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Original article

Lung function and exercise performance assessment after inhalation of naturally essential oils: A descriptive observational study

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Background: Essential oils have gained massive attention. We aimed to assess the effects of inhalation of *Clinopodium serpyllifolium* and *Cymbopogon citratus* volatile oils on exercise performance and lung function.
Methods: Twenty physical education students were randomly assigned into two groups: *Clinopodium serpyllifolium* and *Cymbopogon citratus* (10 participants each). One group was nebulized with *Clinopodium serpyllifolium* oil and the other by *Cymbopogon citratus* oil at a concentration of (0.02 ml/kg of body mass) which was mixed with 2 ml of normal saline before 800 m running tests. Lung function tests were measured using a spirometer pre and post nebulization.
Results: There was a significant increase in Forced Expiratory Volume in the first second and Forced Vital Capacity after inhalation of both oils. The normal spirometry after inhalation of *C. serpyllifolium* oil increased by 30%. A sharp decrease in mild spirometry (50% before to 10% after inhalation of the *C.citratus* oil) was observed. Significant reductions in the means of the running time were observed.
Conclusion: Our findings support the effectiveness of *C. serpyllifolium* and *C. citratus* essential oils on exercise performance and respiratory function parameters.

Keywords: *Clinopodium serpyllifolium, Cymbopogon citratus*, essential oil, lung function, athletic performance.

The plant kingdom is considered an essential part of human being life and it is impossible to live without them. Recently, huge numbers of researchers have been focusing on the phytogenic products due to their importance for solving many pharmacological problems and for investigation of new drugs and other none therapeutic applications.[1, 2] Volatile oils (essential oils) are odorous principles with aromatic odor and most of them are phytogenic compounds that evaporate at ordinary temperature and have been used since ancient times in the manufacturing of perfumes, flavoring agents, food preservatives and medicaments.[3, 4] They are considered secondary metabolic compounds which are biosynthesized in specialized structures for various proposes, such as pollination, protection, communication and signaling between other plants. They have been used pharmaceutically for various aims such as local analgesics (eugenol), decongestants (eucalyptol), expectorants (carvacrol), antiseptics (menthol), anthelmintic (ascaridol), and many of them used as

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flavoring agents in the pharmaceutical industry to get rid of unpleasant odors and tastes of drugs.[5, 6] Many of the volatile oils have been used in folk medicine for the treatment and prevention of various upper respiratory system diseases such as *Clinopodium serpyllifolium* and *Cymbopogon citratus* volatile oils.[7,8] *Clinopodium serpyllifolium* subsp. *fruticosum* (L.) Bräuchler, is a synonym name for *Micromeria fruticosa* (L.) Druce which is a member of the Lamiaceae family and commonly known as White leafed Savory, or White Hyssop, or White micromeria. It is a perennial herbaceous plant which is endemic to Palestine, Syria, Lebanon, Jordan and Turkey, and has grey, egg shaped, opposite leaves and small white flowers on its stems.[9, 10] Its volatile oil contains a mixture of monoterpenoid (+)-pulegone, isomenthol, isomenthone, menthol, pinene limonene, piperitone, and piperitenone oxide, as well as sesquiterpenoids such as caryophyllene and germacrene-D.[11] The aerial parts of the plants are widely used in various Mediterranean regions as infusions and decoctions for the treatment of various diseases including gastrointestinal spasms, diarrhea, ophthalmic inflammations, cardiac disorders, hypertension, open wounds, colds and other respiratory system inflammations.[12, 13] It has been shown that these plant volatile oils exhibited activity against 14 bacteria, three fungi and a yeast, and its methanolic extracts exhibited significant antioxidant activity in DPPH 1,1-Diphenyl-2-picryl-hydrazyl and inhibiting linoleic acid oxidation assays. [14] *Cymbopogon citratus* (DC.) Stapf, commonly known as lemon grass or citronella grass belongs to the Poaceae family which is a perennial herbaceous plant widely distributed throughout the world especially in semi-tropical and tropical regions. Its decoction is used as an antibacterial, antifungal, antipyretic, stomachic, carminative and sedative. Various studies have supported its sedative, CNS depressant, analgesic, antibacterial and antifungal activities.[15 - 19] In the light of this, the present study looks at the inhalation of two species used in folk medicine; *C. serpyllifolium* and *C.citratus* volatile oils in two different groups of male athlete students to assess their exercise performance and lung function using a spirometric pre-and post test assessment design.

