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Aromatherapy with two essential oils from Satureja genre and mindfulness meditation to reduce anxiety in humans

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Abstract

The goal of this study was to verify whether association of aromatherapy with essential oils of *Satureja brevicalyx* or *Satureja boliviana* and mindfulness meditation can reduce anxiety levels in humans. A randomized experimental trial was carried out with 108 participants who were divided into 6 groups, comprising a waiting list control group and five experimental groups. Aromatherapy was carried out by inhalation of essential oils while mindfulness intervention program was focused on “flow meditation”. The anxiety index was evaluated by State-Trait Anxiety Inventory (STAI). Measures were taken two times: pretest and posttest. State and Trait anxiety scores showed a decrease in posttest study phase in comparison with pretest in all experimental groups ($p < 0.005$), especially in those where aromatherapy and mindfulness meditation were used together. All Cohen's d scores were over to 1 that means a large size effect in anxiety variable. Percentages of change showed reductions of anxiety variable ranging between 20% and 47%. All treatments used isolated or associated, may be considered alternative treatment options for anxiety.

Keywords: Anxiety, Aromatherapy, Mindfulness, Essential oils, *Satureja brevicalyx*, *Satureja boliviana*

Graphical abstract

1. Introduction

Anxiety is one of the most prevalent health problems around the world.[1] It encompasses a feeling of intense and indeterminate fear or apprehension with physical signs such as heart palpitations, sweating and tension. Anxiety is a normal reaction in humans because let us to avoid potential threats. However, it can be considered a problem when it becomes associated with cues that are not a real danger and escaping of these feared cues becomes chronic and habitual.[2]

Although patients usually respond well to short-term treatment of medication, the magnitude of improvement on chronic treatment is disappointing; besides anxiolytic drugs produce various side effects

and indiscriminate use.[1] That is why there is a great interest on complementary and alternative medicine treatments such as aromatherapy and mindfulness. The first one is a natural treatment utilizing essential oils as the main therapeutic agents, which are extracted from flowers, leaves, stalks, fruits, seeds, roots and resins.[3] Essential oils have various applications such as massage, inhalation, compress, and baths.[4] Some research teams have found that using aroma inhalation can reduce anxiety, stress and even have the synergistic effect with the drugs used in the treatment of central nervous system disorder.[5, 6, 7] In the other hand, mindfulness meditation, originated from Buddhist Vipassana, has grown in popularity over the past 30 years. Effective interventions based on mindfulness have been developed, such as Mindfulness-Based Stress Reduction (MBSR), Mindfulness-Based Cognitive Therapy (MBTC) and Acceptance-Based Behavioral Therapy (ABBT).[8] Findings support the use of mindfulness meditation to reduce stress and anxiety levels as well as reduce the risk of depressive relapse.[9, 10, 11]

Satureja brevicalyx and *Satureja boliviana* are two species from Satureja genre distributed in South American Andes from southern Peru until Bolivia and northeast Argentina. Both were used from ancient times for Andean people.[12] *S. brevicalyx* is used traditionally as analgesic, anti-inflammatory, antimicrobial and for gastrointestinal conditions. Their leaves are chemically composed by resins, reducing sugars, catechins, sesquiterpene lactones, triterpenes, steroids, saponins, tannins, flavonoids and essential oil. Different laboratories have determined its analgesic, antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, anti-Helicobacter pylori and anxiolytic effects.[13, 14, 15, 16] For its part, *S. boliviana* is traditionally used for colic, altitude sickness, respiratory diseases, rheumatism and migraines. Its leaves are mainly composed by phenolic compounds and essential oil. It was proved its antimicrobial, anti-inflammatory and gastric cytoprotective activities.[17, 18, 19]

The present study was conducted to examine whether aromatherapy with essential oils of *S. brevicalyx* and *S. boliviana* and mindfulness meditation can reduce anxiety levels in humans, as well as explore the synergistic effect of aromatherapy and mindfulness meditation.

2. Material and methods

2.1. Plant material

The leaves of *S. brevicalyx* were collected from Condorcunca mount at 3500 m elevation, located in Quinua district, Ayacucho Region, Peru; while leaves of *S. boliviana* were collected from Sun Island at 4000 m elevation, located in Titicaca Lake, Manco Capac province, La Paz department, Bolivia. The sample collection was conducted in the months of January to February, 2015. Voucher specimens were prepared and identified by Eric Frank Rodríguez Rodriguez, PhD, and deposited at the Herbarium Truxillense (HUT) of National University of Trujillo, under registration numbers 58165 and 58166 respectively.

