.[23] Dopamine, a neurotransmitter stored in prefrontal cortex, is used for regulating working memory.[24] Previous work on monkey with damage of prefrontal cortex demonstrated an impairment of executive function and attention whereas hippocampus played a crucial role in spatial memory by storing information and retrieving memory required for spatial navigation.[25,26] In addition, Spellman and coworkers suggested that the direct hippocampal-prefrontal cortex afferent pathway supported spatial working memory.[27] Since hippocampal acetylcholine correlated with spatial reference memory performance,[28] bilateral damage of the hippocampus could result in the loss of recent memory and inability to learn.[16] Based on this information, working memory evaluations focused on attention and memory parameters by using CDR Computerized Assessment Battery Test for cognitive performance assessment. Our study demonstrated that the odor of orange essential oil could enhance: 1) continuing of attention, 2) speed of memory, and 3) the quality of memory. The findings of this study agree with a previous work which reported the increase of speed of memory in young adults inhaling orange essential oil.[29] However, the age range of the subjects of this work and the previous work are different. Moreover, this work extended the investigation to cover the analysis of chemical constituents of the essential oil by GC-MS, and the effect of the essential oil on mood. A previous study has showed the inhalation of lemon essential oil containing s-limonene attenuates memory impairment induced by scopolamine in rat.[30] Hence, mood and working memory modulations might be due to limonene to alter neurochemical mechanism in the brain, and to be perceived as smell, particularly by the prefrontal cortex and limbic system. Nevertheless, further study would be needed to clarify the active compound which modulates mood and working memory.

**Table 4** Effect of orange essential oil on working memory assessing via CDR Computerized Assessment Battery Test

| Measurement | Group | Pretest baseline score | Posttest baseline score | *t* test | *P* value |
|---|---|---|---|---|---|
| Delayed word recognition (% accuracy) | Placebo | 81.20 ± 2.63 | 83.87 ± 1.38 | 0.466 | 0.643 |
| | Orange | 81.07 ± 1.99 | 87.70 ± 1.34* | -2.312 | 0.026 |
| Delayed word recognition reaction time (ms) | Placebo | 1356.75 ± 107.64 | 1333.67 ± 117.67 | 1.576 | 0.123 |
| | Orange | 1291.97 ± 116.50 | 1168.55 ± 112.83** | 3.558 | 0.001 |
| Delayed picture recognition (% accuracy) | Placebo | 80.11 ± 2.20 | 82.20 ± 1.77 | -1.935 | 0.060 |
| | Orange | 79.80 ± 2.15 | 87.20 ± 1.84*** | -8.286 | 0.000 |
| Delayed picture recognition reaction time (ms) | Placebo | 1455.55 ± 83.45 | 1375.65 ± 77.22 | 1.645 | 0.108 |
| | Orange | 1347.07 ± 108.32 | 1130.34 ± 109.57*** | 5.049 | 0.000 |
| Simple reaction time (ms) | Placebo | 589.13 ± 124.56 | 578.33 ± 135.76 | 1.233 | 0.134 |
| | Orange | 564.45 ± 120.23 | 575.36 ± 112.33 | 1.677 | 0.078 |
| Digit vigilance (% accuracy) | Placebo | 71.02 ± 25.34 | 78.10 ± 23.44 | -1.615 | 0.113 |
| | Orange | 74.69 ± 24.33 | 83.67 ± 19.00* | -2.275 | 0.027 |
| Digit vigilance reaction time (ms) | Placebo | 536.21 ± 125.55 | 540.10 ± 133.45 | -0.401 | 0.690 |
| | Orange | 543.06 ± 132.12 | 549.46 ± 114.57 | -0.896 | 0.375 |
| Choice reaction Time (T1) (% accuracy) | Placebo | 98.85 ± 1.68 | 99.30 ± 1.19 | -1.853 | 0.070 |
| | Orange | 98.92 ± 1.57 | 99.20 ± 1.34 | -0.910 | 0.367 |
| Choice reaction Time (T1) (ms) | Placebo | 838.77 ± 145.67 | 817.94 ± 155.98 | 0.794 | 0.431 |
| | Orange | 830.31 ± 165.45 | 830.16 ± 164.21 | 0.011 | 0.991 |
| Choice reaction Time (T2) (% accuracy) | Placebo | 98.64 ± 2.22 | 90.32 ± 1.18 | -1.981 | 0.054 |
| | Orange | 99.16 ± 1.62 | 99.64 ± 0.87* | -2.129 | 0.038 |
| Choice reaction Time (T2) (ms) | Placebo | 848.44 ± 146.33 | 842.38 ± 154.97 | -0.986 | 0.453 |
| | Orange | 853.15 ± 166.25 | 863.45 ± 155.65 | -0.885 | 0.267 |
| Spatial working memory (% accuracy) | Placebo | 96.88 ± 6.51 | 97.72 ± 4.58 | -1.520 | 0.137 |
| | Orange | 97.48 ± 4.42 | 98.61 ± 3.61* | -2.110 | 0.041 |
| Spatial working memory reaction time (ms) | Placebo | 1289.62 ± 114.61 | 1217.97 ± 117.72 | 1.806 | 0.079 |
| | Orange | 1247.95 ± 81.90 | 1016.21 ± 100.78* | 2.694 | 0.010 |
| Numeric working memory (% accuracy) | Placebo | 97.46 ± 2.82 | 98.19 ± 3.22 | -1.901 | 0.065 |
| | Orange | 99.22 ± 1.68 | 99.46 ± 1.10 | -0.683 | 0.499 |
| Numeric working memory reaction time (ms) | Placebo | 967.33 ± 161.17 | 950.73 ± 187.39 | 1.397 | 0.170 |
| | Orange | 997.06 ± 194.72 | 916.97 ± 117.40 | 1.221 | 0.146 |

Subjects were assessed for continuing of attention, speed of memory, and quality of memory by using paired sample *t* test. Data are presented as mean ± SD (n = 40 / group). *, **, *** P-value < 0.05, 0.01, and 0.001, compared to the placebo-treated group, respectively.

## Conclusion

Inhalation of orange essential oil can enhance alertness and calmness by activating olfactory pathways and modulating limbic system. The essential oil could be a cognitive enhancer. Thus, this oil could be employed to improve working memory during a learning period. However, the brain activity and neurochemistry involved with learning process should be further investigated.


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## Conflict of Interest

None to declare.

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