Materials and methods

Ethical considerations

The study aims, protocols, and the informed consent forms were approved by the Institutional Review Board (IRB) at An-Najah National University (IRB archived number: 11/August/2016). The study was conducted in accordance with the requirements of the declarations of Helsinki. All subjects gave their signed informed consent before they entered into the study. Subjects were not offered any incentives and they were able to withdraw from the study at any time. The data obtained were kept confidential. Subjects were assured that refusal to participate in the study would not affect their future healthcare carrier in any way. Two trained research assistants conducted the face-to-face interviews with the subjects.

Subjects and study design

Twenty male university students from the Faculty of Physical Education, An-Najah National University in Nablus, Palestine volunteered and were randomly assigned into two different groups (10 subjects each) to take part in the experiments using a non-randomized quasi-experimental uncontrolled before-and-after study design for each group. The study was single blind at the level of participants. One group (10 students) was nebulized with *C. serpyllifolium* oil, while the other group (10 students) was nebulized with *C. citratus* oil (0.02 ml/kg of body mass of each oil) mixed with 2 ml of normal saline 5 min before an 800 m running test according to the method validated by Spencer and Gastin, 2001.[16] Traditionally, it was thought that in the middle distance track running the relative energy system was 75% aerobic and 22% anaerobic, and 2 - 3% of the energy came from the creatine-phosphate system. But recently, the aerobic energy system is thought to contribute to significantly to the energy supply during middle distance running and long sprint event. Moreover, the relative anaerobic and aerobic system contributions of the four events (200 m, 400 m, 800 m, and 1500 m). Middle distance running, such as the 800 m event, depend on the aerobic (84%) and anaerobic energy systems, which is greater than traditionally thought, so during this event the need of oxygen increases with the event duration from 200 m to a 1500 m running race.[16] Moreover, the 800 m distance was required to ensure the duration of the effect of the oils (if any) through the inhaled route of administration and the greater distances could have been taken into consideration if the interventions were given orally or by another route of administration However, our estimation was that this distance could be representable of such route of administration, given at the same time, and that the same group conducted the same running pre and post intervention. A washout period of three days (from Sunday to Wednesday) between pre and post tests for each group was given to rule out any fatigue or significant impact on our results of pre and post tests. Lung function tests were measured using a spirometer for each student pre and post nebulization. In each stage, two measurements were assessed and the average was taken. The lung function tests were assessed 3 min after inhalation for both NaCl and the oil. The study was carried out in October, 2016. All subjects we first aligned at a straight horizontal line as the same starting point for all, and at the end of the 800 m another exactly straight horizontal line was drawn. The running moment was given to all at the same point in time and when the subject arrived at the end, his exact running time in seconds was calculated depending on the starting time. This running procedure was adapted for all subjects at the same time.

Instrumentations

During this study, the following instruments were used: ultrasonic-microwave cooperative extractor/reactor (CW-2000, China), balance (Radw ag, AS 220/c/2, Poland), Philips respironics inhalators nebulizers (75644321, China) and Care-fusion spirometer (ME44QY 08563848, UK)

Collection and preparing plant materials

C. serpyllifolium and *C.citratus* plants were collected during its flowering time from the Jerusalem region (Palestine) in April, 2016. Botanical identification was carried out by Pharmacognosist Dr. Nidal Jaradat from the Pharmacognosy and Herbal Products Laboratory, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus. The identification process was conducted using live herbal specimens and photographs from books. Voucher specimens were deposited in the Pharmacognosy and Herbal Products Laboratory under the code numbers: Pharm-PCT-1575 for *C. serpyllifolium* and was Pharm-PCT-2791 for *C. citratus*. To extract volatile oil, the aerial parts of *C. serpyllifolium* and *C. citratus* were separated carefully and then washed twice with distilled water.

Essential oils extraction

The essential oil of *C. serpyllifolium* was extracted using a microwave oven as described by Jaradat, 2016 with some modifications.[15] The power of the microwave oven was set at 1000 W. Clevenger apparatus with a 1L round-bottom flask containing 100g of *C. serpyllifolium* was placed inside the microwave oven. About 500 ml distilled water was then added into the flask containing the powder. The flask was then connected to the Clevenger apparatus. Microwave distillation was carried out three times for 15 min each at 100^{O}C. The obtained volatile oil was collected in a clean beaker and chemically dried. Then the purified essential oil was weighed and stored in tightly-closed amber-colored bottles at 4^{O}C in the refrigerator. The same procedure was repeated for extraction of *C. citratus* essential oil.

