JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/34177

RESEARCH ARTICLE

Effectiveness of Tea Made from *Cissampelos sympodialis* Leaves in the Treatment of Anxiety and Depression: A Controlled Clinical Trial

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Abstract

The species *Cissampelos sympodialis* Eichl. (*Cs*E) is popularly used for its anti-inflammatory, bronchodilator and anti-allergic effects. In preclinical studies, the plant has shown anxiolytic and antidepressant properties, which necessitated a controlled clinical trial to verify its effects in humans. A quasi-randomized study was developed in which the volunteers were divided into three groups: *Cs*E leaves tea (n = 21), auriculotherapy (n = 20) and no intervention (n = 19). The participants in the three groups received weekly sachets of powdered dried leaves of *Cs*E for tea preparation or auriculotherapy or did not receive intervention, respectively. At the beginning and end of the 5-week follow-up, the volunteers completed the following questionnaires: STAI-S, STAI-T, BDI and BAI. The phytochemical analysis of the sachet containing the powdered dried *Cs*E leaves showed the presence of alkaloids, steroids, tannins and flavonoids. In addition, the chromatographic analysis of *Cs*E leaves identified the presence of 7 chemical substances, for instance Malic Acid, α-L-Mannofuranose and Myo-Inositol. The STAI-S, STAI-T, BDI and BAI responses showed that the groups using tea or auriculotherapy showed significant reductions in mean scores in the two-way repeated measures analysis of variance. In the no-intervention group, no significant differences were found on any scale. Applying the post-hoc test, the influence of the group on the initial or final variances of the STAI-S ($p = 0.004$), STAI-T ($p = 0.005$), BDI ($p < 0.0005$) and BAI ($p = 0.011$) data were tested, highlighting that the tea and auriculotherapy led to the decrease in the questionnaire scores post-treatment. The tea of leaves of *Cs*E proved, in this study, to be effective in decreasing the scores of STAI-S, STAI-T, BDI and BAI and could represent a new perspective of complementary treatment for the symptoms of anxiety and depression.

Keywords: Anxiety, Auriculotherapy, *Cissampelos sympodialis* Eichl. (*Cs*E), Medicinal Plants, Traditional Medicine

Clinical trial registration: 24139319.0.0000.8069

List of Abbreviations

*Cs*E - *Cissampelos sympodialis* Eichl.
LD_{50} - Median lethal dose
IL - Interleukin
cAMP - Cyclic adenosine monophosphate
TCM - Tradicional Chinese Medicine
CRAS - Reference Centre for Health Attencion
UFPB - Federal University of Paraíba
GC-MS - Gas Chromatography-Mass Spectroscopy

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Article Received on: 22.06.2023 Revised on: 12.10.2023 Accepted on: 13.10.2023

BSTFA - N,O-bis(trimethylsilyl) triflouro-acetimide
NIST - National Institute of Standards and Technology
STAI - State-Trait Anxiety Inventory
STAI-S - State-Trait Anxiety Inventory-State
STAI-T - State-Trait Anxiety Inventory-Trait
BDI - Beck Depression Inventory
BAI - Beck Anxiety Inventory
SPSS - Statistical Package for the Social Sciences
ANOVA - Analysis of variance
GAD - Generalised anxiety disorder

1. Introduction

The species *Cissampelos sympodialis* Eichl. (*Cs*E) belongs to the Menispermaceae family and is native to the north- and south-eastern regions of Brazil[1]. It is commonly known as 'milona', 'orelha de onça', 'jarrinha' or 'abuteira' and is used in folk medicine to treat symptoms associated with various diseases such as respiratory diseases, allergies and rheumatic diseases[2-4]. The main pharmacological activities of *Cs*E are anti-inflammatory, bronchodilator, anti-allergic, analgesic and immunomodulatory[4-9].

It was verified when investigating the LD_{50} (median lethal dose) of the hydroalcoholic state of *Cs*E leaves, that the administration of doses of up to 5 g/kg orally and 2 g/kg intraperitoneally did not cause deaths in animals, which confers negligible toxicity to the aqueous fraction of the species aforementioned[10]. A clinical study verified that the tea of *Cs*E leaves with boiling water was considered safe and well tolerated for human consumption[11].

The anti-inflammatory activities of *Cs*E, including reducing the levels of interleukin (IL)-10, IL-1β and tumor necrosis factor-α, as well as inhibiting prostaglandin E2 and bradykinin, are beneficial for the treatment of laboratory-induced health conditions related to inflammation, as paw edema, carrageenan-induced peritonitis, acid-induced writhing and pyloric ligation-induced gastric ulcer in animal models, highlighting the need to verify its benefits in humans[7,12]. Some reports of preclinical studies suggest that *Cs*E alkaloids affect the behaviour of rats being treated through reduced food intake or scratching[13,14]. If a positive behavioural effect is observed in humans, *Cs*E species may represent a therapeutic opportunity for mental health.

In studies involving mice, the ethanolic extract of *Cs*E leaves was shown to have an antidepressant effect when the animals were subjected to the forced swim test and the effects induced by reserpine were reversed[15,16]. The inhibitory effect of the ethanolic extract of CsE leaves on the central cyclic nucleotide phosphodiesterase isoenzyme may have antidepressant activity by culminating in the increase of Cyclic Adenosine Monophosphate (cAMP) levels in the brain. Furthermore, cAMP increases bronchodilator agent levels such as methylxanthines, which themselves produce central and antidepressant stimuli[15]. The anxiolytic effect of the plant was demonstrated in a preclinical trial with oral administration of the ethanolic extract of the leaves or the isolated alkaloid (warifitein). It is suggested that antidepressant substances also have anxiolytic properties[17]. Flavonoids, also found in *Cs*E leaves[3], are a substance present in several plants with anxiolytic activity, and may also have psychopharmacological activities[18]. Based on these observations, a clinical trial of *Cs*E leaves was necessary to investigate possible anxiolytic and antidepressant activities.

Auriculotherapy, on the other hand, uses an acupuncture microsystem that acts on the ear by stimulating reflex points to the symptoms felt by the person, making it a simple technique with few adverse effects[19-22]. Two aspects of ear acupuncture are described: one is related to Traditional Chinese Medicine (TCM), which assumes communication between the internal organs and the auricular pavilion via meridians, channels, and/or innervation of the vagus nerve, and the French one by Paul Nogier, who describes the ear as a map resembling that of an inverted foetus, corresponding to the reflex points of the organs of interest[23-25].

Auriculotherapy is a technique that can be used to treat various clinical conditions, such as chronic pain[26-28], and its beneficial effects on depression and anxiety in humans have been described in placebo-controlled clinical trials with other therapeutic approaches and/or without intervention[25,29-31]. The psychogenic effects of auriculotherapy on anxiety and depression are related to the release of neurotransmitters and hormones associated with the reduction of stress-induced biomarkers[30], especially due to the transcutaneous stimulation of the vagus nerve, which can produce a

useful modulatory effect in the treatment of conditions neuropsychiatric[25].

Considering that auriculotherapy is a scientifically based method for reducing the symptoms of anxiety and depression, objects of study in this work, it was decided to use this intervention as a comparison to the effects of tea from the leaves of *Cs*E. In this context, the first clinical trial was conducted to investigate the effects of *Cs*E leaf tea on symptoms related to anxiety and depression. To better test the effects of the proposed intervention, two control groups were used: auriculotherapy and no-intervention control groups.

