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Effect of Sweet Orange Aroma on Experimental Anxiety in Humans

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Abstract

Objectives: The objective of this study was to evaluate the potential anxiolytic effect of sweet orange (*Citrus sinensis*) aroma in healthy volunteers submitted to an anxiogenic situation.
Design: Forty (40) male volunteers were allocated to five different groups for the inhalation of sweet orange essential oil (test aroma: 2.5, 5, or 10 drops), tea tree essential oil (control aroma: 2.5 drops), or water (non-aromatic control: 2.5 drops). Immediately after inhalation, each volunteer was submitted to a model of anxiety, the video-monitored version of the Stroop Color-Word Test (SCWT).
Outcome measures: Psychologic parameters (state-anxiety, subjective tension, tranquilization, and sedation) and physiologic parameters (heart rate and *gastrocnemius* electromyogram) were evaluated before the inhalation period and before, during, and after the SCWT.
Results: Unlike the control groups, the individuals exposed to the test aroma (2.5 and 10 drops) presented a lack of significant alterations ($p > 0.05$) in state-anxiety, subjective tension and tranquillity levels throughout the anxiogenic situation, revealing an anxiolytic activity of sweet orange essential oil. Physiologic alterations along the test were not prevented in any treatment group, as has previously been observed for diazepam.
Conclusions: Although more studies are needed to find out the clinical relevance of aromatherapy for anxiety disorders, the present results indicate an acute anxiolytic activity of sweet orange aroma, giving some scientific support to its use as a tranquilizer by aromatherapists.

Introduction

Anxiety disorders are the most prevalent class of psychiatric disorders in the general population.[1] However, their treatment is still challenging, as the drugs used for the relief of anxiety symptoms can have important side-effects, promote therapeutic dependence, or present a delay in their onset of action.[2] Furthermore, not all patients benefit from the available treatments, and only a few of them have a response near complete recovery.[3]

These facts justify the growing search for alternative or complementary procedures for the relief of anxiety symptoms. Among these procedures, one can find aromatherapy, which is the use of essential oils as an alternative treatment for medical purposes.[4]

According to Charlesworth,[5] about 60% of health complaints in the medical office are stress-related, and aromatherapy could be a great alternative to conventional medication since it has shown positive emotional effects. On the other hand, this therapy still does not have much scientific support.[6,7]

Double-blind, randomized, placebo-controlled clinical trials performed to evaluate the effect of essential oils on anxiety symptoms are gradually starting to appear in the literature (for a systematic review, see Cooke and Ernst[6]). However, in most of these studies, exposure to the essential oil odor was accompanied by massage. This makes it difficult to draw firm conclusions about the essential oil effect, as the massage *per se* is able to reduce anxiety scores.[8] However, in a recent study performed with rats, animals submitted to two different experimental models of anxiety, after being exposed to *Citrus sinensis* aroma, showed less anxiety than animals exposed to air only.[9] This result could not be attributed to massage, previous experience with the aroma, therapeutic relationship, or even to an unspecific effect of any aroma, as animals exposed to *Melaleuca alternifolia* essential oil did not behave differently from control animals. Thus, the probability of *C. sinensis* essential oil having a

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therapeutic action *per se* is quite high. However, whether this aroma has the same effect in humans is yet to be verified.

Bringing all this into consideration, the aim of the present work was to evaluate the potential anxiolytic effect of sweet orange *(C. sinensis*) essential oil inhalation in healthy volunteers submitted to an anxiogenic situation. Moreover, in order to determine the main components of the essential oils administered, a gas chromatography/mass spectrometry analysis was also carried out.

Materials and Methods

Participants

Forty (40) graduate student male volunteers, subjectively healthy, aged between 18 and 30, were selected for inclusion in the study using a structured clinical questionnaire and a translated and adapted version of the State-Trait Anxiety Inventory (STAI)–Part II.[10] Individuals possibly presenting pathologies that could interfere with the results, such as asthma, olfactory problems, psychiatric disorders, or chronic drug use, and individuals with trait-anxiety above 48 points were excluded.

This study was conducted with approval from the Ethics Committee in Research with Humans of the Universidade Federal de Sergipe, Brazil. Written informed consent was obtained from all participants.