Methodology

The subjects were familiarized with the spirometry. Lung function tests (LFTs) were measured using a spirometer for each student pre and post inhalations with *C. serpyllifolium* and *C. citratus* oils. The spirometer is used to differentiate between obstructive and restrictive diseases, and assess the degree of associated changes.[17, 18] Such parameters include Forced Expiratory Volume in the first second (FEV1) and Forced Vital Capacity (FVC). The specificity and sensitivity of the spirometer in the diagnosis of obstructive lung disease are reported as 84% and 92%, respectively.[19] While used in the diagnosis of restrictive lung disease, it has a sensitivity and specificity of 42.2% and 94.3%, respectively.[20] FEV1 is the maximum air volume exhaled with maximal effort in the first second from a position of full inspiration. This value declines less severely with restrictive diseases than obstructive diseases. FVC, on the other hand, is the maximal air volume exhaled with maximal effort from a position of full inhalation.[21], and is reduced by an airflow obstruction and ventilation restriction, which results from lung-exterior factors such as skeletal pains or intrinsic lung disease, especially restrictive ones.[22] In the latter, there is a decline in the lung compliance associated with the presence of partial or diffuse lung fibrosis. These fibrotic changes render the lung smaller and stiffer, leading to a decrease in the FVC. The FEV1/FVC ratio is reduced in obstructive patterns, but it is normal or even increased in restrictive patterns as both the nominator and denominator proportionally change.

The classification criteria for normal, mild and moderate restriction were based on the American thorax society depending on the FEV1 value. Therefore, the severity of any spirometric abnormality was based on the FEV1 (%predicted) as follows: mild >70%, moderate 60-69%, moderately severe 50-59%, severe 35-49% and very severe <35%.

Data collection form

Different sections were included. The first was the demographic section, which contained questions regarding age, gender, education level, health history, smoking status and body mass index. Then, spirometry was performed using a spirometer apparatus. Regular guidelines for spirometer testing were followed.[23] The subjects were seated during the test, with the nose clipped to prevent air leakage through the nares. Three attempts for each subject were allowed, and the best spirograph was selected automatically by the spirometer. Forced spirometry was measured for each subject. For each subject, FEV1, FVC, FEV1/FVC ratios were assessed.

Statistical analysis

All data were entered and analyzed using the Statistical Package for the Social Sciences (SPSS) version 16. The main study outcomes were tested for normality using Shapiro-Wilk significant test and found not to be normally distributed. Qualitative variables have been expressed as frequency tables and bar charts. For non-parametric two-related samples Wilcoxon test was used to test for the differences in the means between pre- and post-inhalers of the continuous outcomes. Data were expressed as mean ± standard deviation (SD).A P - value < 0.05 was considered statistically significant.

Results

Socio-demographic characteristics

All subjects were single. The mean age of the *C. serpyllifolium* group subjects was 19.20 ± 1.23 years; 30% of them were smokers of 1-10 cigarettes per day. In the *C. citratus* group, however, the mean age was 20.70 ± 1.25 years and the majority (60%) were smokers of 1-10 cigarettes per day. Table 1 shows the socio-demographic characteristics details of the studied subjects in each group.

Respiratory parameters assessment

A lung function test showed an improvement on the lung status for the students after their inhalation of the oils. Figure 1 shows that the normal spirometry results were 30%, while after inhalation with *C. serpyllifolium* oil the ratio was 60%. The mild spirometry dropped from 70% before to 40% after inhalation of *C. serpyllifolium* oil. On the other hand, there was also an increase in normal lung status of the students who inhaled *C. citratus* oil (40% before and 60% after). There was also a sharp decrease in mild spirometry (50% before inhalation to 10% after inhalation of the *C. citratus* oil) (Figure 2). Interestingly, Table 2 shows that there is a statistically significant increase in FEV1 and FVC in the post-test students after their inhalation of the *C. serpyllifolium* and *C. citratus* oils. Regarding the FEV1/FVC ratios, there was a strong significant decrease toward normal in *C. serpyllifolium* oil participants, but only a weak and borderline decrease (not significant) toward normal in *C. citratus* oil participants.

Running performance

There was a statistically significant reduction in the mean running time after inhalation of *C. serpyllifolium* oil and *C.citratus* oil among the study participants (P < 0.005 and 0.025, respectively) as shown in Table 3.