2. Materials and Methods

2.1 Study Design

This controlled, quasi-randomized, clinical trial was conducted at the Reference Centre for Health Attencion (Centro de Referência em Atenção à Saúde, [CRAS]) of the Federal University of Paraíba (UFPB), Brazil. Volunteers were divided into groups that received a tea of leaves of *Cs*E Eichl. or auriculotherapy or neither intervention. This study was approved by the Research Ethics Committee of the Centre for Medical Sciences of the Federal University of Paraíba (approval code, CAAE 24139319.0.0000.8069) and the Brazilian Registry of Clinical Trials (UTN code U1111-1287-7367). All participants in this study signed the informed consent form.

2.2 *Cissampelos sympodialis* Eichl. Sachets Design

The plant *Cs*E were cultivated and the leaves were collected in the garden of medicinal plants at the Laboratory of Pharmaceutical Technology Prof. Delby Fernandes de Medeiros, UFPB. The identification and morphological characterisation of *Cs*E were made by Dra. Maria de Fátima Agra. Samples are located in the Lauro Pires Xavier herbarium, at UFPB, by the voucher specimen number Agra 1456 (JPB).

The leaves harvested were dehydrated in a greenhouse with air flowing at 38 ºC for 72 hours and ground in a Harley-type grinder, performing the calculation of average yields. They were pounded, submitted to phytochemical and quality control tests, and then sent to the company responsible for producing the *Cs*E sachets.

2.3 Phytochemical Evaluation of *Cissampelos sympodialis* Eichl.

The phytochemical trial of *Cs*E tea identified the presence of alkaloids, steroids, tannins and flavonoids. The characterization of the classes of chemical substances present on the leaves of CsE was made before sachets production.

2.4 Gas Chromatography-Mass Spectroscopy (GC-MS) for Compound Identification in *Cissampelos sympodialis* Eichl. Tea

The sample (200 µL of tea) was derivatized with 200 µL N,O-bis(trimethylsilyl)triflouro-acetimide (BSTFA) with trimethylchlorosilane and catalyzed by heating, in a closed flask, with methanol, until boiling for 1 hour.

The GC-MS used in this study was an Agilent gas chromatograph model 8860 CG System (Agilent, Santa Clara, CA) with mass selective detector model 5977B GC/MSD. The GC was operated in the split (50:1) injection mode with an injection (0.5 µL) port temperature of 250ºC. The carrier gas was helium and the flow rate was maintained at 1.0 mL/min. An initial oven temperature of 50ºC. The temperature was then ramped at a rate of 3ºC/min to a final temperature of 290ºC. The total analysis time was 80 minutes including a 4 minutes solvent delay. An Agilent 19091S-433 capillary column was used (30 m x 250 µm x 0.25 µm). Quadrupole mass spectrometry was used for detection (EI: 70 eV, Scan: 40 – 550 m/z, 230ºC). Data were acquired and processed with the software GC/MSD (Agilent). The limitations of GC-MS involve the need for volatile and thermally labile components capable of withstanding the allotment conditions of the gas chromatograph[32].

The identification and analysis of volatile compounds derived from tea were identified by the National Institute of Standards and Technology (NIST) database. The efficacy of the derivatisation agent BSTFA-TMS was assessed during this study, and we identified seven compounds.

2.5 Patients and Population

Volunteers were recruited while waiting for medical attention at CRAS based on the offer to participate in the study. They were informed about the spontaneity of contribution and the right to withdraw their participation at any stage of the study.

Individuals who experienced symptoms related to anxiety and depressed mood were included in the study if they were at least 18 years old and had the physical and mental capacity to talk to the interviewers, follow the intervention protocols, and complete the questionnaires. Individuals who were affected by anxiety and/or depression disorders could participate in this study if they were not undergoing psychopharmacological treatment, which could interfere with the observed results. The exclusion criteria were as follows: i) pregnant women, nursing mothers and individuals with kidney or liver disease, ii) individuals taking psychotropic drugs, iii) patients who refused to receive any of the interventions or sign the informed consent form, iv) and individuals whose auricle had anatomical abnormalities that made it difficult to locate the auricular points.

2.6 Trial Design and Interventions

Data collection and follow-up were conducted in individual sessions between January 2020 and September 2022. First, volunteers were informed of the risks and benefits of the study and informed consent was obtained. They were assigned to one of three groups: tea of *Cs*E leaves (G1); auriculotherapy (G2); control/no intervention (G3). Due to the pandemic, our study was quasi-randomised. We decided to allocate the volunteers according to a follow-up schedule. The first 21 volunteers were allocated in G1, the following 21 in G2 and the last 21 in G3. The follow-up of one participant from G2 and two from G3 was lost.

All groups were followed up weekly for 5 weeks by individual supervision of a medical doctor associated with the Brazilian Medical College of Acupuncture (Colégio Médico Brasileiro de Acupuntura), with an initial and a final questionnaire completed during the first and fifth sessions, respectively. If needed, participants could call the medical team of CRAS, including the psychiatrist who was part of the research team any time before, during or after the follow-up.

For the G1 patients, a total of 21 sachets containing 1 gram of the powdered dry leaf of the plant *Cs*E were delivered at each session, of which 3 sachets were to be consumed daily, before the three main meals, until the next appointment. The total daily dose administered, based on the popularly used dose, with a wide safety range relative to preclinical toxicity studies. The volunteers prepared the tea by pouring boiling water (approx. 150 mL) over the sachet for 10 to 15 min in standardised porcelain cups distributed by the researcher, followed by the removal of the sachet and consumption of the tea.

G2 volunteers underwent weekly asepsis, inspection and palpation of the ear pavilion, followed by applying ear seeds to the acupuncture points, which were attached with micropore tape. Participants were instructed to stimulate each ear point at least 3 times per day. The auriculotherapy protocol used followed the method suggested by TCM, using areas already researched for anxiety and depression: 'Shen Men'[29,33,34], 'Kidney'[35-37], 'Sympathetic'[34,38,39], 'Anxiety'[35,39,40] and 'Heart'[35,41,42]. Each week, the ear seeds were removed from the ear in question and placed on the corresponding contralateral ear. The G3 participants, on the other hand, received no intervention. They continued with the weekly monitoring session and answered the questionnaires.

2.7 Questionnaires and Outcome Measures

The State-Trait Anxiety Inventory (STAI) was used to classify anxiety[43]. The STAI has one segment that represents anxiety as a state (STAI-S), highlighting a transient situation related to a recent stressful situation, and another that reflects anxiety as a trait (STAI-T) or a more stable occurrence. The STAI has 20 items and takes into account a minimum score of 20 and a maximum score of 80, with scores of 20–40 denoting a 'low level of anxiety', 40–60 denoting a 'medium level of anxiety', and 60–80 denoting a 'high level of anxiety'[43,44].

The Beck Depression Inventory (BDI) and the Beck Anxiety Inventory (BAI) were also used as they are internationally recognised for assessing symptoms of depression and anxiety[45,46]. Both questionnaires consist of 21 items about how the person has felt in the last week, with depression and anxiety scores ranging from 0 to 63 points each. The scoring classification of BDI for depression symptoms takes into account a total score of 0–13 for the minimal range (normality), 14–19 for mild, 20–28 for moderate, and 29–63 for severe depressive symptoms.