Anxiogenic condition

The video-monitored Stroop Color-Word Test, as standardized by Teixeira-Silva et al.,[11] was used to elicit anxiety in the volunteers. In short, this test consists of presenting a board to the participant with 100 of the color-naming words blue, yellow, red, green, and violet organized randomly in a 10×10 matrix. Each word is printed in a color different from its meaning (for example, the word "red" printed in yellow ink). This board corresponds to the "Color-Word" card of the Stroop test.[12] To perform the task, the subject has to say, as quickly as possible and in the sequence presented, the names of the colors being seen (i.e., the color of the ink), but not the colors designated by the words. The task has to be performed in 2 minutes (maximum), and any errors are signaled with a bell. Skipping a color's sequence, hesitation in saying the color, and saying the color's "word" instead of its "ink" are all considered to be errors. The whole test is videoed and presented to the subject on a monitor during the test.

Instructions were given to the subject using a CD recording, which led them to believe that a group of professionals, located in another room, were observing them and would evaluate their performance.

Psychologic measurements

State-Trait Anxiety Inventory (STAI)[13]. This instrument is divided into two sections, each having 20 questions. The first subscale measures state anxiety, the second measures trait anxiety. The range of scores, for each subscale, is 20–80, the higher the score, the higher the anxiety. A validated Portuguese version of the STAI was used.[10] A cutoff score for high trait anxiety was derived from Gama et al.[14]; it provides normative data for male university students from our city. The mean anxiety score for this normative group was 39.6, and the standard deviation (SD) was 9.2. The cutoff between high and medium trait anxiety was set 1 SD above the mean, with the result that scores greater than 48 were classified as high anxiety and, therefore, met the exclusion criteria.

Visual Analogue Mood Scale (VAMS)[15]. This scale is composed of 16 pairs of opposite adjectives. Each pair is separated by a 10-cm line on which the subject is requested to mark the point that best represents his feelings. The 16 items are distributed into four factors: (1) tranquilization; (2) mental sedation; (3) physical sedation, and (4) other feelings and attitudes. The range of values, for each factor, is 0–40 cm. A Portuguese version, translated and validated by Zuardi and Karniol[16] and adapted by Del Porto et al.,[17] was used.

Physiologic measurements

Heart rate. Heart rate is derived from two active Ag/AgCl electrodes placed across the chest, and one ground Ag/AgCl electrode placed on the abdomen.

Electromyogram of the gastrocnemius muscle. This is derived from two active Ag/AgCl electrodes placed on the gastrocnemius muscle (part of the fight/flight response) of the nondominant leg.

The skin was cleaned with a mixture of alcohol/ether (90:10, vol/vol) before placement of the electrodes.

The recordings were made using a computerized system for monitoring physiologic responses (I-330-C2+ Physiological Monitoring System, J&J Engineering, USA).

All tests were performed in a quiet room maintained at a temperature between 23°C and 25°C (73.4°F and 77.0°F).

Treatments

Test aroma. The test aroma consisted of essential oil of sweet orange *(C. sinensis*, BioEssência, Brazil), 2.5, 5, or 10 drops ($SO_{2.5}$, SO_5, SO_{10}).

Control aroma. The control aroma was essential oil of tea tree *(M. alternifolia,* BioEssência, Brazil), 2.5 drops (TT).

Nonaromatic control. The nonaromatic control was distilled water, 2.5 drops (H_2O).

All treatments were administered by inhalation. Each drop corresponded to 25 μL added to a surgical mask, which was worn by the volunteers for 5 minutes.

A randomized double-blind design was followed. For this, a random distribution of the subjects to the experimental groups ($n = 8$) was made, using a draw, by a researcher who had no direct participation in the test sessions. In addition, the volunteers were told that they would inhale nontoxic substances extracted from plants, which could or could not have a smell. The term "aromatherapy" was never used. A control aroma, proven not to change anxiety levels in rats,[9] was used to confirm that the observed results were not due to unspecific effects of any aroma.

Throughout the entire study, the volunteers were kept ignorant of the therapeutic use of the essential oils being evaluated and about the real purpose of the Stroop test, which was to induce anxiety. Therefore, even if some volunteers could recognize the orange smell, they had no idea how the aroma was supposed to affect their performance in the test.