Table 1. Socio-demographic characteristics of the study population given *C. serpyllifolium* and *C. citratus* oils (N=10 for each group).

Variables	***C. serpyllifolium* oil group**	***C. citratus* oil group**
	n (%)*	
Gender		
-Male	10 (100)	10 (100)
Marital status		
-Single	10 (100)	10 (100)
Smoking status (cigarette/day)		
-Non-smokers	4 (40)	4 (40)
-1-10	3(30)	6 (60)
-11-20	3(30)	0 (0)
Age (year)	19.20 ± 1.23**	20.70 ± 1.25**
Weight (kg)	72.80 ± 5.88**	71.90 ± 9.07**
Height (meter; m)	1.79 ± 0.036**	1.74 ± 0.044**
BMI (kg/m^2)	22.59 ± 2.08**	23.40 ± 2.12**

*Data is presented as frequency (percent) (N=10). **Mean ± standard deviation.

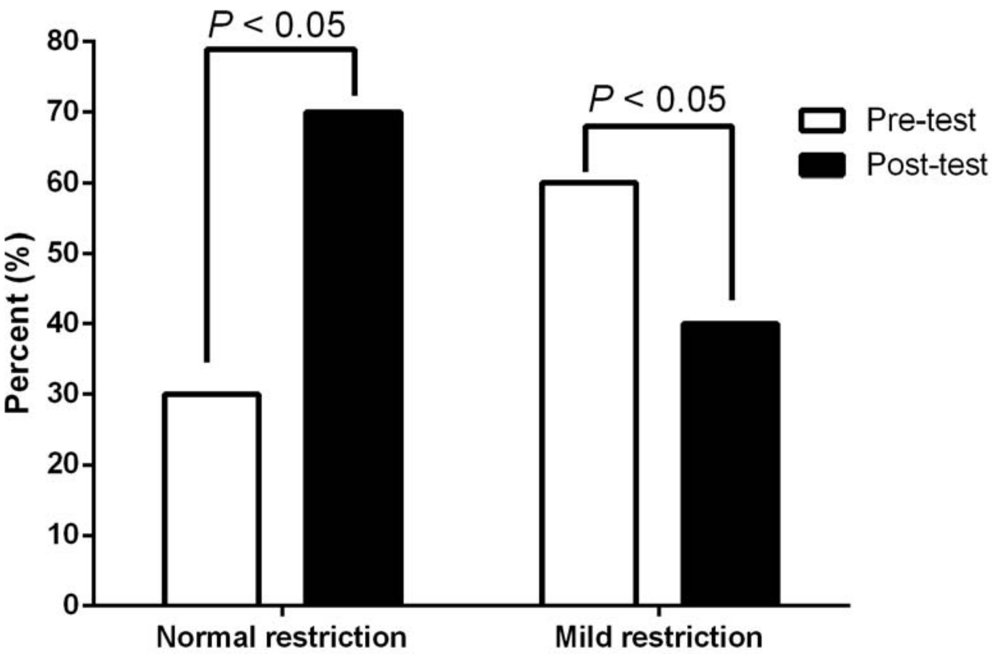

Figure 1. Respiratory parameters for participants before and after inhalation of *C. serpyllifolium* oil (as percent)