2.8 Statistical Analysis

Statistical analysis was conducted using the Statistical Package for the Social Sciences statistical software version 26 (SPSS Inc., Chicago, IL, USA). The normality of the data was assessed using the

Shapiro–Wilk test and the homogeneity of variance with the Lavene test. Numerical descriptive data from the questionnaires were presented as mean and standard deviation. One-way Analysis of Variance (ANOVA) and the Kruskal-Wallis test were used to assess the statistical difference between groups in the preliminary analysis of questionnaire scores. To assess the statistical difference between the initial and final scores of the questionnaires of each group and between groups, the two-way repeated measures ANOVA was performed, followed by the Sidak post-hoc test to observe the group effect on the initial and final variation with $p < 0.05$.

3. Results

3.1 Phytochemical Evaluation and GC-MS Results

Four samples of the powdered dry leaves of CsE were evaluated, using average concentrations of 30 g (Table 1). Phytochemical screening was also carried out with the sachet containing 1 g of powdered dry leaves, using for analysis 1, 4, 6 and 15 sachet units. The presence of alkaloids, steroids, tannins and flavonoids was observed in lower levels than those described in Table 1, however, the amounts were increasing and directly proportional to the concentrations used.

Chromatographic analysis of the *Cs*E leaves tea identified the presence of 7 chemical substances, described in Figure 1. Malic Acid, one of the substances present, has potential anti-inflammatory activity, reduction of stress-related symptoms, inhibition of superoxide anion and is related to the reduction of neuroinflammation revealed in preclinical studies[47,48]. α-L-Mannofuranose, in turn, seems to be associated with reduced osmotic stress[49]. Myo-Inositol was associated with antidepressant activity in experimental models of depression and a possible role in stress resistance pathways evidenced in studies with plants[50,51]. The 1,5-Anhydroglucitol is an important substance with potential use for monitoring glycemic control, and may also interfere with cardiovascular risk[52]. Lastly, D-ribose is used as an anti-fatigue medication and appears to have a role in improving the quality and life expectancy of diabetic patients[53]. MS profiles are shown in Supplementary Figures S1-S7 of chemical substances (BSTFA-derivatized *Cs*E Tea).

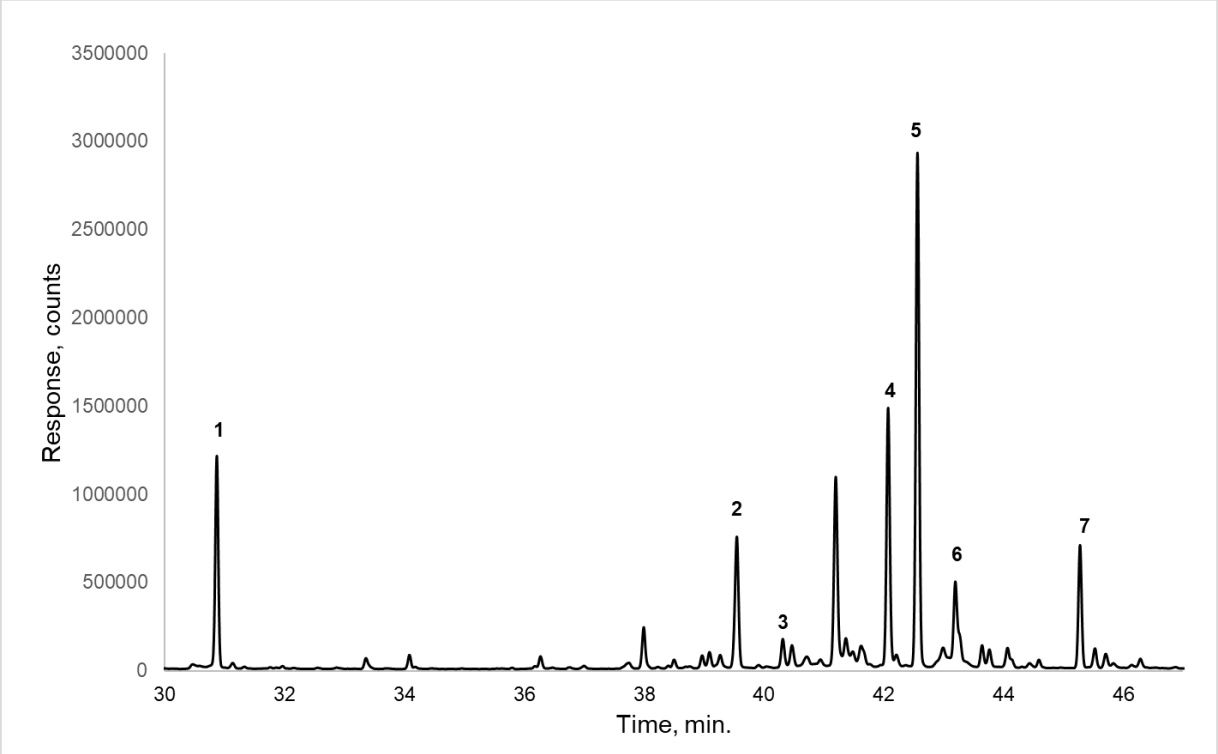
Table 1. Phytochemical screening of leaf samples of *Cissampelos sympodialis*

	Test	Sample results			
		1º	2º	3º	4º
Alkaloids	Bouchardat	++	+	+	+
	Mayer	++	+	++	++
	Dragendorff	+++	++	+++	+++
	Silico-tungsten acid	++	-	+	+
Steroids	Steroids	++	+	++	++
Tannins	Gelatin $_{0.5\%}$	+	++	+	++
	$FeCl_{3\ 2\%}$ (25%)	-	-	+	+
	$FeCl_{3\ 2\%}$ (50%)	++	+	++	+
	$FeCl_{3\ 2\%}$ (100%)	++	++	++	++
Flavonoids	Magnesium-tape	+	+	++	++
	Fluorescence	++	++	++	++

*The presence and amount of substance class is demonstrated with (+) and the absence with (-).

