Effects of *Satureja brevicalyx* essential oil inhalation on coping premenstrual syndrome

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ABSTRACT

Introduction: The aim of this research was to evaluate the effects of *Satureja brevicalyx* essential oil inhalation on premenstrual syndrome. **Methods:** The oils were extracted by hydro-distillation using a modified Clevenger-type apparatus and chemical composition was performed by Gas chromatography-mass spectrometry (GC–MS). Furthermore, a quasi-experimental study was conducted, where 61 participants were divided in two groups, comprising an experimental with essential oils and a control group with no treatment. Main psychological symptoms were assessed with the used of Zung Self-Rating Anxiety Scale (SAS) and Zung Self-Rating Depression Scale (SDS) as well as total score of premenstrual syndrome was evaluated with Premenstrual Syndrome Scale (PMSS). **Results:** chemical analysis showed that linalool (17.6%) was the main component. All variables showed a decrease in posttest study phase in comparison with pretest in experimental group (p<0.05). A large size effect was observed for anxiety (d = 1.25; Δ = 1.35). Besides, moderate changes were found for depression (d = 0.79; Δ = 0.81) and premenstrual syndrome total score (d = 0.62; Δ = 0.56). **Conclusion:** The essential oil of *Satureja brevicalyx* exerts a potencial use, especially for the treatment of psychological symptoms of premenstrual syndrome. **Key words:** Essential oils, Premenstrual syndrome, Anxiety, Depression, *Satureja brevicalyx*.

INTRODUCTION

Premenstrual syndrome (PMS) is a monthly pattern of symptoms which occur approximately one week before menstruation in women and lessen with the beginning of the new menstrual cycle.¹ This syndrome includes psychological symptoms such as mood swings, anger, irritability, lack of concentration, anxiety, depression, sleep difficulties, between others.² Besides, physical symptoms such as headache, fatigue, appetite changes, gastrointestinal symptoms, etc., are present.³ The intensity of these symptoms may vary between women, affecting their quality of life.⁴

Epidemiological data affirm that 90% of females of reproductive age are usually impacted by mild to acute premenstrual symptoms, among them, about 20% to 40% are affected for PMS.⁵ Additionally, prevalence among university students is over 50%.⁶ Indeed, an investigation found that PMS affects quality of life students, particularly components related to mental and environmental health.⁷

Pharmacological treatments focus on the use of antidepressants, anxiolytic agents, hormonal therapy, contraceptives, and anti-inflammatory drugs; however, risk of adverse events is frequent, and drugs only palliative the symptoms which cannot be stopped totally.⁸ In this situation, medicinal plants may play a key role in PMS treatment, since are women who commonly used medicinal herbal products.⁹

Essential oils (EOs) have been used in complementary and alternative medicine (CAM) for the prevention and treatment of diseases.¹⁰

Aromatherapy is a component of CAM, which involves the inhalation of EOs, some of them used to counteract gynecological ailments.² In fact, literature confirmed that EOs relief multiple symptoms of PMS.¹¹⁻¹³ These natural volatile oils derived from herbal medicine are considered inexpensive, safe, useful, as well as easy to obtain.¹⁴

Satureja brevicalyx is a specie properly from South America Andes, and grows from southern Peru, Bolivia, and northern Argentina.¹⁵ Its leaves were used traditionally as infusion to relief gastrointestinal problems and menstrual disorders.¹⁶ Evidence shows that EOs of *S. brevicalyx* have anxiolytic,¹⁷ neuroprotective¹⁸ and antibacterial potential;¹⁹ besides this essential oil has no genotoxic effect, appropriated to use in humans.²⁰ Therefore, the aim of this research was to evaluate the effects of Satureja brevicalyx essential oil inhalation on premenstrual syndrome.

MATERIALS AND METHODS

Plant material

The leaves of *Satureja brevicalyx* were collected from Huamanguilla district, Huanta Province, Ayacucho Region, Perú, at 3800 masl; The sample collection was conducted in the months of January and February 2022. Voucher specimens were prepared and identified by Segundo Leiva Gonzales, Biol, and deposited at the Herbarium Antenor Orrego (HAO) of Antenor Orrego University.