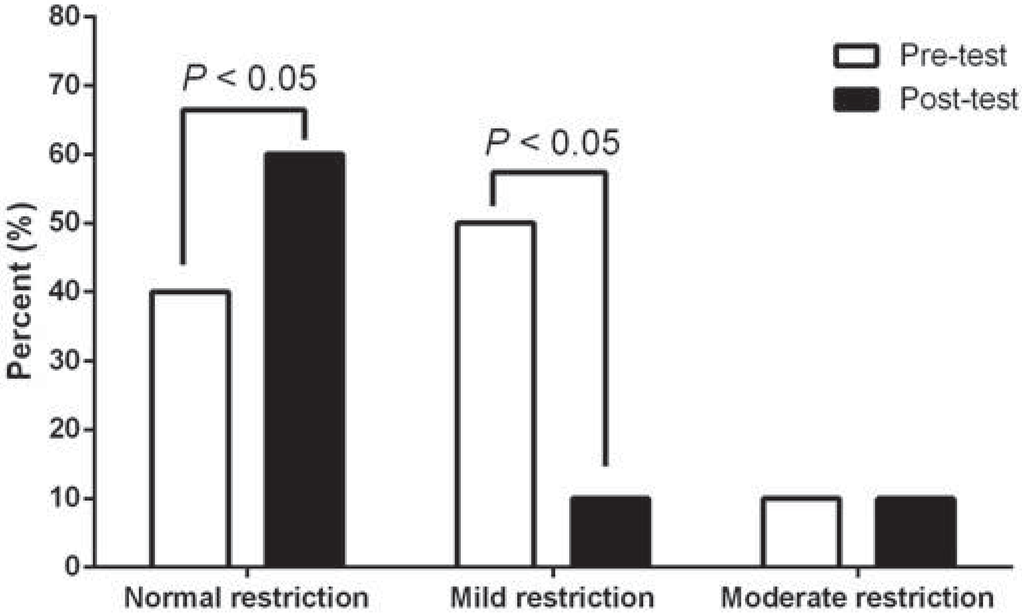

Figure 2. Respiratory parameters for participants before and after oil inhalation of *C. citratus* oil (as percent)

Table 2. Respiratory parameters of the post- and pre-inhaler measurements for the study outcomes of the participants given *C. serpyllifolium* (n=10) and *C. serpyllifolium* oils (n=10) when running an equal distance of 800 m before and after.

Outcome Variables	C. serpyllifolium oil group			*C. citratus* oil group		
	Mean ± SD*	Mean difference ± SD	*P* - value§	Mean ± SD*	Mean difference ± SD	*P* - value§
FEV1						
- Post-inhaler	4.76 ± 0.24		**0.005**ƚ	4.62 ± 0.22		**0.009**ƚ
- Pre-inhalers	4.06 ± 0.57	0.70 ± 0.69		3.89 ± 0.64	0.73 ± 0.57	
FVC						
- Post-inhaler	5.64 ± 0.31		**0.005**ƚ	5.54 ± 0.29		**0.005**ƚ
- Pre-inhalers	4.29 ± 0.63	1.35 ± 0.75		4.37 ± 0.47	1.17 ± 0.38	
FEV1/FVC						
- Post-inhaler	84.00 ± 1.88		**0.005**ƚ	83.90 ± 0.32		0.092
- Pre-inhalers	96.90 ± 8.90	-12.90 ± 10.16		89.80 ± 10.83	-5.90 ± 10.91	

*SD, standard deviation; FEV1, Forced Expiratory Volume in the first second; FVC, Forced Vital Capacity. FEV1/FVC, Ratio of FEV1 over FVC. §P - value for the mean differences of the Two-related samples Wilcoxon test. ƚ Significant *P*-values.

Table 3. Changes in exercise performance (time in seconds) for the participants given *C. serpyllifolium* (n=10) and *C. citratus* oils (n=10) when running an equal distance of 800 m before and after oil inhalations.

Outcome Variables	*C. serpyllifolium* oil group			*C. citratus* oil group		
	Mean ± SD*	Mean difference ± SD	*P* - value§	Mean ± SD*	Mean difference ± SD	*P* - value§
Time (sec)**						
- Post-inhaler	188.40 ± 13.29			171.90 ± 14.36		
- Pre-inhalers	206.60 ± 13.79	-18.20 ± 13.60	**0.005**ƚ	179.00 ± 16.15	-7.10 ± 7.75	**0.025**ƚ

* SD, standard deviation; §P - value for the mean differences of the Two-related samples Wilcoxon test. ƚ Significant *P* - values.
**Data in the table is expressed as seconds.