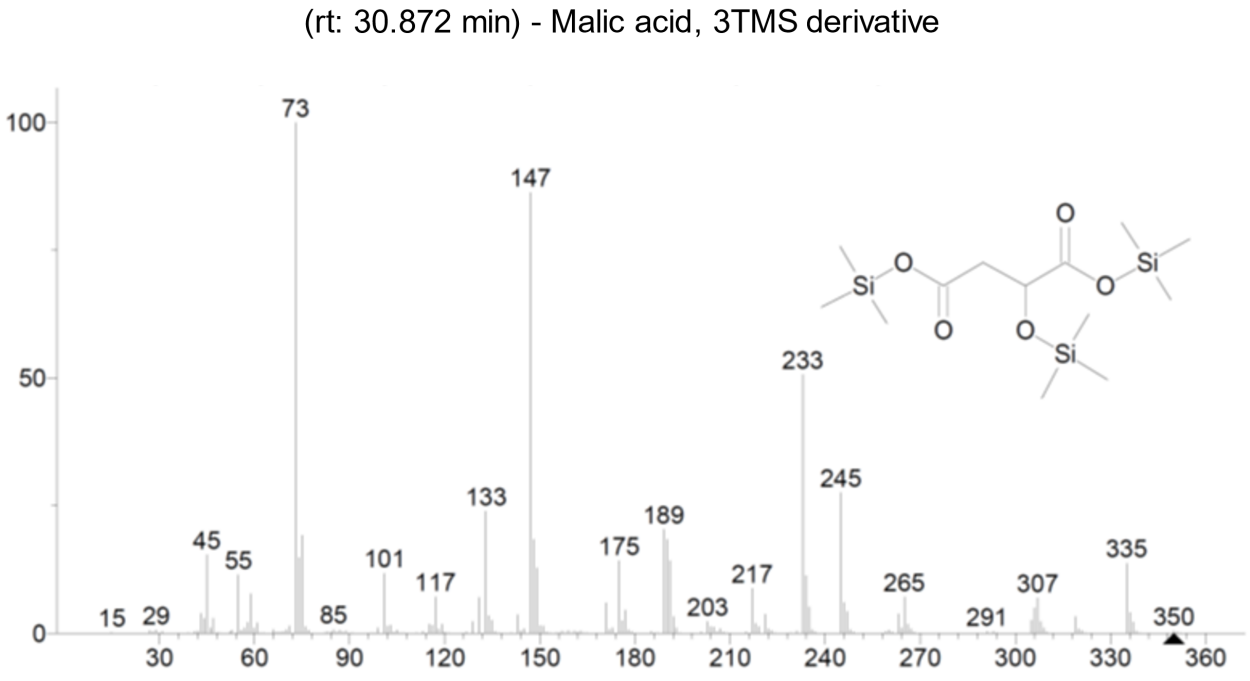
3.2 Clinical Trial Results

The total number of participants included and evaluated in this study was 60. None of the volunteers requested a psychiatry consultation due to a mental change or psychosocial distress as a result of this study. No adverse effects were reported during the follow-up period due to the use of the tea or auriculotherapy. The mean scores with standard deviations for G1 (n = 21), G2 (n = 20) and G3 (n = 19) obtained before and after each questionnaire are shown in Table 2. Normality and homogeneity of variance tests of each questionnaire are disposed of in Supplementary Table 1. The mean ages of the groups were 42.19±13.62, 44.25±17.59, and 30.95±17.66, respectively. Of the total sample at the beginning of the study, based on the STAI scores, 48.3% (n = 29) had a low level of anxiety, 48.3% (n = 29) had a medium level of anxiety, and only 3.3% (n = 2) had a high level of anxiety. Considering BDI scores, 76.7% (n = 46) of patients had a minimal range of depressive symptoms, 15% (n = 9) had mild depressive symptoms,

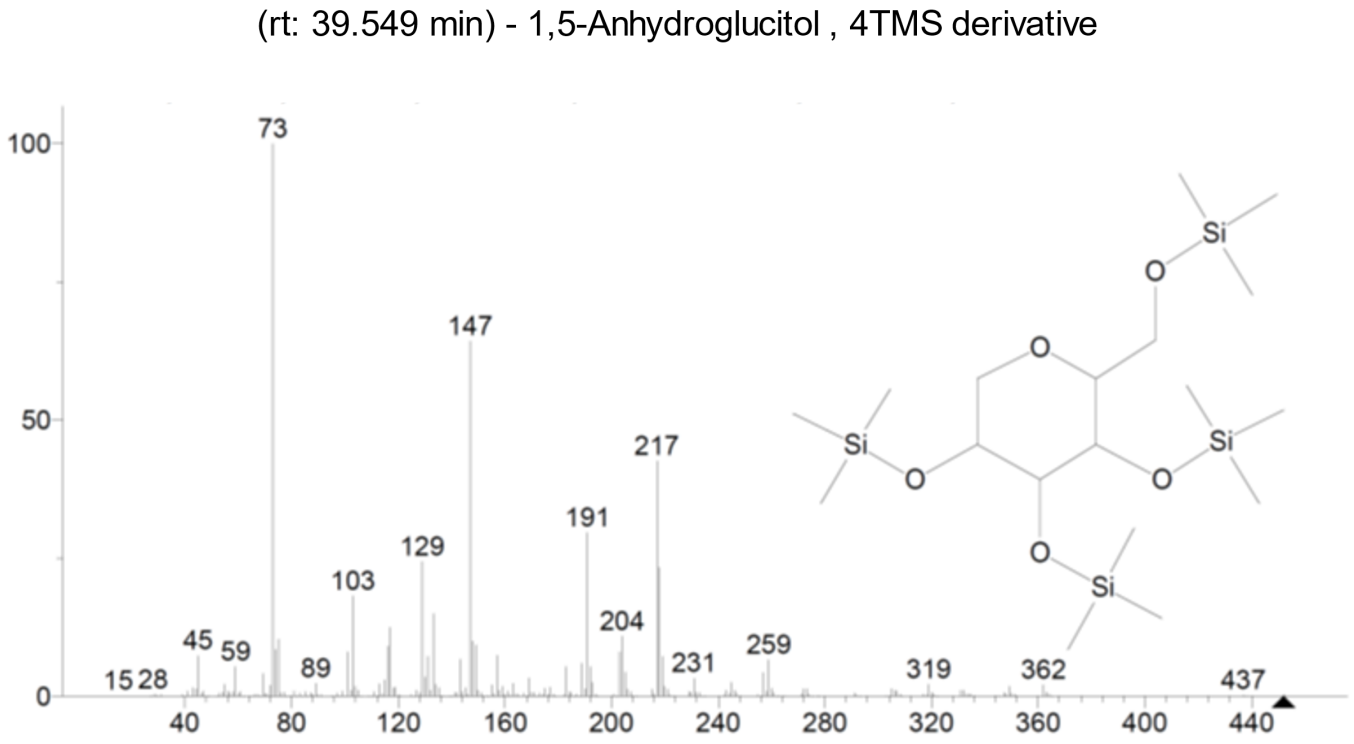


1: Malic acid, 3TMS derivative; 2: 1,5-Anhydroglucitol, 4TMS derivative; 3: Lactulose, octakis(trimethylsilyl) ether (isomer 1); 4: α-L-Mannofuranose, 6-deoxy-1,2,3,5-tetrakis-O-(trimethylsilyl); 5: Myo-Inositol, 6TMS derivative; 6: D-Allofuranose, pentakis(trimethylsilyl) ether; 7: D-Ribose, 4TMS derivative.