It is worth mentioning that both of the essential oils used are considered to be safe. Sweet orange oil is on the U.S. Food and Drug Administration's GRAS (Generally Regarded as Safe) List,[18] while tea tree oil is generally considered to be nontoxic and nonirritant, although it may cause sensitization in some individuals.[19]

Procedure

The selected volunteers attended the laboratory on 2 consecutive days. The first day was used for adaptation, and the second for the actual test.

Adaptation day. The subjects were taken to the experiment room, which was already organized and equipped with the necessary apparatuses for the execution of the test. Subsequently, the subjects were submitted to the psychologic evaluations and then to 5 minutes of physiologic recordings.

Test day. The subjects were again taken to the experiment room, where they rested for 5 minutes, after which the adaptation procedure from the first day was repeated. The psychologic and physiologic data collected were labeled as the "Pretreatment" experimental phase. Subsequently, the volunteers were conducted to another room, in order to receive one of the treatments. Here, they watched a researcher pick up one of three identical amber flasks, identified as A, B, or C, and pipette 62.5, 125, or 250 μL of its contents onto a surgical mask, which was gently attached to their face using just the top straps, so as to allow some air circulation under the mask. Immediately after the inhalation period (5 minutes), the mask was removed, put into a sealed container and taken out of the room. The volunteers were then asked the following questions: (1) Could you smell anything on the mask? (2) Can you identify what you smelled? and (3) Did you find the smell pleasant, unpleasant, or neither? They were then taken back to the experiment room and submitted to the anxiogenic condition as follows: Immediately before being given the test instructions, the participant was submitted to psychologic and 30 seconds of physiologic evaluations. These data were labeled as the "Before" experimental phase. After listening to the recorded instructions, the participant then performed the task, during which his physiologic measurements were recorded. After 50 words, a pause was made for a second set of psychologic evaluations. These new data were labeled as the "During" experimental phase. Immediately following the evaluations, the test was restarted and continued up to the last color or until the end of the scheduled time. The participant then rested for 5 minutes, after which all the physiologic and psychologic parameters were again evaluated. This final set of data was labeled as the "After" experimental phase.

The room where the aromas were administered to the volunteers was left ventilated for about 2 hours between tests.

Gas chromatography/mass spectrometry (GC/MS) analysis of the essential oils

GC/MS analysis was performed as described in a previous article.[9]

Statistical analyses

The data collected during the adaptation phase were not analyzed as this phase was only intended to habituate the participants to the environment and apparatus that would be used on the following day.

All the psychologic data obtained during the test were analyzed by Friedman's analysis of variance (ANOVA), for each treatment group, followed by Tukey-type test for *post hoc* comparisons.

All the physiologic data obtained were analyzed using a two-factor ANOVA for repeated measures followed by Tukey's test for *post-hoc* comparisons.

The STAI-Trait scores used in the selection process were analyzed by Kruskal-Wallis ANOVA.

The familiarity and hedonicity of the aromas were also compared by Kruskal-Wallis ANOVA. For this, the subjects who recognized the smell scored "1" and those who did not recognize the smell scored "0". In the same way, the subjects who found the smell pleasant scored "2," the ones who found the smell unpleasant scored "0," and the ones who found the smell neither pleasant nor unpleasant scored "1."

All significance tests were two-tailed and were performed at the 5% significance level.

Results

Aroma perception

The aroma perception scores obtained for each treatment group are shown in Table 1.

The ANOVA revealed no significant differences among the treatment groups regarding either familiarity ($p = 0.47$) or hedonicity ($p = 0.32$) of the aromas.

In the groups SO and TT, all the volunteers sad "yes" to the question "Could you smell anything on the mask?", while in the group H_2O, only 2 subjects said "yes" to the same question, although they could not identify the smell nor did they find it either pleasant or unpleasant.

Psychologic measurements

Data are presented as median ($\tilde{x}$) and interquartile range (Q_1–Q_3).