2.2. Essential oils extraction

The powdered plant material (100 g) of the leaves of *S. brevicalyx* and *S. boliviana* were hydrodistilled for 4 h using a modified Clevenger-type apparatus. Then the oil was dried over anhydrous sodium sulfate and stored in the refrigerator at 4 °C for further use in experiments.[20]

2.3. Determination of essential oil composition

The essential oils were analyzed by gas chromatography–mass spectrometry (GC/MS) using Hewlett-Packard 6890/5972 GC/MS system with the following conditions: fused silica HP-5 column, carrier gas He (1.1 ml/min), temperature programme: 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). The identification of essential oil constituents was accomplished by visual interpretation, comparing their retention indices 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).[21, 22]

2.4. Study design and sample

An experimental study with measures at pretest–posttest was conducted, using five experimental groups and a waiting-list control group. 108 participants, between 25 and 45 years old (mean age = 31.5 years) took part of this study. Participants were randomly divided into 6 groups of 18 participants (9 male and 9 female) comprising a wait-list (WL) control group, experimental group 1 (EG1) treated with mindfulness meditation program, experimental group 2 (EG2) treated with aromatherapy based on *S. brevicalyx* essential oil, experimental group 3 (EG3) only treated with aromatherapy based on *S. boliviana* essential oil, experimental group 4 (EG4) treated with mindfulness mediation program and aromatherapy with Satureja brevicalyx essential oil, and finally experimental group 5 (EG) treated with mindfulness mediation program and aromatherapy with *S. boliviana* essential oil.

2.5. Study procedure

A free meditation and aromatherapy course was offered through local press to recruit participants. 121 people were enrolled and 108 took part in this research (Inclusion criteria included participants men and female between the ages of 18–45 and they were required to have a State-Trait Anxiety Inventory score of greater than 20 in both scales; meanwhile exclusion criteria were participants with previous practice of meditation, tai chi or yoga, psychiatric treatment and pregnancy).

18 participants for each group were randomized. After the control and intervention groups were formed, an anxiety self-report instrument was administered (pretest) and filled by all participants. 5 schedules were disposed for each intervention group (2 in the morning and 3 in the afternoon). Control group (WL) participants were informed they were going to take the course after 2 weeks due to schedule was full. Psychotherapy room (4 × 4 m size) of Integral Psychotherapy Center was used for experiments. Windows were closed hermetically during stimulus administration and participants sat in ergonomic chairs forming a circle. EG1 participants were treated with the mindfulness intervention program focused on “flow meditation” where attention is focused in breathing in abdomen area while a mantra is repeated.[23] EG2 participants were treated with aromatherapy with *S. brevicalyx* essential oil. 5 environmental diffusers were used for administrating oil by inhalation. These were placed one in each corner of therapy room and one in the middle of the circle of participants. The essential oil dose required to saturate the experimental room was 2 drops of 2% essential oil = 0.1 mL.[16] EG3 participants were treated with aromatherapy with *S. boliviana* essential oil and EG4 and EG5 participants were treated at once with aromatherapy with *S. brevicalyx* and *S. boliviana* essential oils respectively and mindfulness meditation program. All groups had 30 min intervention sessions from Monday to Saturday during two weeks (12 sessions). After that, an anxiety self-report instrument was administered (posttest) to all participants (Fig. 1).

When the entire intervention program finished, all participants were informed about the investigation program goals and signed a consent form in which confidentiality and anonymity were guaranteed. This investigation was performed in accordance to the Declaration of Helsinki.

2.6. Instruments

To evaluate anxiety, the State-Trait Anxiety Inventory (STAI) was used which consists of two self-report scales measuring two distinct types of anxiety: state (actual levels of intensity and anxiety states) and trait (selects individuals who vary in their tendency to react to psychological stress with varying degrees of intensity). Both scales consist of 20 statements. The part that regards trait describes how the subjects generally feel, while the part that regards state describes how they feel at a given moment. This tool is one of the most widely used scales for the evaluation of anxiety in normal population and, to a lesser extent, psychiatric patient.[4, 24] Validations and reliability coefficients for local population were found in a previous study.[16]

2.7. Data analysis

Means and standard deviations (SD) were found; as well as Mann–Whitney U test was used to determine significant differences between WL group and intervention groups, while Wilcoxon test for paired samples was used to determine significant differences between the study phases. These tests were chosen because data did not conform to the normal distribution. Cohen's d and percentage change were calculated between

pretest and posttest scores. All statistical analysis was performed using SPSS v.20.0 (IBM Corp., Armonk, NY, USA).