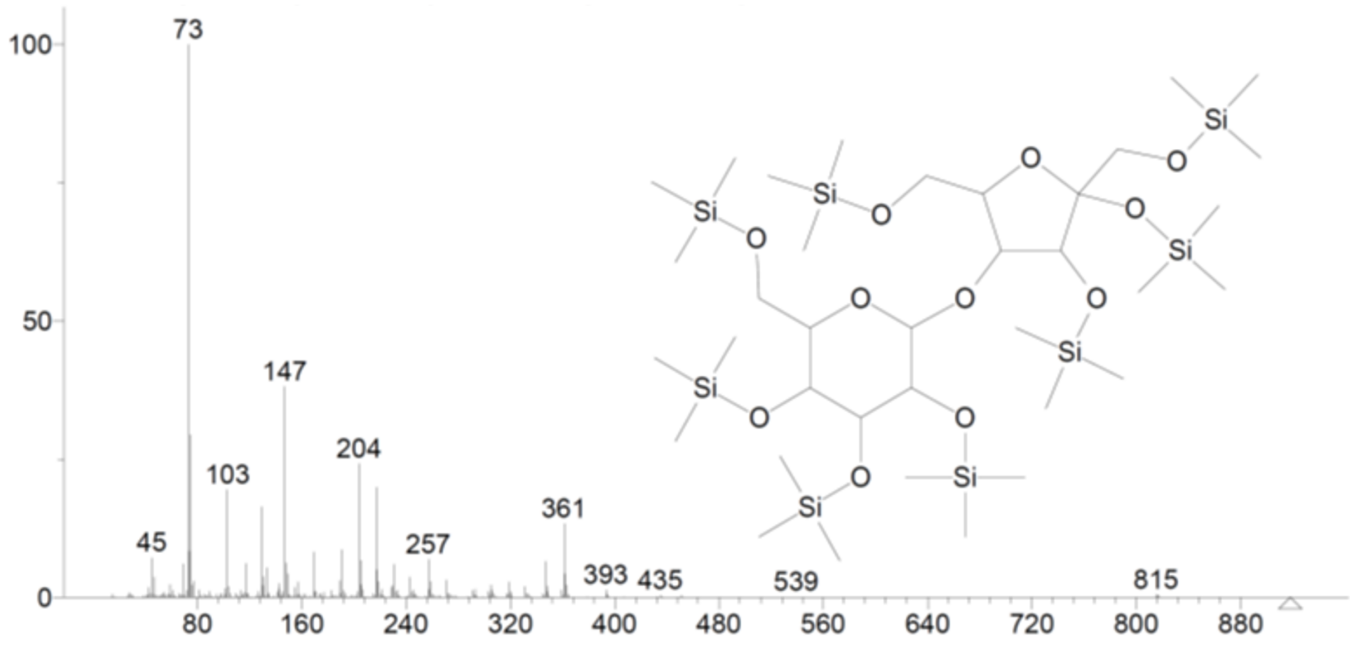
Figure 1. GC-MS chromatogram of BSTFA-derivatized CsE tea.



Supplementary Figure S1. MS profile of BSTFA-derivatized *Cs*E tea – malic acid.

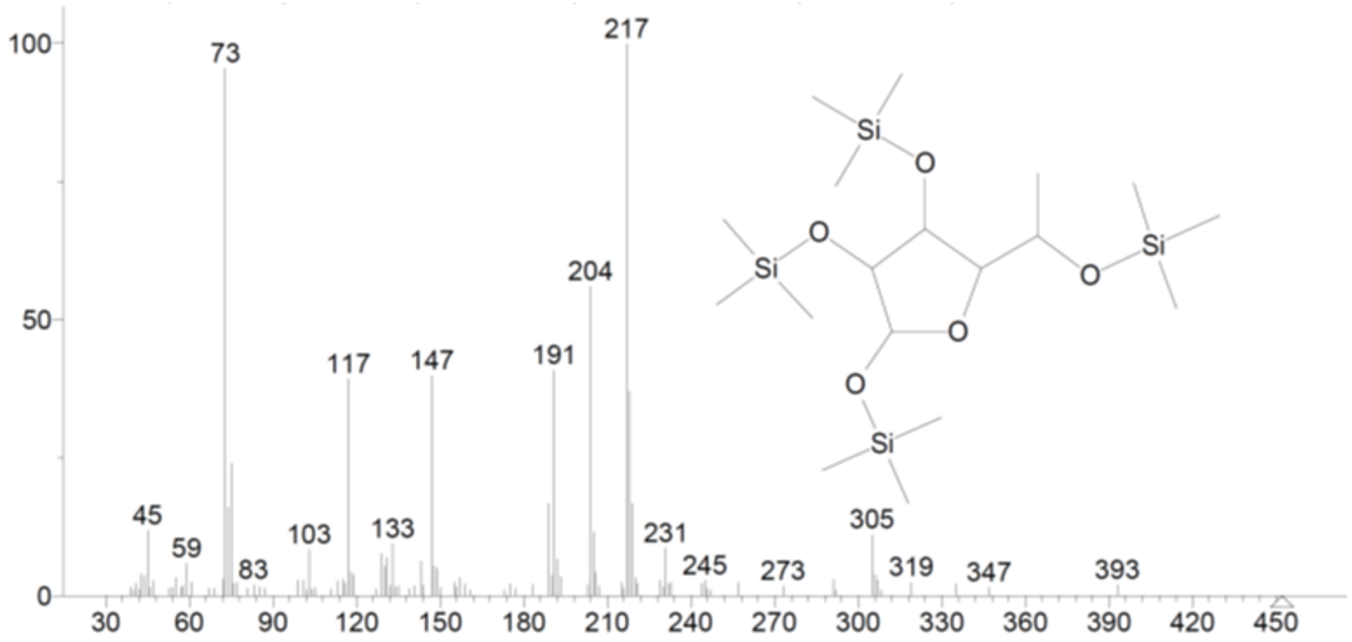


Supplementary Figure S2. MS profile of BSTFA-derivatized *Cs*E Tea – 1,5-anhydroglucitol.



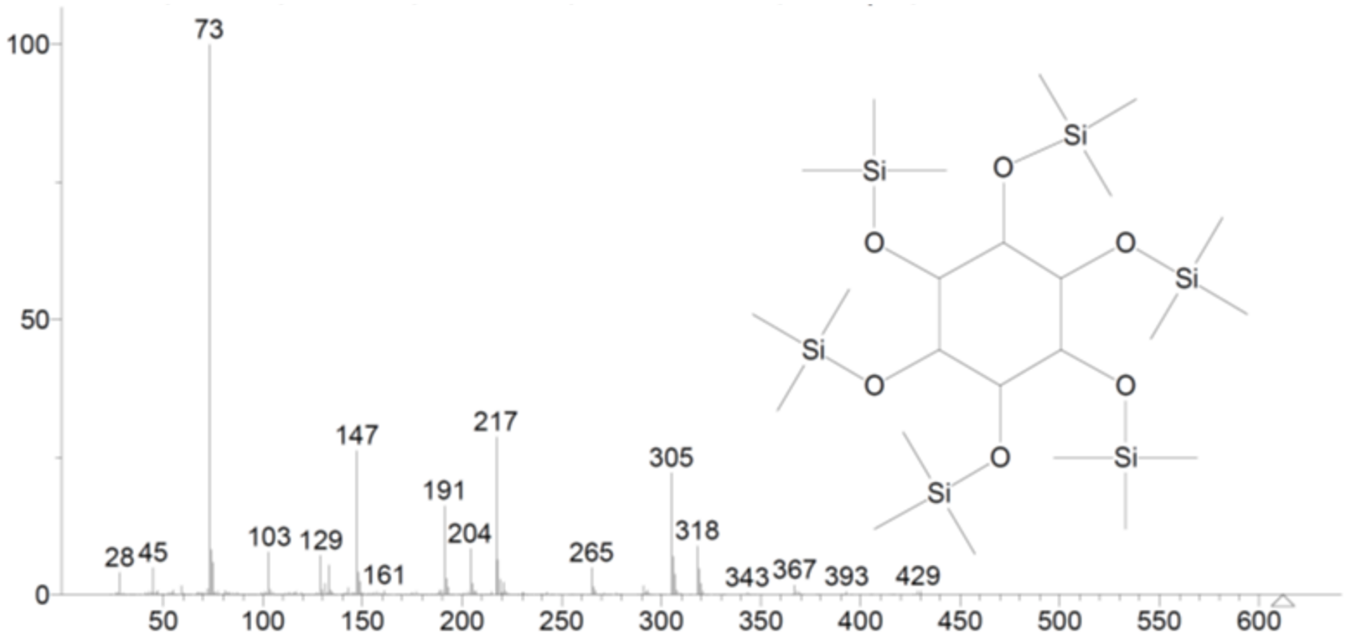
Supplementary Figure S3. MS profile of BSTFA-derivatized *Cs*E tea – lactulose.