Table 1. Aroma Perception

	Aroma perception scores	
Treatment	*Familiarity (Can you identify what you smelled?)*	*Hedonicity (Did you find the smell pleasant, unpleasant or neither?)*
$SO_{2.5}$	0.5 (0.0–1.0)	2.0 (1.8–2.0)
SO_5	1.0 (1.0–1.0)	2.0 (2.0–2.0)
SO_{10}	1.0 (0.0–1.0)	2.0 (1.0–2.0)
TT	1.0 (0.0–1.0)	2.0 (1.8–2.0)

Data are presented as median and interquartile range (Q_1–Q_3). $SO_{2.5}$, SO_5, SO_{10} represent essential oil of sweet orange 2.5, 5, or 10 drops.

Familiarity scores: Subjects who identified the smell scored "1" and those who did not scored "0".

Hedonicity scores: Subjects who found the smell pleasant scored "2", the ones who found the smell unpleasant scored "0", and the ones who found the smell neither pleasant or unpleasant scored "1".

SO, essential oil of sweet orange; TT, essential oil of tea tree.

ANOVA results are described here, while *post hoc* results are shown in Table 2.

It is worth calling attention to the fact that the treatment's effect is revealed by the lack of significant alterations in the observed parameters throughout the test.

STAI-trait. Kruskal-Wallis ANOVA found no significant differences in trait-anxiety ($p = 0.79$) among treatment groups [$\tilde{x}$ (Q_1–Q_3): $SO_{2.5} = 34.0$ (31.0–38.0); $SO_5 = 32.0$ (30.8–38.0); $SO_{10} = 33.5$ (29.8–35.8); TT = 35.5 (32.8–37.3); $H_2O = 36.0$ (33.0–37.5)].

STAI-state. Friedman's ANOVA revealed no significant differences among experimental phases for the groups $SO_{2.5}$ ($p = 0.063$) and SO_{10} ($p = 0.098$), although differences were found for groups SO_5 ($p < 0.001$), TT ($p = 0.001$) and H_2O ($p = 0.005$).

VAMS–tranquilization. Friedman's ANOVA revealed no significant differences among experimental phases for the group SO_{10} ($p = 0.062$), although differences were found for groups $SO_{2.5}$ ($p = 0.015$), SO_5 ($p = 0.003$), TT ($p = 0.007$), and H_2O ($p = 0.018$).