Essential oils extraction

The leaves were washed with distilled water to remove dust. Subsequently, samples were dried using a forced

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air circulation stove at a temperature of 40°C for 24 h. After, samples were milled, and the powdered plant material (100 g) were placed in a round bottom flask with 1000 ml distilled water, and then connected to a modified Clevenger-type apparatus. Hydrodistillation was completed for 4 h after boiling. Then the oil was dried over anhydrous sodium sulphate and stored in a refrigerator in amber glass vials at 4°C for further use in experiments.²¹

Determination of essential oil composition

Gas chromatography-mass spectrometry (GC/MS) was performed using a Hewlett-Packard 6890/5972 GC/MS system under the following conditions: fused silica HP-5 column, carrier gas He (1.1 ml/min), temperature program: 3 °C/min from 60 °C to 240 °C; the injection port temperature was 250 °C; detector temperature was 280 °C. Ionization of the sample components was performed in the EI mode (70 eV). Finally, visual interpretation was used to identify the essential oil constituents, comparing their retention indices (RI) and mass spectra with literature data, by computer library search (HP Chemstation computer library NBS75K.L, NIST/EPA/NIH Mass Spectral Library 2.0 and Mass Finder 3 Computer Software and Terpenoids Library).^{22,23}

Study design and sample

A quasi-experimental study with pretest and posttest design was conducted in a sample of 61 participants, where 31 conformed the experimental group (EG) treated with aromatherapy based on *Satureja brevicalyx* essential oil and a control group (CG) conformed by 30 participants.

Instruments

Premenstrual Syndrome Scale (PMSS): This is a 5-point Likert type scale consisting of 40 items, scored from 1 to 5 (1=never, 2=rarely, 3=sometimes, 4=very often, 5=always).²⁴ For the present investigation, the validity and reliability test for the local population were determined using the item-test method, finding values above 0.35 for each item, in addition, the reliability coefficient of 0.91 was found using the split-half method.

Zung Self-Rating Anxiety Scale (SAS): This scale consists of 20 items, scored each one from 1 to 4 (1= non or a little of the time, 2 = some of the time, 3 = good part of the time, 4 = most of the time).²⁵ Validity and reliability test for the local population were determined in a previous study.²⁶

Zung Self-Rating Depression Scale (SDS): This scale consists of 20 items, constructed based on the clinical diagnostic criteria used to characterize depressive disorders. Each item is scored from 1 to 4 (1= none or a little of the time, 2 = some of the time, 3 = good part of the time, 4 = most of the time).²⁷ Validity and reliability test for the local population were determined in a previous study.²⁶

Study procedure

A quasi-experimental study was conducted with female health sciences students from a university in Trujillo, Perú. All participants were students enrolled in two sections (A and B) of the same course. Section A was selected as CG and Section B as EG. The criteria for inclusion were the willingness to participate in the study, regular menstruation for at least two years and no use of vitamin supplements when entering the study, not using hormonal medications for at least 2 months prior to the study, the length of menstruation days between 24 and 35 days, no underlying diseases such as thyroid disease, diabetes, or mental illness, not currently taking any psychotherapy nor using any support group or relaxation technique, and not taking any psychiatric medication. Women with changes in the menstrual cycle, stressful event during the investigation period, and sensitivity to the essential oils used were excluded from the study. The aromatherapy was administered by

inhalation where each participant received an amber bottle containing 10 ml of Satureja brevicalyx essential oil at 0.05% concentration. Two menstruation periods were analyzed; pretest was administered before EOs administration at the first period. Aromatherapy begins ten days before menstruation, applied twice a day at the same hour (in the morning and at night), and it was stopped when period had started. Posttest was administered at the beginning of the second menstruation period. The EOs application was according to the methodology proposed by Heydari et al.,11 with some modifications; where the participants were instructed to pour five drops of EOs onto a sterile eye pad to be uniformly absorbed, sit in a quiet and comfortable place, position the pad 30 cm away from the nose and inhale the odor during five minutes with normal breathing. All participants were informed about the investigation program goals and signed a consent form in which confidentiality and anonymity were guaranteed. The study protocol was approved by Institutional Review Board (IRB). Besides, this investigation was performed in accordance with the Declaration of Helsinki.