(rt: 42.074 min) - α-L-Mannofuranose , 6-deoxy-1,2,3,5-tetrakis-O-(trimethylsilyl)-

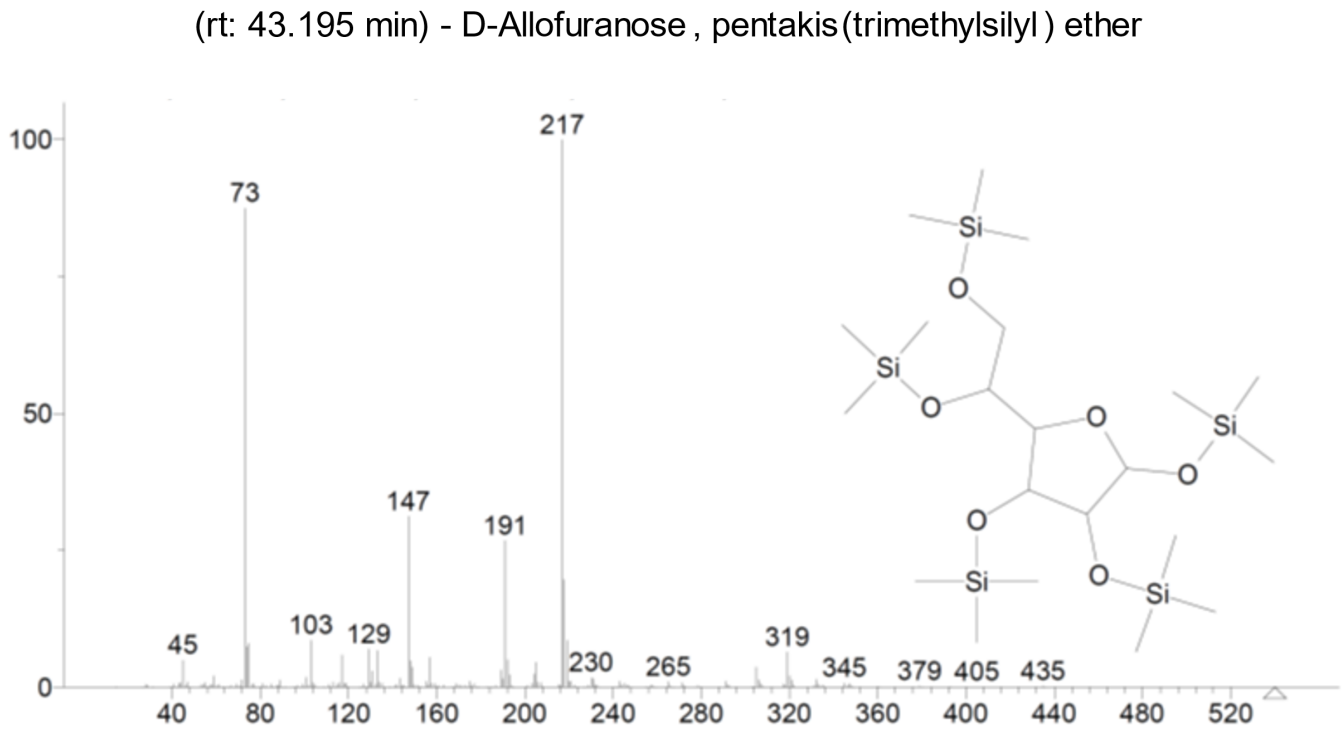


Supplementary Figure S4. MS profile of BSTFA-derivatized *Cs*E Tea – α-L-mannofuranose.

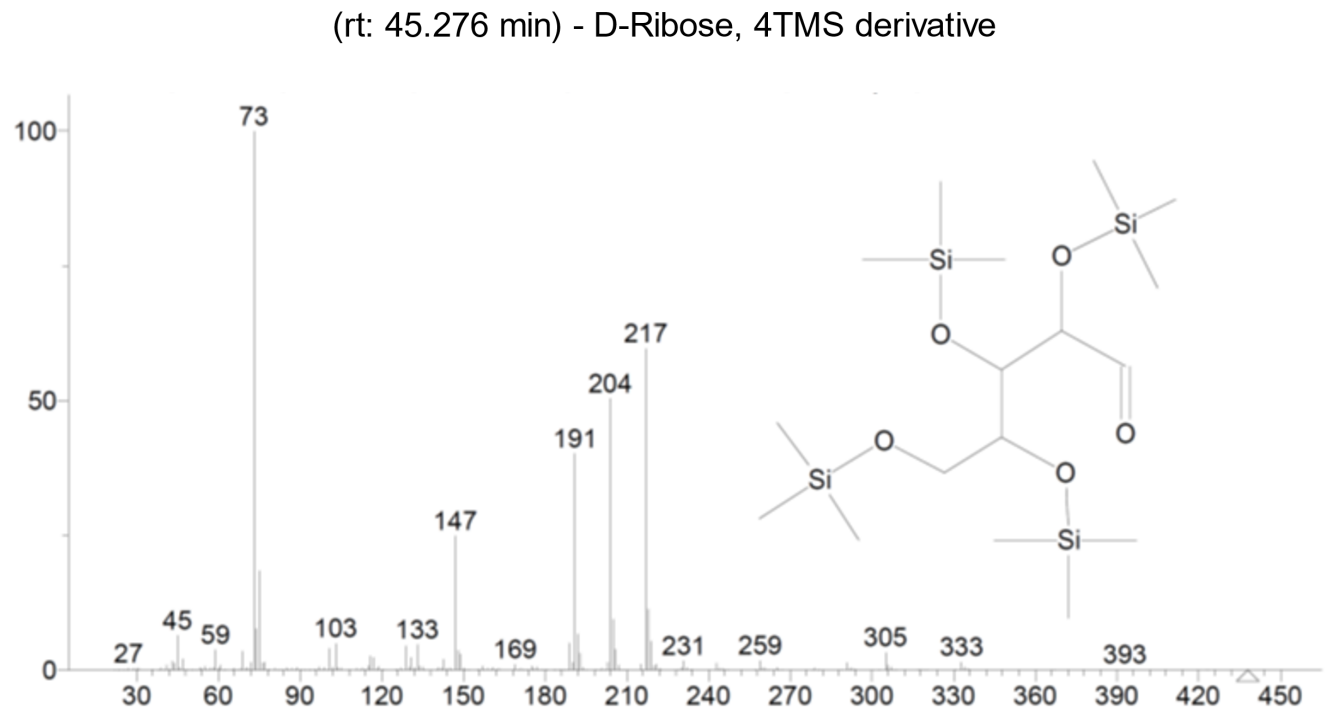
(rt: 42.564 min) - Myo-Inositol, 6TMS derivative



Supplementary Figure S5. MS profile of BSTFA-derivatized *Cs*E tea – myo-inositol.