3. Results

The results obtained in Table 1 shows the samples analyzed by GC/MS. From the oil of *S. brevicalyx* 39 constituents were identified, representing 97.6% (area percent) of the total oil, among which linalool (21.1%), menthone (12.3%), geranyl acetate (11.2%), pulegone (10.4%), isomenthone (8.1%), bicyclogermacrene (7.3%), β-caryophyllene (6.5%) and p-cimene (5.3%) were the major components. From the oil of *S. boliviana* 37 constituents were identified, representing 97.2% of total oil content, which major components were linalool (12.8%), menthone (10.7%), pulegone (9.7%), bicyclogermacrene (8.7%), geranyl acetate (8.6%), germacrene D (7.8%), p-cimene (6.4%) and carvacryl acetate (5.2%).

Table 2 presents socio-demographic and clinical data of participants in study, where there was homogeneity in gender with the same amount of men and women. Majority of participants were between 25 and 35 years old (n = 85; 75%) and the rest between 36 and 45 years old (n = 27; 25%). 6 people (5%) attend to high school, 47 (44%) were undergraduate students, 32 (30%) were graduated and 23 (21%) were postgraduate. In relation with their marital status, most of participants (n = 68; 63%) were unmarried, 29 (27%) were married, 10 (9%) were divorced and just 1 participant was widowed. Finally, majority of participants never attended to treatment (n = 95; 88%), 13 (12%) attended to psychological treatment and no one attended to psychiatrist for pharmacological treatment.

Table 3 shows the mean score and SDs for anxiety based on STAI, where all experimental groups present differences in posttest scores in comparison with waiting list group (EG1, EG2, EG4 and EG5: $p < 0.005$; EG3:$p < 0.05$). State and Trait anxiety scores show a decrease in posttest study phase in comparison with pretest in all experimental groups ($p < 0.005$), showing a change in STAI scores after the intervention. Meanwhile WL group show a slight increase in posttest scores in comparison with pretest scores ($p > 0.05$). It also can be observed that when mindfulness and aromatherapy are used together anxiety levels are lower than when these therapies are used by themselves (EG1 = 27.22; EG2 = 28.17; EG3 = 30.28; EG4 = 23.78; EG5 = 25.67).

Regarding the amount of change in the mean scores at posttest, it is observed that all Cohen's d scores are over to 1 that means a large size effect in anxiety variable. Finally, percentages of change between pretest and posttest measures show reductions of anxiety variable ranging between 20% and 47%, both state anxiety and trait anxiety. All these results show important decrease in anxiety scores (Table 4).

4. Discussion

Some studies refer that young adults and adults as well as more educated people are more likely to be interested in alternative treatments.[25] This is in concordance with our study where majority of participants were adults with a good educational level. In Peru, only 20% of urban population had access to university education.[26] This is considered one of the limitations because there were no participants that represent low educational levels and our study does not show how these therapies can work in a different population. Besides, participant number may be not enough to generalize results. Other limitation is that due to homogeneity sample, correlations between Socio-demographic and clinical data with anxiety scores are not shown.

Participants show a decrease in anxiety scores after intervention. Trait scores were lower than state scores in all groups. This is because state anxiety is related to temporary situations that changes every moment.[27] Besides, findings of this study show a decrease in anxiety scores in all experimental groups after intervention in comparison with WL group, especially in those where aromatherapy and mindfulness were administered together (EG4 and EG5), what suggests a synergy effect that was also noted in an previous study.[16] In this sense, another investigation also found that aromatherapy enhances the use of mindfulness meditation in anxiety treatment.[5]

In the case of treatment using only essential oils, EG2 (*S. brevicalyx*) showed relatively more reduction of anxiety scores than EG3 (*S. boliviana*). This is consistent with EG4 results where essential oil of *S.*

brevicalyx is used together with mindfulness meditation. According to data studies, anxiolytic effects of essential oils are attributed to linalool, a component that has dose-dependent effects on central nervous system, including sedation, hypnotic, and anxiolytic effects.[28, 29, 30] In this research the highest amount of linalool was observed in the oil of *S. brevicalyx* (21.1%) while in the oil of *S. boliviana* the amount of linalool was 12.8% of the total oil content. It means that differences in anxiety scores could be due to linalool content, although further studies are needed to prove this hypothesis.