Table 2. Summary of the Psychologic Results

		Experimental phase			
Parameter	*Treatment*	*Pretreatment*	*Before*	*During*	*After*
STAI-state (points)	**$SO_{2.5}$**	**30.5 (25.0–32.5)**	**26.5 (23.5–31.5)**	**37.0 (30.0–40.2)**	**33.0 (30.0–34.0)**
	SO_5	31.5 (30.3–33.0)	28.5 (26.5–31.0)[a]	36.5 (30.3–41.3)[b]	31.5 (28.5–35.3)[b,c]
	SO_{10}	**32.5 (30.7–36.2)**	**32.0 (27.5–33.2)**	**39.0 (35.2–41.2)**	**33.0 (32.5–34.5)**
	TT	30.0 (27.0–33.2)	28.0 (24.0–29.2)[a]	36.5 (31.7–39.0)[b]	28.5 (26.2–34.2)[b,c]
	H_2O	32.5 (30.5–36.0)	32.5 (30.5–35.8)	41.0 (32.3–43.3)[b]	36.0 (30.3–42.3)[b,c]
Tranquilization (cm)	$SO_{2.5}$	34.4 (31.9–37.3)	35.9 (33.0–37.9)[a]	25.8 (23.8–30.7)[b]	33.1 (30.9–34.9)[b,c]
	SO_5	33.8 (32.3–35.6)	35.1 (33.4–38.5)[a]	31.0 (24.0–33.0)[b]	32.1 (27.8–36.5)[b,c]
	SO_{10}	**34.3 (25.1–38.1)**	**32.1 (29.0–38.8)**	**23.3 (21.5–30.4)**	**33.6 (24.0–37.8)**
	TT	35.0 (29.7–36.8)	35.6 (31.4–36.1)	27.0 (23.3–30.6)[b]	34.3 (29.4–36.2)[c]
	H_2O	31.8 (27.8–35.2)	29.2 (27.3–33.5)[a]	26.8 (19.3–28.7)[b]	29.0 (23.2–30.1)[b,c]
Tranquilization: tense/ relaxed item (cm)	**$SO_{2.5}$**	**8.7 (7.6–9.3)**	**9.1 (8.8–9.2)**	**6.2 (4.2–7.9)**	**8.1 (7.3–8.4)**
	SO_5	8.6 (8.2–9.6)	8.6 (7.9–9.5)	7.1 (5.8–8.1)[b]	7.8 (7.4–8.9)[b,c]
	SO_{10}	**8.1 (6.3–10.0)**	**8.6 (7.3–9.9)**	**6.0 (4.4–6.7)**	**8.4 (6.2–9.5)**
	TT	8.5 (7.0–9.4)	8.9 (7.9–9.3)[a]	5.9 (4.0–7.9)[b]	8.6 (7.9–9.2)[b,c]
	H_2O	8.4 (8.0–9.6)	7.9 (7.3–8.8)[a]	7.0 (3.7–7.5)[b]	6.6 (4.7–7.8)
Mental sedation (cm)	**$SO_{2.5}$**	**12.2 (7.2–17.7)**	**11.3 (4.7–13.6)**	**9.1 (6.5–10.5)**	**10.1 (6.1–12.4)**
	SO_5	**9.5 (5.0–14.0)**	**9.6 (6.2–14.9)**	**9.2 (5.4–11.7)**	**10.7 (7.6–12.7)**
	SO_{10}	**11.1 (9.9–12.2)**	**14.0 (9.2–20.2)**	**9.4 (3.2–13.1)**	**8.6 (3.4–15.5)**
	TT	**10.3 (7.5–14.0)**	**8.3 (5.3–11.9)**	**10.5 (6.8–13.5)**	**8.7 (5.0–12.0)**
	H_2O	**12.8 (6.4–16.9)**	**7.0 (4.8–13.0)**	**9.9 (7.6–13.3)**	**10.6 (6.4–12.6)**
Physical sedation (cm)	**$SO_{2.5}$**	**12.1 (7.8–14.5)**	**5.7 (5.0–9.6)**	**6.2 (4.2–8.2)**	**9.4 (7.0–10.9)**
	SO_5	**6.2 (5.5–10.8)**	**7.2 (5.8–11.0)**	**7.8 (5.8–11.6)**	**7.1 (5.7–13.0)**
	SO_{10}	8.6 (2.4–12.5)	9.3 (2.3–15.6)[a]	7.3 (0.7–11.2)[b]	7.7 (1.2–12.9)[b,c]
	TT	**8.0 (6.7–10.6)**	**7.2 (5.8–12.1)**	**8.6 (6.5–12.1)**	**7.1 (4.5–11.0)**
	H_2O	**10.9 (6.5–14.0)**	**7.6 (3.6–13.8)**	**8.0 (5.5–10.3)**	**7.9 (3.1–10.1)**
Physical sedation: lethargic/energetic item (cm)	**$SO_{2.5}$**	**3.6 (1.6–4.6)**	**2.1 (1.5–2.6)**	**1.8 (1.3–2.1)**	**2.3 (1.7–3.0)**
	SO_5	**2.8 (1.2–3.7)**	**2.9 (1.8–4.2)**	**2.9 (1.9–4.5)**	**2.7 (1.3–3.8)**
	SO_{10}	3.2 (2.1–3.5)	3.1 (1.7–3.8)	1.7 (0.2–2.9)[b]	1.5 (0.4–3.4)[b,c]
	TT	**2.6 (1.5–4.3)**	**2.5 (2.1–3.6)**	**2.5 (1.2–4.1)**	**1.7 (0.9–3.1)**
	H_2O	**3.4 (1.9–4.9)**	**2.4 (1.5–3.1)**	**1.9 (1.2–3.1)**	**2.2 (0.9–3.1)**
Others feelings and attitudes (cm)	**$SO_{2.5}$**	**4.2 (3.3–6.5)**	**4.1 (3.1–7.3)**	**4.5 (3.7–7.5)**	**3.6 (3.5–5.9)**
	SO_5	**4.1 (2.9–6.3)**	**4.4 (2.0–7.6)**	**5.2 (2.0–8.2)**	**5.7 (3.2–7.4)**
	SO_{10}	5.8 (2.7–7.3)	7.5 (4.0–10.1)[a]	7.3 (3.2–10.3)	6.7 (2.6–9.9)[c]
	TT	**4.5 (3.0–10.3)**	**4.4 (3.2–9.7)**	**7.1 (5.1–9.2)**	**5.5 (3.2–8.8)**
	H_2O	**5.8 (3.5–11.7)**	**9.2 (4.1–11.3)**	**7.7 (4.2–10.9)**	**7.1 (3.6–11.5)**
Others feelings and attitudes: withdrawn/ gregarious item (cm)	**$SO_{2.5}$**	**0.7 (0.3–1.6)**	**0.7 (0.4–1.5)**	**1.1 (0.6–1.7)**	**0.8 (0.3–1.3)**
	SO_5	**0.5 (0.4–1.6)**	**0.5 (0.2–0.9)**	**1.0 (0.6–1.2)**	**0.9 (0.4–1.3)**
	SO_{10}	1.2 (0.2–1.9)	1.5 (0.3–2.2)[a]	1.7 (0.4–3.0)[b]	1.3 (0.2–2.6)[c]
	TT	**1.4 (0.7–1.8)**	**1.2 (0.4–1.9)**	**1.8 (1.2–2.3)**	**1.5 (0.7–2.2)**
	H_2O	**1.8 (0.5–2.9)**	**1.8 (0.9–2.8)**	**1.7 (0.6–2.3)**	**1.5 (0.6–2.3)**