Supplementary Figure S6. MS profile of BSTFA-derivatized *Cs*E tea – D-allofuranose.



Supplementary Figure S7. MS profile of BSTFA-derivatized *Cs*E tea – D-ribose.

Table 2. Means and standard deviations scores before and after the interventions

Questionnaire*	Total	Tea	Auriculoterapy	Control	p-value
	(*n* = 60)	(*n* = 21)	(*n* = 20)	(*n* = 19)	
Initial STAI-S score	42.43 ± 10.7	42.43 ± 9.57	46 ± 13.4	38.68 ± 7.38	0.101[a]
Final STAI-S score	33.82 ± 9.04	31.10 ± 7.18	33.90 ± 9.74	36.74 ± 9.64	0.159[b]
Initial STAI-T score	42.27 ± 10.91	40.95 ± 8.37	46.95 ± 12.98	38.79 ± 9.76	0.49[a]
Final STAI-T score	34.50 ± 9.35	31.5 ± 5.62	34.8 ± 10.06	38 ± 9.135	0.034[b]
Initial BDI score	9.35 ± 7.58	7.57 ± 3.97	14.05 ± 8.85	6.37 ± 7.12	0.001[b]
Final BDI score	4.88 ± 5.51	3.57 ± 4.68	4.55 ± 4.85	6.68 ± 6.69	0.101[b]
Initial BAI score	8.9 ± 7.46	8.81 ± 4.96	12.45 ± 9.53	5.26 ± 5.57	0.006[b]
Final BAI score	4.83 ± 5.16	4.29 ± 3.74	5.1 ± 5.92	5.16 ± 5.86	0.967[b]

*Quantitative variables were presented by mean and standard deviation. [a]: One-Way ANOVA; [b]: Kruskal-Wallis test.

Supplementary Table 1. Normality and homogeneity of variance tests of the instruments used in this study

Questionnaire	Normality	Homogeneity of Variance
	Shapiro-Wilk Test	Levene Test
Initial STAI-S score	0,074	0,018[a]
Final STAI-S score	0,028	0,544[b]
Initial STAI-T score	0,056	0,176[a]
Final STAI-T score	0,011	0,092[b]
Initial BDI score	<0.0005	0,142[b]
Final BDI score	<0.0005	0,589[b]
Initial BAI score	<0.0005	0,147[b]
Final BAI score	<0.0005	0,525[b]

[a]:p-value based on mean for normal distribution samples; [b]: p-value based on median for non-normal distribution samples.

3.3% (n = 2) had moderate depressive symptoms and 5% (n = 3) had severe depressive symptoms.

3.3 STAI-S

The results found when assessing the STAI-S before and after between the groups are shown in Table 3. There was a significant decrease in the mean STAI-S scores for the initial-final variation in G1 ($p < 0.0005$) and G2 ($p < 0.0005$) but no difference in G3 ($p = 0.331$). Sidak's

Table 3. Verification of differences between the groups with statistical significance and the effect of the intervention group in the initial and final variations in STAI-S

Two-Way Repeated Measures ANOVA - STAI-S		
Parameter	F (df model and error)*	p-value
Initial tea *vs* final tea		<0.0005
Initial auriculotherapy *vs* final auriculotherapy		<0.0005
Initial control *vs* final control		0.331
Initial auriculotherapy *vs* initial tea		0.499
Initial auriculotherapy *vs* initial control		0.033
Initial tea *vs* initial control		0.529
Final auriculotherapy *vs* final tea		0.698
Final auriculotherapy *vs* final control		0.847
Final tea *vs* final control		0.172
Effect of group on initial *vs* final variation (Sidak post-hoc test)	[F(2, 36) = 6.424]	0.004

*F: F-statistic; df: degrees of freedom. *vs*: *versus*.

post-hoc test showed that there was a group effect on the initial-final variation in the STAI-S anxiety scores (F(2, 36) = 6.424; p = 0.004).

3.4 STAI-T

The results of the initial and final STAI-T scores are presented in Table 4. The STAI-T mean scores showed a significant decrease for groups G1 (p < 0.0005) and G2 (p < 0.0005), while that of group G3 showed no difference after the follow-up period (p = 0.636). Sidak's post-hoc test showed that the initial-final variation in STAI-T anxiety scores was influenced by the group of the volunteer (F (1.47, 26.62) = 7.421; p = 0.005).

3.5 BDI

Table 5 shows the results for the initial and final BDI of the groups. The group that used the *Cs*E leaves tea and the group that received auriculotherapy had significantly different initial and final BDI scores (p < 0.0005; p < 0.0005). In the non-intervention group, no significant changes were found between the initial and final mean scores (p = 0.646). Sidak's post-hoc test showed that there was a group effect on the initial and final variations in BDI scores for depressed mood (F (1.32, 23.75) = 7.421; p < 0.0005).

Table 4. Verification of differences between the groups with statistical significance and the effect of the intervention group on the initial and final variations in the STAI-T scores

Two-Way Repeated Measures ANOVA - STAI-T		
Parameter	**F (df model and error)***	**p-value**
Initial tea *vs* final tea		<0.0005
Initial auriculotherapy *vs* final auriculotherapy		<0.0005
Initial control *vs* final control		0.636
Initial auriculotherapy *vs* initial tea		0.127
Initial auriculotherapy *vs* initial control		0.034
Initial tea *vs* initial control		0.932
Final auriculotherapy *vs* final tea		0.34
Final auriculotherapy *vs* final control		0.781
Final tea *vs* final control		0.015
Effect of group on initial *vs* final variation (Sidak post-hoc test)	[F(1.47, 26.62) = 7.421]	0.005

*F: F-statistic; df: degrees of freedom. *vs*: *versus*.

Table 5. Verification of differences between the groups with statistical significance and the effect of the intervention group on the initial and final variations in BDI

Two-Way Repeated Measures ANOVA - BDI		
Parameter	**F (df model and error)***	**p-value**
Initial tea *vs* final tea		<0.0005
Initial auriculotherapy *vs* final auriculotherapy		<0.0005
Initial control *vs* final control		0.646
Initial auriculotherapy *vs* initial tea		0.047
Initial auriculotherapy *vs* initial control		0.026
Initial tea *vs* initial control		0.83
Final auriculotherapy *vs* final tea		0.953
Final auriculotherapy *vs* final control		0.549
Final tea *vs* final control		0.375
Effect of group on initial *vs* final variation (Sidak post-hoc test)	[F(1.32, 23.75) = 7.421]	<0.0005

*F: F-statistic; df: degrees of freedom. *vs*: *versus*.

3.6 BAI

Table 6 presents the results of the initial and final BAI of the groups. G1 had significantly different initial and final mean scores (p < 0.0005), as did G2 (p = 0.002). G3, on the other hand, could not demonstrate any changes in the initial-final interval (p = 0.834). Sidak's post hoc test showed that there was a group effect on the change in BAI anxiety scores before and after follow-up (F [1.19, 21.58] = 7.105; p = 0.011).