Meanwhile with respect to mindfulness meditation (EG1), scores present a bit more reduction of anxiety levels after treatment, in comparison with EG2 and EG3. This is consistent with other research teams that found a diminution of state, trait, or overall anxiety after treatments using mindfulness meditation.[16, 31, 32] This is due to individuals at practicing mindfulness may learn to keep a relaxed mind and focus on present moment, cultivating an attitude of acceptance and patience toward unpleasant emotions and thoughts that may arise.[32]

IG4 presents the major percentages of changes of anxiety variable and then IG5 what reveal the feasibility to use mindfulness and aromatherapy together in anxiety treatment. In this way few studies considering the two variables together have been carry out to date and although the results of this investigation cannot be generalized to other settings due to the small and no representative sample; aromatherapy and mindfulness emerging as a effective treatment option for anxiety, besides Andean essential oils of *S. brevicalyx* and *S. boliviana* may be a new alternative to be used in aromatherapy for anxiety. Nevertheless, future research is needed to help us gain better understanding of synergy effect of aromatherapy and mindfulness meditation.

5. Conclusion

Aromatherapy based on essential oil of *S. brevicalyx* and *S. boliviana* as well as mindfulness meditation, used isolated or associated, may be considered alternative treatment options for anxiety.

Conflicts of interest

All authors have no conflicts of interest to declare.

Footnotes

Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

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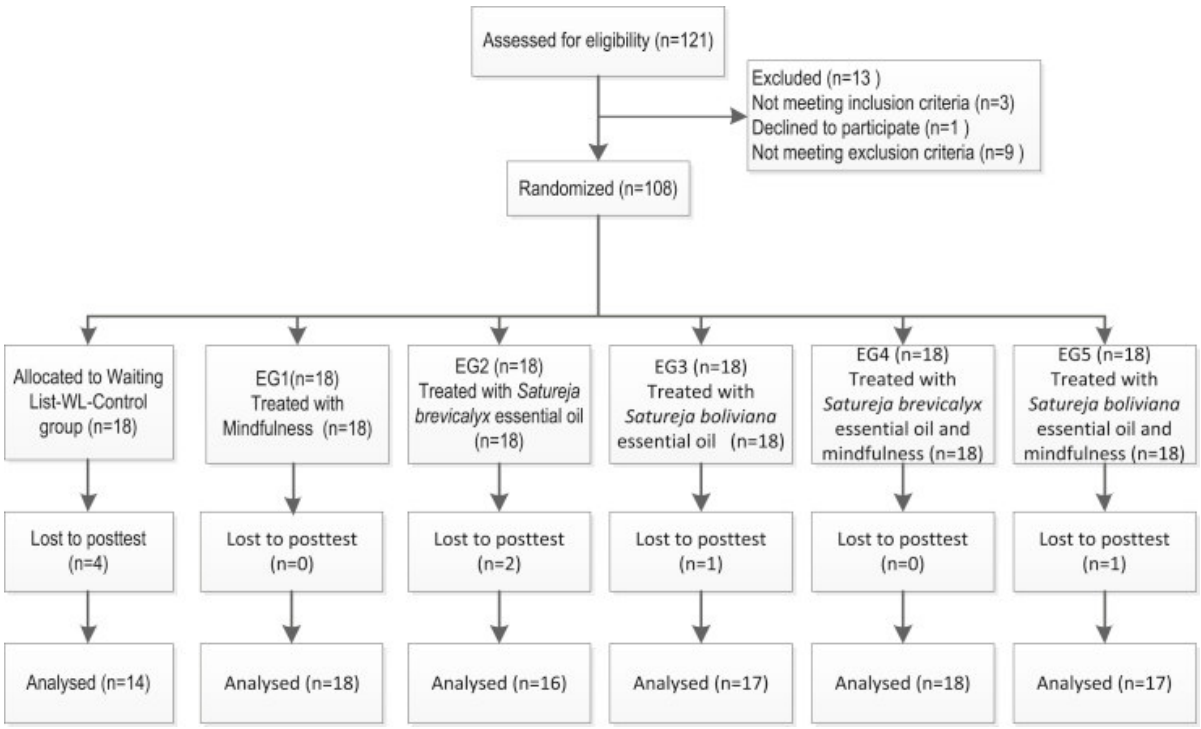
Figures and Tables

Fig. 1



Flow chart of the study.

Table 1

Main chemical constituents (%) of the essential oils of *Satureja brevicalyx* and *Satureja boliviana*.