Data are presented as median and interquartile range (Q_1–Q_3). The treatments in bold are those that showed no significant differences in state-anxiety among the experimental phases. $SO_{2.5}$, SO_5, SO_{10} represent essential oil of sweet orange 2.5, 5, or 10 drops.

[a]Significantly different from "Pretreatment."

[b]Significantly different from "Before."

[c]Significantly different from "During."

[a,b,c]$p < 0.05$.

VAMS–tranquilization: Tense/relaxed item. Friedman's ANOVA revealed no significant differences among experimental phases for groups $SO_{2.5}$ ($p=0.078$) and SO_{10} ($p=0.126$), although differences were found for groups SO_5 ($p=0.044$), TT ($p=0.003$), and H_2O ($p=0.003$).

VAMS–mental sedation. Friedman's ANOVA revealed no significant differences among test phases for all of the groups [$SO_{2.5}$ ($p=0.327$); SO_5 ($p=0.985$); SO_{10} ($p=0.060$); TT (p = 0.832); and H_2O ($p=0.369$)].

VAMS–physical sedation. Friedman's ANOVA revealed no significant differences among test phases for the groups $SO_{2.5}$ ($p=0.615$), SO_5 ($p=0.403$), TT ($p=0.138$), and H_2O ($p=0.271$). However, differences were found for the group SO_{10} ($p=0.009$). Separate analyses of the individual items of this category, for this group, showed differences among test phases for the lethargic/energetic item ($p=0.008$).

VAMS–other feelings and attitudes. Friedman's ANOVA revealed no significant differences for the groups $SO_{2.5}$ ($p=0.145$), SO_5 ($p=0.814$), TT ($p=0.110$), and H_2O ($p=0.618$). However, differences were found for the group SO_{10} ($p=0.027$). Separate analyses of the individual items of this category, for this group, showed differences among test phases for the withdrawn/gregarious item ($p=0.002$).

Physiologic measurements

Data are presented as mean and standard error of the mean in Table 3.

Heart rate. The ANOVA revealed that the interaction between test phase and treatment was not significant ($p=0.641$); therefore the two main effects were analyzed. The test phase effect was significant ($p<0.001$), in that the heart rate was greater "During" in relation to "Before" ($p<0.001$). The treatment effect was not significant ($p=0.525$).

Gastrocnemius electromyogram. The ANOVA revealed that the interaction between test phase and treatment was not significant ($p=0.653$); therefore the two main effects were analyzed. The test phase effect was significant ($p<0.001$), in that the muscular tension was greater "During" in relation to "Before" ($p=0.001$). The treatment effect was not significant ($p=0.126$).

CG/MS analysis. The volatile composition of sweet orange and tea tree essential oils is shown in Tables 4 and 5, respectively.

