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CLINICAL SIGNIFICANCE OF *THYMUS SERPYLLUM* L. ESSENTIAL OIL IN PATIENTS WITH SYNCOPE

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Dysautonomia, reflecting autonomic nervous system dysfunction, is frequently observed in patients with syncope, particularly those with orthostatic hypotension. *Thymus serpyllum* L. (wild thyme) has demonstrated antioxidant, anti-inflammatory, and cardiometabolic properties in preclinical studies. This study aimed to evaluate the effects of *T. serpyllum* essential oil on autonomic function and selected biochemical markers in patients with syncope. This prospective, single-center study included 15 patients with a history of syncope who received a capsule formulation of *T. serpyllum* essential oil for three months. Autonomic function was assessed using beat-to-beat heart rate and blood pressure variability analysis, 24-hour Holter ECG monitoring, and ambulatory blood pressure monitoring. Biochemical parameters were evaluated at baseline and after treatment. After three months, the beat-to-beat analysis showed increased LFnu values and a higher LF/HF ratio, along with decreased HF values, indicating a shift toward sympathetic predominance. No significant changes were observed in 24-hour heart rate or blood pressure parameters. Biochemical analyses revealed significant reductions in AST and C-reactive protein levels after treatment. In this pilot study, three-month supplementation with *T. serpyllum* essential oil was associated with changes in autonomic balance and favorable reductions in selected inflammatory and biochemical markers in patients with syncope. Further and larger placebo-controlled studies are required to confirm these findings and clarify their clinical significance.

Keywords: syncope, autonomic nervous system, *Thymus serpyllum* essential oil, neurocardiology

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INTRODUCTION

Dysautonomia, defined as a dysfunction of the autonomic nervous system (ANS), encompasses a heterogeneous group of disorders characterized by variable clinical presentations. It may result from isolated ANS impairment or form part of a broader multisystem failure (1). This diversity arises from the ANS's critical role in the involuntary innervation of all non-motor end organs and its close interconnection with the central nervous system (CNS), where the boundaries between the two are not clearly delineated (2).

Among the most common clinical manifestations of dysautonomia are disorders of orthostatic intolerance, including orthostatic hypotension (OH), both initial and delayed forms, and postural orthostatic tachycardia syndrome (POTS). These conditions are frequently accompanied by symptoms such as dizziness, near-syncope, blurred vision upon standing, and transient loss of consciousness, i.e., syncope (3-5). Regarding syncope specifically, reflex syncope has been diagnosed in 56-73% of patients, whereas orthostatic hypotension accounts for 1-10% of cases (6).

In addition to ANS dysfunction in neurodegenerative diseases (such as Parkinson's disease and multiple system atrophy), dysautonomia has been extensively studied in patients with diabetes mellitus (DM). Notably, patients with DM and cardiac autonomic neuropathy (CAN) exhibit an associated five-year mortality rate of 25-50% (7). The development of autonomic neuropathy in DM is believed to result from inflammation induced by advanced glycation end products (AGEs) and the activation of the hexosamine, protein kinase C, and polyol pathways, coupled with increased oxidative stress (7). Ziegler et al. confirmed elevated oxidative stress by employing various biochemical markers in patients with diabetic CAN compared to a control group (8).

A wide range of pharmacological and natural agents with antioxidant properties have been identified that can counteract the adverse effects of these processes. Among them, *Thymus serpyllum* L. (wild thyme) has garnered significant attention due to its potent antioxidant effects demonstrated in both in vitro and in vivo studies (9). In addition to its essential oil constituents, *T. serpyllum* contains numerous bioactive compounds, including thymol, carvacrol, flavonoids, rosmarinic acid, phenolic acids, and terpenoids, which are considered largely responsible for its antioxidant, anti-inflammatory, and cardiometabolic activities (10,11). Experimental studies have shown that T. serpyllum extracts exhibit pronounced free-radical scavenging activity, inhibition of lipid peroxidation, metal-chelating properties, and protection against oxidative damage to biological molecules (12,13).