Composition	RI	*S. brevicalyx*	*S. boliviana*
α-Pinene	939	0.5	3.0
Camphene	954	–	4.2
Sabinene	975	0.3	0.8
β-Pinene	979	0.1	0.5
1-Octen-3-ol	979	0.1	0.6
3-octanol	991	0.1	t
p-cimene	1014	5.3	6.4
1,8 Cineole	1023	0.9	1.8
Limonene	1030	0.8	0.9
Y-Terpinene	1053	0.4	0.4
Linalool	1085	21.1	12.8
Menthone	1157	12.3	10.7
Isomenthone	1163	8.1	0.5
cis-isopulegone	1177	2.4	–
Isopulegone	1185	t	2.3
trans-isopulegone	1188	1.2	–
α-terpineol	1195	0.5	0.3
Pulegone	1237	10.4	9.7
Geraniol	1252	–	0.2
Piperitone	1253	0.1	1.3
Thymol	1291	1.1	0.6
Carvacrol	1299	1.3	3.4
Thymol acetate	1329	1.5	–
Bicycloelemen	1330	0.4	–
Neryl acetate	1342	t	0.2
Piperitenone	1348	0.2	0.1
Geranyl acetate	1387	11.2	8.6
β-caryophyllene	1424	6.5	–
Aromadendrene	1432	0.6	t
α-humulene	1455	0.2	0.6
Germacrene D	1472	0.5	7.8
Bicyclogermacrene	1493	7.3	8.7
Germancrene B	1560	0.1	0.3
Spathulenol	1578	0.9	1.9
Caryophyllene oxide	1583	0.3	1.5
Viridiflorol	1583	–	0.5
(E)-Nerolidol	1587	0.1	t
cis-Isolongifolanone	1619	–	0.1
Isoespatulenol	1629	0.1	t

Composition	RI	*S. brevicalyx*	*S. boliviana*
Cubenol	1641	t	0.1
α-cadinol	1652	–	1.1
14-Hydroxy-b-caryophyllene	1657	0.1	t
α-bisabolol	1680	–	t
Oplopanone	1733	–	0.1
Carvacryl acetate	1885	0.6	5.2
Total identified (%)		97.6	97.2

RI, Retention index; t = traces (<0.1%); –, not detected.

Table 2

Socio-demographic and clinical data of participants in study.

Socio-demographic data	Frequency	%
Gender		
Male	54	50
Female	54	50
Age(yr)		
25–35	81	75
36–45	27	25
Level of education		
High school	6	5
Undergraduate	47	44
Graduate	32	30
Postgraduate	23	21
Marital status		
Married	29	27
Unmarried	68	63
Divorced	10	9
Widowed	1	1
Anxiety treatment provided		
Psychological	13	12
Pharmacological	0	0
None	95	88

Table 3

Group differences of anxiety variable according to State-Trait Anxiety Inventory (STAI).

Groups	Pretest		Posttest		p-Value[b]
	Mean	SD	Mean	SD	
WL					
State anxiety	37.00	±9.65	37.56	±9.33	0.197
Trait anxiety	27.83	±8.78	27.94	±8.43	0.706
EG1					
State anxiety	37.06	±9.31	27.22	±5.00	0.000**
p-Value[a]	0.962		0.000**		
Trait anxiety	27.56	±7.80	19.50	±3.52	0.000**
p-Value[a]	0.787		0.001**		
EG2					
State anxiety	37.83	±9.92	28.17	±6.89	0.001**
p-Value[a]	0.751		0.001**		
Trait anxiety	28.56	±8.82	20.22	±5.56	0.001**
p-Value[a]	0.703		0.003**		
EG3					
State anxiety	37.94	±8.32	30.28	±7.41	0.000**
p-Value[a]	0.861		0.017*		
Trait anxiety	29.06	±7.44	22.28	±6.34	0.000**
p-Value[a]	0.656		0.034*		
EG4					
State anxiety	37.56	±9.28	23.78	±7.81	0.000**
p-Value[a]	0.812		0.000**		
Trait anxiety	29.33	±9.35	16.06	±5.49	0.000**
p-Value[a]	0.600		0.000**		
EG5					
State anxiety	37.67	±9.60	25.67	±7.97	0.000**
p-Value[a]	0.911		0.000**		
Trait anxiety	28.83	±9.57	18.17	±6.01	0.000**
p-Value[a]	0.691		0.001**		

*p < 0.05, **p<0.005.

[a]p-Value is calculated by Mann–Whitney U test between groups.

[b]p-Value is calculated by Wilcoxon test between study phases.

Table 4

Cohen's d and pretest–posttest percentages of change in intervention groups.

	Cohen's d Posttest	% of change Pretest–Posttest
EG1		
State anxiety	8.05	−26.55
Trait anxiety	7.62	−30.77
EG2		
State anxiety	6.68	−25.55
Trait anxiety	6.31	−29.18
EG3		
State anxiety	5.04	−20.20
Trait anxiety	4.43	−23.33
EG4		
State anxiety	9.34	−36.69
Trait anxiety	9.74	−47.73
EG5		
State anxiety	7.99	−31.86
Trait anxiety	7.79	−36.99