Discussion

The aim of this work was to evaluate the potential anxiolytic effect of sweet orange *(C. sinensis)* essential oil in healthy volunteers submitted to an anxiogenic situation. The experimental model of anxiety, as employed here, was shown to be valid, since the subjects from the control groups behaved as expected,[11] significantly increasing their anxiety signs/symptoms during the Stroop task.

The obtained results showed the anxiolytic properties of sweet orange essential oil through the lack of a significant increase in state-anxiety and tension, and the lack of a significant decrease in tranquility, presented by the individuals exposed to the aroma. Of the two doses (2.5 and 10 drops) that demonstrated anxiolytic activity, the higher one seemed to be better at preventing anxious symptoms, as its results were further from reaching statistical significance and were observed in a greater number of parameters. It is reasonable to question the fact that the intermediate dose failed to produce an effect. However, this activity profile was observed before, when the same aroma was tested in animals.[9] This lack of a dose/effect relation is common when dealing with a mixture of compounds instead of a pure substance.

Interestingly, the observed anxiolytic effects were not followed by sedative/hypnotic effects. On the contrary, at the highest dose, sweet orange oil made the volunteers feel more energetic while performing the test. They also felt more introverted. It is tempting to speculate that these two symptoms together mean that the subjects were more concentrated on the task, which would be a welcome side-effect, especially as tranquilizers tend to cause concentration loss. Nevertheless, this should be further investigated.

The GC/MS analysis determined that limonene corresponded to 54.48% of the volatile components of our sweet orange oil sample. Previous studies, performed with rodents, using essential oils that were practically pure limonene (>97%),[9,20] have shown very clear anxiolytic effects,

Table 3. Summary of Physiologic Results

Parameters	*Treatment*	*Experimental phase*			
		Pretreatment	*Before*	*During*	*After*
Heart rate (beats/min)	$SO_{2.5}$	76.7±3.5	70.5±3.2	108.8±9.4	77.7±3.7
	SO_5	71.5±4.5	69.9±3.6	95.7±7.6	72.1±5.1
	SO_{10}	76.5±5.2	74.9±5.3	107.1±10.8	74.5±5.0
	TT	80.3±7.0	79.5±7.4	127.1±20.8	75.1±4.9
	H_2O	69.5±2.9	69.9±3.9	103.1±10.3	70.6±2.5
Electromyogram of the gastrocnemius muscle (μV)	$SO_{2.5}$	1.1±0.3	0.9±0.2	2.3±0.5	0.9±0.3
	SO_5	3.1±1.0	2.1±0.6	3.7±0.8	1.7±0.7
	SO_{10}	1.5±0.4	0.8±0.2	3.6±1.5	1.0±0.3
	TT	1.0±0.2	1.7±0.5	2.2±0.6	1.6±0.5
	H_2O	2.7±0.7	1.8±0.4	4.0±0.6	2.5±0.5

Data are presented as means±standard error of the mean.
$SO_{2.5}$, SO_5, SO_{10} represent essential oil of sweet orange 2.5, 5, or 10 drops. TT, essential oil of tea tree.

Table 4. Volatile Composition of *Citrus sinensis* Essential Oil

Peak	*RT (min)*	*Compounds*	*(%)*	*RI*
1	9.958	Limonene	54.48	1027
2	13.342	*trans-p*-Mentha-2,8-dien-1-ol	2.00	1120
3	13.892	*cis-p*-Menth-2,8-dienol	2.97	1135
4	15.458	NI	0.83	1177
5	16.100	NI	1.18	1195
6	16.233	NI	2.04	1198
7	16.942	*trans*-Carveol	5.59	1218
8	17.425	*cis*-Carveol	1.98	1231
9	17.842	Carvona	7.65	1243
10	19.017	NI	0.68	1275
11	19.517	NI	1.12	1289
12	21.475	Limonene-1,2-diol	13.47	1345
13	23.558	NI	4.67	1405
14	24.842	NI	1.34	1444
		Total	100.00	

RT, retention time; RI, retention índex; NI, nonidentified compounds.

indicating this monoterpene as the responsible constituent for the anxiolytic activity of citrus oils. Therefore, it is possible that, in the present study, a purer sweet orange oil sample (around 90% limonene) could have led to more robust results. It is also possible that the duration of exposure to the essential oil needs adjustments, as aromatherapists' recommendations vary from a few breaths to a few minutes. For these reasons, future studies must be designed in order to optimize the posology of sweet orange aroma for the relief of anxiety symptoms.