Wild thyme essential oil is widely used in the food industry as a flavoring agent and preservative, and its antimicrobial, anti-inflammatory, hepatoprotective, and cardiometabolic properties have been well-documented, particularly in rodent models (90. Recent reviews have further highlighted its immunomodulatory, antioxidant, and potential cardiovascular protective effects, which have been attributed to the synergistic action of multiple phytochemicals rather than a single active constituent (9,10). Notably, the hydrosolic (secondary) fraction of *T. serpyllum* essential oil demonstrates enhanced antibacterial and antifungal activity, and contains a markedly higher proportion of phenolic compounds than the primary, water-insoluble fraction (9).

Furthermore, using an aqueous extract of *T. serpyllum*, Mihailović-Stanojević et al. demonstrated a significant reduction in blood pressure and peripheral vascular resistance in hypertensive rats via a nitric oxide-independent mechanism, without affecting hemodynamic parameters in normotensive rats, alongside confirming its strong antioxidant capacity (14). Similarly, Miloradović et al. reported blood pressure normalization and reduction in oxidative stress in rats following a bolus injection of thyme extract (15). Additional experimental studies have shown that *T. serpyllum* preparations may reduce oxidative stress through flavonoid- and rosmarinic acid-mediated mechanisms while simultaneously exerting anti-inflammatory effects (10). The major constituents of the essential oil, including thymol and carvacrol, have also been associated with antioxidant, antimicrobial, and vasoregulatory properties (9,10). Although animal studies suggest that *T. serpyllum* may exert beneficial effects on the cardiovascular system in humans, further clinical research is required to validate these findings and clarify their clinical significance.

The primary aim of this study was to investigate the effects of a tablet formulation of *T. serpyllum* essential oil on autonomic nervous system function parameters and selected biochemical markers in patients presenting with syncope, with measurements obtained at baseline and three months after treatment.

METHODS

Study design and participants

This prospective study included 15 patients over the age of 18 who were evaluated at the Neurocardiology Laboratory of the Cardiology Clinic, Institute for Cardiovascular Diseases "Dedinje," between December 2024 and March 2025. The inclusion criterion was a documented history of syncope, defined as a transient loss of consciousness (TLOC) due to cerebral hypoperfusion, characterized by rapid onset, short duration, and spontaneous complete recovery (3). Exclusion criteria included: cardiogenic etiology of syncope; confirmed epilepsy as the cause of TLOC (diagnosed by a neurology specialist); orthostatic hypotension resulting from volume depletion or medication use; and the presence of primary autonomic neuropathy (e.g., multiple system atrophy, pure autonomic failure, Parkinson's disease).

Demographic data, including age and sex, were collected for all participants, alongside detailed medical histories covering comorbidities (hypertension, diabetes mellitus, thyroid disorders, systemic diseases, etc.) and the presence of OH, verified during a head-up tilt test and defined as a progressive and sustained fall in systolic blood pressure from baseline of >20 mmHg, a diastolic drop of >10 mmHg, or a decrease in systolic blood pressure to <90 mmHg (3). Each participant underwent: (1) beat-to-beat blood pressure monitoring in the supine position for 5 minutes; (2) 24-hour Holter ECG monitoring; (3) ambulatory blood pressure monitoring; and (4) venous blood sampling for laboratory analyses, including standard biochemical and hematological parameters (C-reactive protein, AST, ALT, GGT, urea, creatinine, uric acid, glucose, total cholesterol, LDL and HDL fractions, and triglycerides). All measurements were obtained at baseline and after three months of treatment with the tablet formulation of *T. serpyllum* essential oil.

All participants were informed of the study's purpose and the administration protocol for the investigational product. They received detailed instructions regarding dosage and timing. Written informed consent was obtained from each participant prior to enrollment. No additional medications such as statins, beta-blockers, calcium channel blockers, or any other agent known to affect the autonomic nervous system or the biochemical parameters under investigation were introduced during the study period.

The study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Institute for Cardiovascular Diseases "Dedinje" (protocol code 6472, issued on 11 December 2024).

Study Protocol

Plant material of *Thymus serpyllum* L. was cultivated in Bavanište, Serbia (44°48′59.99″ N, 20°52′59.99″ E). Voucher specimens were deposited in the Herbarium of the Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia (voucher numbers 302 and 311).