Data analysis

Data were presented as mean \pm standard deviation (SD). Differences in sociodemographic and clinical data from participants were analyzed using the Pearson Chi-Square and Likelihood-ratio tests. Mann– Whitney U test was used to determine significant differences between groups, while Wilcoxon test was used to determine significant differences between the study phases, in which p < 0.05 was considered statistically significant. These tests were used because data did not conform to the normal distribution. Cohen's D, Glass's delta and Percentage Change were calculated between pretest and posttest scores. Statistical analysis was performed using SPSS v.25.0 (IBM Corp., Armonk, NY, USA) and Prism 8 software (GraphPad, CA, USA).

RESULTS

Table 1 presents the results from samples analyzed by GC/MS, in which 33 components were identified in the essential oil, representing 96.9% (area percent) of total oil content, where the major constituents were linalool (19.6%), pulegone (11.7%), menthone (10.5%), isomenthone (7.8%), carvacrol (6.5%) bicyclegermacrene (6.2%), β -caryophylene (5.8%), p-cimene (4.1%) and geranyl acetate (3.9%).

Table 2 shows socio-demographic data of analyzed undergraduate women students, where most participants in CG were between 18 and 25 years old (n=24;67.7%), so were in EG (n=26;70.3%). Besides, 26 to 38 years old students were minority in both groups (CG: n=12;33.3% and EG: n=11;29.7%). These results show no statistically significant differences (p>0.05), using the Chi square test. In relation to their marital status, the majority, both in CG (n=34;94.4%) and EG (n=36;97.3%) were single, only few participants were married (CG:n=2;55.6% and EG:n=1;2.7%); finding no difference between groups conformation, showing no statistically significant differences (p>0.05), using Likelihood ratio test.

Figures 1, 2 and 3 show anxiety, depression, and PMS scores where CG and EG have similar outcomes at pretest, showing no statistical differences (p>0.05). All these results were calculated by Mann–Whitney U test. In relation with study phases, CG scores in posttest phase showed a slight increase in all variables, however these were not large enough to make a significant difference, therefore CG scores did not show differences between pretest and posttest (p>0.05). In the case of EG, all variables showed a decrease in posttest phase in comparison with pretest phase, showing statistical difference between study phases (p<0.05). All these results were calculated by Wilcoxon test.

In addition, Table 3 shows the scores obtained by the Cohen's D and Glass's delta test, as well as the percentage of change found; where values between 0.5 and 0.1 show moderate changes, as is the case of

Table 1: Main chemical constituents (%) of "Satureja brevicalyx" essentia	L
oil.	

Composition	RI	%
α-Pinene	936	0.4
Sabinene	975	0.5
β-Pinene	980	0.2
Myrcene	991	0.4
p-cimene	1010	4.1
1,8 Cineole	1025	2.6
Limonene	1033	1.7
Y-Terpinene	1050	0.5
Linalool	1078	19.6
Menthone	1142	10.5
Isomenthone	1165	7.8
cis-isopulegone	1180	2.0
trans-isopulegone	1189	1.3
α -terpineol	1198	0.6
Pulegone	1235	11.7
Piperitone	1251	t
Thymol	1293	2.1
Carvacrol	1300	6.5
Thymyl acetate	1325	1.4
Neryl acetate	1344	0.1
Piperitenone	1352	0.3
Geranyl acetate	1387	3.9
β –caryophyllene	1423	5.8
Aromadendrene	1436	0.5
α -humulene	1458	0.6
Germacrene D	1475	0.3
Bicyclogermacrene	1499	6.2
Germancrene B	1562	0.1
Spathulenol	1576	2.5
Caryophyllene oxide	1588	t
Isoespatulenol	1629	0.4
14-Hydroxy-b-caryophyllene	1661	t
Carvacryl acetate	1884	2.3
Total identified (%)		96.9

RI= Retention index; t= traces (<0.1%)

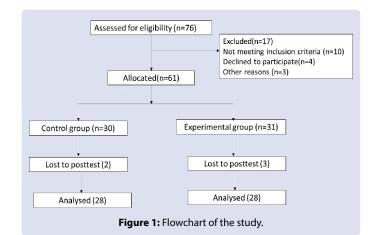
Table 2: Socio-demographic data of participants.