Nevertheless, the present study makes it clear that sweet orange essential oil has anxiolytic properties in humans, corroborating a previous study by Lehrner et al.,[21] in which female patients exposed to this oil, diffused in a waiting room before a dental procedure, showed lower levels of state anxiety compared to control patients who were exposed to air only.

Table 5. Volatile Composition of *Melaleuca alternifolia* Essential Oil

Peak	*RT (min)*	*Compounds*	*(%)*	*RI*
1	6.575	α-Tujene	0.58	924
2	6.800	α-Pinene	2.17	931
3	8.208	β-Pinene	0.55	976
4	8.600	Myrcene	0.65	988
5	9.550	α-Terpinene	6.97	1016
6	9.825	*p*-Cymene	5.30	1024
7	9.983	Limonene	1.02	1027
8	10.100	1,8-Cineole	4.31	1031
9	11.050	γ-Terpinene	17.69	1058
10	12.017	Terpinolene	2.76	1084
11	15.633	Terpinen-4-ol	49.83	1082
12	16.108	α-Terpineol	3.79	1194
13	23.992	(E)-Caryophyllene	0.37	1417
14	24.617	Aromadendrene	1.19	1437
15	25.325	Allo-aromadendrene	0.49	1458
16	26.350	Viridiflorene	0.59	1489
17	26.500	NI	0.37	1494
18	27.217	δ-Cadinene	0.95	1517
19	29.350	Globulol	0.42	1585
		Total	99.26	

RT, retention time; RI, retention índex; NI, nonidentified compounds.

It could be argued that Lehrner's results were only due to unspecific effects of any aroma, as his study did not use any aromatic control and tested only a single oil dose, which was not very precise. The results presented here serve to rule out this possibility, as tea tree essential oil did not show any anxiolytic activity. Still, it could be argued that tea tree was not a very good aromatic control, as it may not have been as familiar to the volunteers as the orange smell, and familiarity might have provided comfort to the subjects. However, statistical analysis showed no differences between the orange and tea tree essential oils in this respect. This result was probably due to the fact that tea tree essential oil has an astringent, camphorous scent, similar to eucalyptus and other medicinal plants. Thus, despite the fact that the volunteers may not have known exactly what the scent was, it was not unfamiliar to them. Another characteristic of the oils that could have interfered with the results is their hedonicity, as pleasant aromas may induce a state of well-being in people. But again, there were no significant differences between orange and tea tree, with both aromas generally being considered pleasant. Therefore, it is reasonable to believe that the anxiolytic effects observed in this study were due to sweet orange essential oil *per se*, with the strongest argument in favor of this idea being the fact that not all doses of this oil were effective.

Regarding the physiologic parameters, there was no prevention of the stress response to the Stroop test in any of the treatment groups. However, this result does not weaken the case for sweet orange aroma as an anxiety reducer, since diazepam, a classic anxiolytic drug, is also unable to prevent physiologic alterations provoked by anxiogenic situations.[11,22,23]

Nevertheless, it is worth pointing out that this research does present some sample-related limitations. First, all subjects were graduate students, under 30 years of age. This was not only because it was convenient to recruit these volunteers, but also because the model of anxiety used was validated for this population.[11] Still, it would be valuable to test the effects of sweet orange aroma in subjects with different levels of education and in different age groups. Second, only men were selected for this study and, despite the fact that the anxiolytic effect of sweet orange aroma in women has been shown before,[21] it would be interesting to test women in the conditions presented here, especially if the menstrual cycle was controlled. Third, the sample size was rather small and, although it was large enough to give the experiment sufficient power to detect statistical differences, future studies with larger sample sizes could reinforce the results.

Conclusions

In conclusion, although more investigations are necessary in order to clarify the clinical relevance of sweet orange essential oil as an anxiety treatment, the present work has strengthened the idea that this oil has anxiolytic properties that cannot be accredited to expectancy biases, massage, or therapeutic relationship which, according to some

authors,[8,24] could be responsible for most of the aromatherapy outcome.

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