Essential oil from *T. serpyllum* was extracted using a semi-industrial distillation apparatus (SP-130) based on water and steam distillation principles. The isolated essential oil is a light yellow liquid with an aroma characteristic of the *Thymus genus*, and was subsequently subjected to inverse molecular distillation for further refinement.

The primary carrier for the highly purified essential oil was lyophilized honey. Acacia honey, rich in natural monosaccharides (approximately 70% combined glucose and fructose), was selected due to its favorable nutritional profile and stability during processing. Lyophilization was performed in three stages: freezing, primary drying, and secondary drying (desorption). Initially, the honey was frozen at temperatures ranging between -40 °C and -60 °C. During primary drying, sublimation occurred under reduced pressure and temperature, enabling the direct transition of ice into water vapor. Secondary drying, conducted at room or slightly elevated temperatures, removed residual moisture that did not crystallize during freezing.

Through this process, the lyophilized honey achieved stability, enabling long-term storage and preservation. After removing moisture, the honey was ground to eliminate lumps, creating a uniform, fine-grained powder that also served as a filler for capsules.

The final formulation consisted of 500 mg HPMC capsules containing 50 mg of *T. serpyllum* essential oil and 450 mg of lyophilized acacia honey as the carrier. Patients were instructed to take one to two capsules daily according to the treatment protocol.

The chemical composition of the essential oil was determined by gas chromatography–mass spectrometry (GC–MS) and ATR-FTIR analysis. GC-MS analysis was performed using an Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA) equipped with 5975C MSD and FID detectors. For chromatographic separation, a weakly polar HP-5MS (5% diphenyl- and 95% dimethyl-polysiloxane, 30 m × 0.25 mm, 0.25 µm film thickness) capillary column was used (Agilent Technologies, Santa Clara, CA, USA). EO samples were dissolved in dichloromethane at a concentration of 10 µL/mL and were injected in 1:10 split mode. Helium was used as the carrier gas at a constant pressure condition (16.255 psi). The oven temperature was programmed to increase from 60 °C to

300 °C at a rate of 3 °C per minute, followed by a final hold for 10 min. Mass spectra were obtained using the electron ionization (EI) mode across a scan range of 40–600 m/z, with the ion source maintained at 230 °C, the quadrupole set to 150 °C, and a solvent delay of 3 min.

Data processing was carried out using MSD ChemStation (Agilent Technologies, Santa Clara, CA, USA). Compound identification was performed by matching the experimental mass spectra with commercial libraries (NIST 17, Wiley 7, and Adams 4) using AMDIS 32 software (v2.73) along with the NIST search program (v2.3). Retention indices (RIs) were calculated using a homologous series of n-alkanes (C8–C32) and compared with the literature values. Relative abundances of the detected compounds were determined from GC-FID chromatograms.

ATR-FTIR analysis of *T. serpyllum* L. EO samples was recorded using an IRAffinity-1 FTIR spectrometer (Schimadzu, Kyoto, Japan). The spectra were recorded in the spectral range from 400 to 4000 cm−1 with a resolution of 4 cm−1 (256 scans). Afterwards, preprocessing including baseline correction, normalization, and smoothing was performed using the Spectragryph v1.2.15 software (Menges, 2018).

GC–MS analysis identified thymol (50.47%), p-cymene (23.56%), linalool (5.84%), 1,8-cineole (4.98%), carvacrol (3.18%), and caryophyllene oxide (2.28%) as the principal constituents of the essential oil. The complete chemical composition of the investigated *T. serpyllum* essential oil is presented in Supplementary Table S1.

Beat-to-beat heart rate and blood pressure variability were measured using the Task Force® Monitor system, which performs spectral analysis of heart rate and blood pressure via an autoregressive methodology, providing parameters such as power spectral density (PSD), frequency bands (VLF, LF, HF), baroreceptor reflex sensitivity (BRS), and baroreceptor effectiveness index (BEI) (16,17).

A 12-lead electrocardiograph was used for 24-hour Holter ECG monitoring, and recordings were reviewed by trained analysts. After manual artifact removal and correction of beat classifications, data were analyzed to determine mean heart rate and RR intervals. Heart rate variability (HRV) in the time domain was assessed using three standard parameters: SDNN, RMSSD, and PNN50.