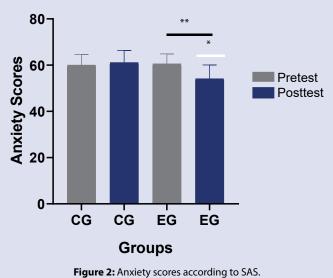
Socio-demographic data	CG	EG	Total	p-Value
Age(yr)				
18-25	24 (67.7%)	26 (70.3%)	50 (68.5%)	0.740^{a}
26-38	12 (33.3%)	11(29.7%)	23 (31.5%)	
Marital status				
Single	34 (94.4%)	36 (97.3%)	70 (95.9%)	0.536 ^b
Married	2(5.6%)	1 (2.7%)	3 (4.1%)	

^ap-value is calculated by Pearson Chi-Square test ^bp-value is calculated by Likelihood-ratio test

Table 3: Cohen's D, Hedges' G and Percentage Change in intervention groups.

• •			
Group	Cohen's d Posttest	Glass's ∆ Posttest	% Of change Pretest-Posttest
EG			
Anxiety	1.25	1.35	-11.45
Depression	0.79	0.81	-10.07
PMS	0.62	0.56	-6.38





rigure 2. Anxiety scoles according to SAS.

*p < 0.05, calculated by Mann–Whitney U test **p < 0.05, calculated by Wilcoxon test between study phases

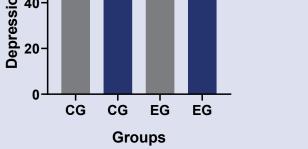
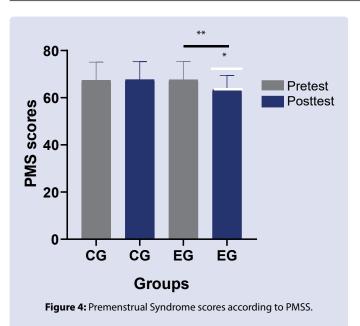


Figure 3: Depression scores according to SDS.

*p < 0.05, calculated by Mann–Whitney U test

**p < 0.05, calculated by Wilcoxon test between study phases



^{*}p < 0.05, calculated by Mann–Whitney U test **p < 0.05, calculated by Wilcoxon test between study phases

depression (d = 0.79; Δ = 0.81) and PMS (d = 0.62; Δ = 0.56); however, it was observed a large size effect for anxiety (d = 1.25; Δ = 1.35). Finally, percentages of change show an anxiety decrease of 11.45%, as well as reduction in depression and PMS of 10.07% and 6.38% respectively.

DISCUSSION

PMS is a clinical condition where women present persistent somatic and mental symptoms.²⁸ Treatment options are varied and include pharmacological interventions for both, somatic and psychological symptoms.²⁹ Nevertheless, long-term side effects and to avoid risk of abuse make possible new treatment alternatives such as aromatherapy. Thus, results point that the main component of Satureja brevicalyx essential oil is linalool what is in accordance with investigations performed in the same Andean region,17 although the amount of linalool is less than the one found in literature, this component is still the main. Indeed, samples were obtained in the beginning of winter and after bloom stage where linalool content decrease.^{30,31} In addition, some components such as pulegone, menthone, isomenthone may also be considered majoritarian, which is in coincidence with other research.¹⁹ However, there are some differences in the percentages of the composition, which may be due to phenological factors. In fact, research in Satureja species show that EOs composition variations may be attributed to seasonal variation, geographical origin, genetic variation, growth stages of the plant, part of plant utilized, postharvest drying and storage, among others.^{32,33}

Concerning socio-demographic data of female students, the results are in concordance with some studies which confirm that university population in Peru is conformed mostly for young and single.³⁴ In this case, sample was constituted by health science students, and it is known that these have a good acceptation towards CAM.³⁵ Therefore, it is necessary larger samples, as well as to verify if similar data could be found in other student populations in order to generalize results.