Discussion

Our findings showed that the essential oils isolated from *C. serpyllifolium* and *C. citratus* enhanced athletic performance and lung function. The results showed that there is a significant increase in FEV1 and FVC in the post-test students after their inhalation of the *C. serpyllifolium* and *C. citratus* oils. Moreover, the running times for each group were significantly decreased in both the *C. serpyllifolium* and *C. citratus* nebulized groups. As previously mentioned, natural plants' essential oils have been traditionally used in the treatment of various physiological and psychological disorders.[24] Most of the essential oils are considered to be safe and their safety had been monitored in different ways. They have been used from ancient times in perfumery, cosmetics and food industries.[25] Most of these studies focused on the *peppermint plant* but unfortunately, and according to the best of authors' knowledge, there were no previous studies about *C. serpyllifolium* and *C. citratus* oils inhalations on lung function and on the athletic performance. We have found no previous studies that were conducted regarding to *C. citratus* in relation to exercise and/or lung function performance. In the meanwhile, all previous studies conducted on these two plants were *in vitro* tests. However, *C. citratus* has shown an effect against dental caries [26], anti-malarial effects [27], and anti-bacterial effects. [28] Another study showed a positive effect of *C. citratus* leaves on some serum components and the antioxidant status without any beneficial effect on growth performance and carcass traits in broiler chickens. [29] Regarding *C. serpyllifolium*, however, results from mice models suggested that the aqueous extract of *Micromeria fruticosa* (*C. serpyllifolium*) has both anti-inflammatory as well as, gastro-protective activities. The authors concluded that this plant could be used as an alternative or supplementary herbal remedy for the treatment of inflammatory diseases, especially when combined with strong anti-inflammatory medications that have ulcerogenic side effects such as non-steroidal anti-inflammatory drugs (NSAIDs). [30] Another study found that both the oil and the aqueous extract of *M. fruticosa* exhibited marked anti-tumor activities against Human Colon Tumor cells (HCT) and Mammary Carcinoma F7 (MCF7). The study concluded that an extract of *M. fruticosa* has a remarkable inhibitory activity in non-inflammatory reactions, as well as inflammatory pain. [31] The use of essential oils in medicine began in the ancient Egyptian Era, and has continued ever since [32, 33]. One of the most popular parts of complementary and alternative medicine is considered to be aromatherapy, which depends only on essential oils utilization for the treatment of various diseases and this branch has spread worldwide, despite the lack of scientific basis for the effectiveness of essential oils. [34] On the other hand, the long history of essential oils usages in medicine and pharmacy suggests that they may indeed be effective. The odor of essential oils is believed to be important for their effectiveness in treating various illnesses. [35] Future study is needed to demonstrate oral supplementation of *C. serpyllifolium* and *C. citratus* essential oils to improve athletic performance and post-exercise recovery; another future objective would be to clarify their mechanism of action and to explain their physiological and pharmacological effects.

Our study has some limitations. This study is limited by the lack of control group for the nebulizer (0.9% NaCl only). There was neither placebo or control experiments within subjects nor a randomized (subjects/sequence) placebo control experiment. However, no control groups have been proposed in order to ensure that the intervention reaches the maximum number of students that agreed to participate. In addition, the design could also be another source of limitations. However, we are aware that in the design we used, secular trends or sudden changes make it difficult to attribute observed changes to the intervention. Further limitations of this design could be the occurrence of over-estimation of the effects of the intervention. By using this design, however, we were able to measure performance before and after the introduction of the intervention in the same study group and the observed differences in performance were assumed to be due to the intervention. This study design has advantages in that it is relatively simple to conduct and participants had the same characteristics pre- and post-test. Additionally, it is superior to observational studies. Furthermore, due to a lack of resources and the difficulty of circumstances in our country and university, we found this the most robust design possible to minimize bias and maximize generalizability. The small sample size might also be a source of limitation that could have affected the study's statistical power. We are fully aware of this, but we were not able to recruit more due to voluntary participation. Therefore, we found it sufficient to split the twenty students ten and ten in each group in order to run this study. We also did not conduct between group changes as we did not aim to perform comparison between the two groups with the different oil interventions but we only aimed to assess the outcome for each intervention in the same group pre-and post-test. As we have indicated above, we used a quasi-experimental uncontrolled before-and-after study design and we did not aim to compare between the two groups; therefore, differences in smoking or any other variations between groups were not taken into consideration in the analysis as we have measured the changes for each intervention separately among the same group pre-and post-test. There was no dietary control in this study. However, we believe that those are informative groups of students who might have the same weekly dietary intake level and we asked them to restrict any energy stimulator intakes during the week of the study. Therefore, dietary intake could have minimal effects on our findings.

Conclusions

This is the first study that explored the effects of inhalation of *C. serpyllifolium* and *C. citratus* essential oils on exercise performance and lung function. Our findings support the effectiveness of *C. serpyllifolium* and *C. citratus* essential oils on the exercise performance and respiratory function parameters. However, our conclusion and the generalizability of our results should be interpreted with caution due to small sample size and lack of control groups, randomization or masking. We recommend further investigations to explain the mechanism of actions for these two essential oils on exercise performance and respiratory gas exchange parameters.

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Conflict of interest

The authors, hereby, declare no conflict of interest.

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