Ambulatory blood pressure was recorded over 24 hours using an oscillometric device, with measurements obtained every 15 minutes during daytime hours. Analyses included systolic and diastolic blood pressure values for the entire 24-hour period and separately for daytime and nighttime intervals.

Statistical Analysis

All results are reported as counts and percentages, means with standard deviations, or medians with interquartile ranges (25th-75th percentiles), depending on the data type. The distribution of variables was assessed using the Shapiro-Wilk test and Q-Q plots. Paired measurements were compared using the paired-samples t-test for normally distributed data or the Wilcoxon signed-rank test for non-normally distributed data. Statistical analyses were performed using SPSS software (version 26.0), with a significance threshold set at $p < 0.05$.

RESULTS

Demographic data and comorbidities of the study population are presented in Table 1. The majority of patients were female and over 40 years of age. In addition to syncope, most patients also exhibited orthostatic hypotension. Hypertension, diabetes mellitus, and other comorbidities were present in fewer than 20% of patients.

The results of the beat-to-beat analysis before and three months after treatment are presented in Table 2.

LFnu was significantly higher following treatment (Figure 1), whereas HF expressed in ms^2 decreased. The LF/HF ratio (normalized units) was also significantly higher after treatment (Figure 2). Although baroreceptor parameters were lower after three months, the differences were not statistically significant.

Table 1. *Demographic data and comorbidities of the study population*

	N = 15
Demographic data	
Age (mean ± SD)	43.8 ± 17.80
Female (n,%)	13 (72.2%)
Comorbidities	
Syncope (n,%)	15 (100%)
OH (n,%)	13 (83.3%)
HTA (n,%)	3 (16.7%)
DM (n,%)	2 (11.1%)
Hashimoto thyroiditis (n,%)	1 (5.6%)
IBS (n,%)	0
Asthma (n,%)	0
COPD (n,%)	0
Hyperlipoproteinemia (n,%)	0

SD – Standard deviation; OH – Orthostatic hypotension; SOH – Syncope and OH; HTA – Hypertension; DM – Diabetes Mellitus;; IBS – Irritable Bowel Syndrome; COPD – Chronic Obstructive Pulmonary Disease

Table 2. *Beat-to-beat blood pressure and heart rate parameters of study population, before and 3 months after therapy*

	Before therapy N = 15 (n,%)	After 3 months N = 15	p value
Beat to beat Heart rate and Blood pressure (5 minutes recording)			
HR (bpm) (mean ± SD)	72.4 ± 10.9	73.6 ± 11.9	0.391[a]
SBP (mmHg) (mean ± SD)	106.7 ± 9.3	103.9 ± 8.3	0.348[a]
DBP (mmHg) (mean ± SD)	72.7 ± 6.4	71.6 ± 8.7	0.615[a]
MAP (mmHg) (mean ± SD)	82.7 ± 7	82.2 ± 8.1	0.813[a]
Short term HRV parameters			
LFnu (%) (mean ± SD)	60.8 ± 17.6	70.3 ± 11.4	0.019[a]
PSD (ms2) (Mdn (IQR))	685 (301 – 1211)	524 (335 – 1288)	0.9290[b]
VLF (ms2) (Mdn (IQR))	168 (49 – 361.5)	120 (44 – 362)	0.9170[b]
LF (ms2) (Mdn (IQR))	206 (146 – 426)	274 (139.5 – 355.5)	0.8070[b]
HF (ms2) (Mdn (IQR))	126.5 (61 – 462.5)	94 (68 – 229.3)	0.0500[b]
LF/HF (nu) (Mdn (IQR))	1.9 (.7 – 3.6)	2.3 (1.7 – 5)	0.0130[b]
LF/HF (ms2) (Mdn (IQR))	2 (.7 – 3.8)	2.2 (1.5 - 4)	0.1580[b]
Baroreceptors parameters			
BRS (ms/mmHg) (Mdn (IQR))	14 (7.5 – 20)	10 (6 – 15.8)	0.6740[b]
BEI (%)(Mdn (IQR))	113 (34.6 – 173.5)	61.3 (30.5 – 133)	0.2850[b]