According to the evidence, aromatherapy is an effective tool to manage PMS symptoms, in fact, anxiety, depression, and physical symptoms and total score of PMS decrease after odor plants application.² This is in accordance with the results expressed in this investigation where all variables showed a decrease after intervention in EG, especially in

anxiety which revealed the greatest effect size and percentage of change. Indeed, some studies have found that PMS emotional symptoms decrease after aromatherapy intervention.^{36,37}

It is known that linalool has sedative properties through an antagonistic action on glutamatergic receptors such as N-Methyl-D-aspartate receptors (NMDARs).³⁸ Nevertheless, the major mechanism is associated to inhibitory activity on Ca²⁺ influx mediated by voltage-gated calcium channels, probably by binding and inhibiting the channel complex.³⁹ In this sense, opening Ca²⁺ channels produce anxiety, and their blockade attenuates anxiety.⁴⁰ In addition, natural products with a large amount of pulegone have been used as sedatives,⁴¹ however evidence points out that anxiolytic-like effect of this component is related to a synergism or an additive effect between pulegone and other compounds.⁴² Besides, monoterpenes as menthol, menthone, carvone and their derivates exert anxiolytic effects explained by modulation of serotonergic or dopaminergic function.⁴³

It should be mentioned that depression is also considered a PMS emotional symptom, which showed a moderate effect size, however its percentage of change was close to that of anxiety. This may be explained by the presence of monoterpenes as linalool which have demonstrated antidepressant activity exerted through the postsynaptic 5-HT_{1A} receptors, in addition, these compounds interact with the adrenergic system through receptors as α_2 .⁴⁴ Besides, it is known that neuroplastic changes in depression is mediated by neuroimmune processes where proinflammatory cytokines are involved as well as lack of endocannabinoid receptor CB2 that exacerbates stress-induced neuroinflammatory responses.⁴⁵ In this, sense, β –caryophyllene may play a role in the modulation of depression in relation with the suppression of inflammatory response, modulating the expression of CB2 and ameliorating neurotrophic inflammatory changes in the hippocampus.⁴⁶

PMS outcomes also showed a moderate size effect, although with a minor percentage of change in comparison with depression. In fact, PMS score was measured with PMSS, a scale which considers psychological symptoms, but also physiological and behavioral symptoms. In the case of psychological and behavioral ones, the effect may be explained by the interaction between the EOs volatile compounds and brain areas as limbic system, hypothalamus and hippocampus which manages conscious thought processes and emotions,² as well as mechanisms explained above at speaking of anxiety and depression. In the other hand, a dysregulated response to inflammation may be a factor in the pathogenesis of premenstrual disorder as PMS.47 In this regard, linalool displays anti-inflammatory properties, interacting with cyclooxygenase-1 and cyclooxygenase-2 which prevent prostaglandin activity, reducing pain, too.48 Furthermore, Pulegone exerts antiinflammatory effects, through the inhibitory mechanisms of inducible nitric oxide synthase (iNOS), cyclooxygenase (COX-2), nuclear factor kappa B (NF-KB), mitogenactivated protein kinases (MAPK) as well as up-regulation of nuclear factor erythroid 2-related factor 2 (Nrf2)/ heme oxygenase (HO)-1.49 Likewise, other monoterpenes such as menthone and carvacrol exert anti-inflammatory effects by reducing the production of inflammatory mediators.^{50,51} In fact, PMS pathophysiology is not clear; in this way, further research is needed, especially to determine the mechanisms of action involved in the physiological symptoms of PMS.

CONCLUSION

In accordance with this research, *Satureja brevicalyx* essential oil exert a large effect size for anxiety, as well as moderate effect size in depression and total score of PMS. In this sense, this essential oil offers a potential use, especially for the treatment of psychological symptoms of PMS.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

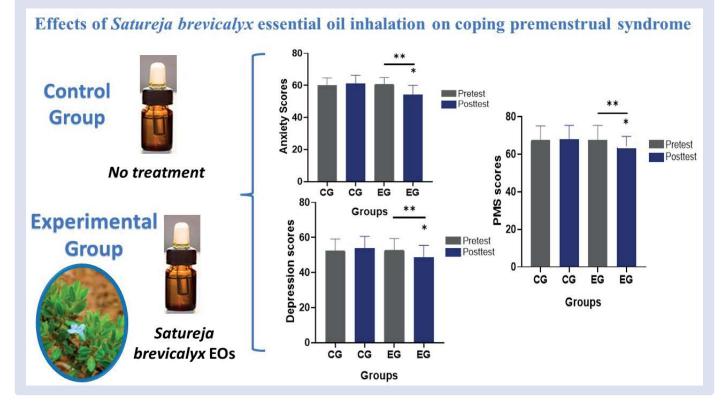
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GRAPHICAL ABSTRACT

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