Table 6. Verification of differences between the groups with statistical significance and the effect of the intervention group on the initial and final variations in BAI

Two-Way Repeated Measures ANOVA - BAI		
Parameter	**F (df model and error)***	**p-value**
Initial tea *vs* final tea		<0.0005
Initial auriculotherapy *vs* final auriculotherapy		0.002
Initial control *vs* final control		0.834
Initial auriculotherapy *vs* initial tea		0.372
Initial auriculotherapy *vs* initial control		0.033
Initial tea *vs* initial control		0.195
Final auriculotherapy *vs* final tea		0.987
Final auriculotherapy *vs* final control		1
Final tea *vs* final control		0.977
Effect of group on initial *vs* final variation (Sidak post-hoc test)	[F(1.19, 21.58) = 7.105]	0.011

*F: F-statistic; df: degrees of freedom. *vs*: *versus*.

4. Discussion

The initial and final mean scores of the group receiving the tea of *Cs*E leaves were not significantly different relative to the auriculotherapy group, although a decrease in anxiety scores was observed before and after treatment. The results of this study demonstrate the potential of the *Cs*E species to reduce anxiety scores. In the group receiving the tea of *Cs*E leaves, a significant decrease in BDI scores was observed before and after the intervention, also indicating a possible antidepressant effect of the species.

On assessing the effect of the group on the variation in initial and final scores, the group to which the participant was assigned was crucial for all questionnaires assessed in determining whether the scores obtained decreased or not. Both the auriculotherapy and *Cs*E leaf tea groups showed decrease in questionnaires scores, which was not observed in the control group.

In Brazil, acupuncture plays an important role in public health. There is an official medical education programme, and the country is among the top 10 producers of publications on this practise[54,55]. The increasing number of studies on acupuncture and the greater interest of the academic community demonstrate that the practice is not limited to somatic or neuroinflammatory-related pathologies[56]. The results of the present study on auriculotherapy are consistent with previous studies showing its efficacy in reducing anxiety relative to placebo or control groups[37,44]. Other studies using the same instrument to measure anxiety, the STAI, also observed a significant reduction in scores after treatment[35,57].

Another prospective study involving patients undergoing auricular acupressure treatment who were examined over 4 weeks also observed a reduction in scores for the BAI and BDI inventories[58]. The perceived reduction in the BDI can be explained by the evidence that transcutaneous auricular vagus nerve stimulation is safe and effective for relieving depressive symptoms[25]. Therefore, the findings on the effect of ear acupuncture are supported and consistent with the theoretical framework, confirming the auriculotherapy group of this study as an appropriate modality for evaluating the effect of tea from the leaves of *Cs*E.

These findings may be of interest to public health, as species of the genus *Cissampelos* have a wide geographical distribution globally, while *Cs*E is already commonly used for the treatment of various diseases, proving that society has access to the plant[4,7,11]. The results of the study on the anti-anxiety properties of *Cs*E leaf tea could provide a perspective for public health intervention, as it is readily available, affordable and minimally toxic.

The anti-allergic and immunoregulatory properties of the *Cs*E plant found in preclinical studies[59,60] seem to support its use in folk medicine. The use of medicinal plants is recommended by the World Health Organisation (WHO), especially because of their widespread use in developing countries[61]. Brazil, in turn, has a health monitoring agency that regulates and maps medicinal plants and their pharmacological effects, promotes their use and assesses their quality[62]. This work is of national importance as it provides evidence that may lead to new uses of milona.

As this is the first clinical trial to use *Cs*E leaves to evaluate possible anxiolytic and antidepressant activities, the present results need to be related to studies

that have used other plants for the same purpose. Most studies using plants to treat anxiety and depression are not phase III trials, although it has been observed that these symptoms are alleviated, despite not being as efficient as with some psychotropic drugs[63-65]. In the present study, no comparison was made with gold standard drugs for the treatment of the above mental pathologies despite the use of auriculotherapy and non-intervention as controls.

Plants with scientifically proven antidepressant and antianxiety effects include *Matricaria chamomilla* (*M. chamomilla*) and plants of the genus *Passiflora*, of which *Passiflora incarnata* (*P. incarnata*) is the main representative[66,67]. *P. incarnata* is considered to have beneficial effects on generalised anxiety disorder (GAD) and is available on the market in various formulations[67].

In a double-blind clinical trial with the use of 260 mg of *P. incarnata* 30 minutes before surgery, compared with taking midazolam 15 mg, a similar anti-anxiety effect was observed with no statistical differences in blood pressure, heart rate and oxygen saturation after ingestion[68]. In another double-blind placebo-controlled clinical trial in which *P. incarnata* tea was taken daily for one week, no significant difference was found between the tea and placebo groups when the STAI-S was evaluated[69]. In this study, there was a decrease in anxiety scores in the group using the tea of powdered leaves of species *Cs*E, and a comparison was made with an intervention already established in the literature; no significant difference was found between the final scores for the two interventions.

M. chamomilla has sedative, antinociceptive and anxiolytic effects that have been described in pre-clinical studies[70]. However, there does not appear to be sufficient evidence for the treatment of anxiety when assessed in clinical trials using the BAI, the Hospital Anxiety-Depression Scale and other methods of score measurement, despite being known for its supposed sedative effects[71]. In a randomised placebo-controlled clinical trial, long-term use of chamomile was found to significantly reduce symptoms of GAD, but there was no significant difference between the BAI scores for the chamomile and placebo groups[72]. Again, the effects of the tea of *Cs*E leaves were different; it reduced the BAI, which is different from the no-intervention group and similar to the auriculotherapy group.

The limitations of this study are mainly related to the heterogeneity of the sample, established by the scores of the questionnaires used. This is attributed to the coronavirus disease pandemic, which changed the time of data collection and the conduct of the study. To standardise the approach to the volunteers, each session was conducted by only one member of the team, which helped to extend the research during the pandemic.

Another factor that may have contributed to the heterogeneity mentioned above is the different mean ages of the groups, which occurred due to changes in the age range attended by CRAS associated with the return to face-to-face activities at the university where the study was developed. It is important to emphasize that the method of allocation of volunteers was according to the period in which they attended the CRAS and were invited to participate, without a formal randomization process, making the study quasi-randomized. Despite the limitations, the results can represent a new perspective on mental health treatment, with high availability, low toxicity and affordable cost.

Future studies should involve a sizeable quantity of professionals qualified to deliver the interventions, thus reducing the time required to develop the research. A larger number of patients and controlling pharmacological therapeutic approaches are necessary to consolidate the use of *Cs*E species in the treatment of anxiety and depression.

5. Conclusions

The present study revealed that the tea of *Cs*E leaves reduced the STAI-S, STAI-T, BDI and BAI questionnaire scores with statistical significance. The tea and auriculotherapy groups showed similar reductions in the scores of the instruments used, which was not observed in the no-intervention group.

6. Acknowledgements

We thank the Reference Centre for Health Attencion (Centro de Referência em Atenção à Saúde [CRAS]) and the researchers of the Federal University of Paraíba for encouraging the development of this research. We also thank the volunteers who contributed to the execution of this study.

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