HR – heart rate; bpm – beat per minute; SBP – systolic blood pressure; mmHg – millimeters of mercury; DBP – diastolic blood pressure; MAP – mean arterial pressure; LFnu – normalized low frequency of HRV ; PSD – power spectral density of HRV; VLF – VLF – very low frequency component of HRV; LF - low frequency component of HRV ; HF – high frequency component of HRV; LFnu/HFnu – normalized low frequency/ normalized high frequence ratio of HRV; LF/HF – low frequency/high frequency ratio of HRV; BRS – Baroreflex sensitivity; ms/mmHg – millisecond per millimeters of mercury; BEI - baroreflex sensitivity index; [a] – Paired Samples T test; [b] – Wilcoxon signed rank test

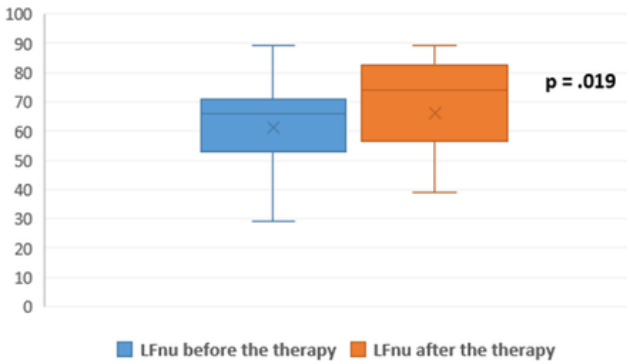

Figure 1. *Changes of LFnu after three months of therapy with Thymus serpyllum essential oil.*
For comparison, Paired samples T test was used.

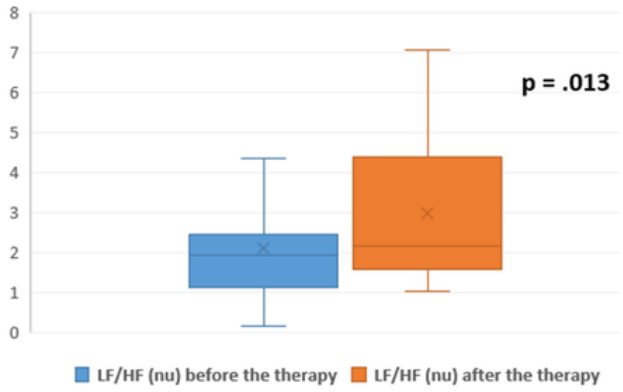

Figure 2. *Changes of LF/HF ratio expressed in normalized units after three months of therapy with Thymus serpyllum essential oil.*
For comparison, the Wilcoxon signed-rank test was used.

The 24-hour Holter ECG and ambulatory blood pressure parameters before and three months after treatment are presented in Table 3. Although time-domain HRV parameters and blood pressure values (systolic and diastolic) were higher at the end of treatment, the differences were not statistically significant.

The biochemical parameters before and three months after therapy are presented in Table 4. Levels of AST and C-reactive protein were significantly lower after the three-month treatment period (Figure 3 and Figure 4). No other statistically significant differences were observed.

Table 3. *24H Holter ECG and Ambulatory blood pressure parameters of the study population, before and 3 months after the therapy*

	Before therapy N = 15 (n,%)	After 3 months N = 15	p value
24h Holter ECG monitoring			
RRI (ms) (mean ± SD)	809.5 ± 125.4	838.2 ± 109	0.135[a]
HR (bpm) (mean ± SD)	79 ± 12.3	75.4 ± 7.5	0.348[a]
SDNN (ms) (mean ± SD)	135.8 ± 28.6	145.6 ± 31.8	0.170[a]
RMSSD (ms) (mean ± SD)	31.8 ± 14.3	33.4 ± 11.9	0.636[a]
PNN50 (%) (mean ± SD)	10.6 ± 9.9	12.8 ± 9.8	0.418[a]
AMBP (24h)			
SBP (mmHg) (mean ± SD)	111.4 ± 8.8	116.9 ± 8.7	0.091[a]
SBP day (mmHg) (mean ± SD)	114.4 ± 10.8	121.7 ± 8.9	0.128[a]
SBP night (mmHg) (mean ± SD)	102.6 ± 9.6	106.9 ± 8.4	0.244[a]
DBP (mmHg) (mean ± SD)	68.4 ± 8.3	71.7 ± 5.4	0.135[a]
DBP day (mmHg) (mean ± SD)	70.5 ± 9.5	74.6 ± 5.9	0.153[a]
DBP night (mmHg) (mean ± SD)	62 ± 7.8	63.2 ± 6.3	0.609[a]

RRI – RR interval; HR – Heart rate; ms – milliseconds; bpm – beats per minute; SDNN – Standard deviation of normal intervals; RMSSD –root mean square of successive differences; PNN50 – percentage of pairs of successive normal intervals that differ by more than 50 ms; SBP – Systolic blood pressure; DBP – Diastolic blood pressure; SD – standard deviation; [a] – paired samples t test

Table 4. *Biochemical Parameters of the study population, before and 3 months after the therapy*

	Before therapy N = 15 (n,%)	After 3 months N = 15	p value
Urea (mmol/L) (mean ± SD)	4.4 ± 1.1	4.5 ± 0.9	0.692[a]
Creatinine (µmol/L) (Mdn(IQR))	77.5 (70.5 – 84)	74 (72 – 85.5)	0.4800[b]
Glucose (mmol/L) (mean ± SD)	4.9 ± 0.5	4.9 ± 0.8	0.937[a]
AST (U/L) (Mdn(IQR))	25 (20 – 31)	22 (20 - 24)	0.0400[b]
ALT (U/L) (Mdn(IQR))	18 (16.5 – 39.5)	19 (14.5 – 26)	0.1720[b]
GGT (U/L) (Mdn(IQR))	13.5 (12 – 21.8)	14.5 (10.3 – 22.3)	0.4650[b]
Uric acid (µmol/L) (mean ± SD)	269.7 ± 74.7	253.9 ± 69.2	0.1430[b]
CRP (mg/L) (Mdn(IQR))	1.6 (0.8 – 3.4)	1 (0.8 – 1.4)	0.0080[b]
Triglycerides (mmol/L) (mean ± SD)	1.5 ± 0.6	1.3 ± 0.4	0.199[a]
Cholesterol (mmol/L) (mean ± SD)	5.9 ± 1	5.9 ± .9	0.952[a]
HDL (mmol/L) (mean ± SD)	1.4 ± 0.4	1.5 ± 0.4	0.392[a]
LDL (mmol/L) (mean ± SD)	3.9 ± 0.8	3.8 ± 0.9	0.522[a]

AST - Aspartate Aminotransferase; ALT - Alanine Aminotransferase; GGT - Gamma-Glutamyl Transferase; CRP – C reactive protein; HDL – High-Density Lipoprotein; LDL – Low-Density Lipoprotein; mmol/L - millimoles per liter; µmol/L - micromoles per liter; U/L - Units per Liter; mg/L – milligrams per liter; SD – Standard deviation; Mdn – Median; IQR – Interquartile range; [a] – Paired Samples T test; [b] – Wilcoxon signed rank test

DISCUSSION

The findings of this study indicate an enhancement of sympathetic tone with concurrent parasympathetic withdrawal, as recorded by beat-to-beat monitoring, without significant changes in blood pressure during either the 5-minute recordings or ambulatory blood pressure monitoring. In addition, decreased levels of AST and C-reactive protein were observed in patients with syncope treated with *T. serpyllum* essential oil.

As presented in Table 1, the majority of the participants in our study were female. This aligns with research, as it is, in fact, well established that the incidence of syncope is higher in women, who tend to experience syncopal episodes over longer periods of their lives and have more frequent episodes as compared to male patients (18). It is also notable that this study population was relatively healthy, with few significant comorbidities (Table 1).

One of the key observations, as shown in Table 2, is the increase in LFnu values and the LF/HF ratio of HRV, accompanied by a reduction in HF. Both LFnu and LF (ms^2), although the latter did not reach statistical significance, are

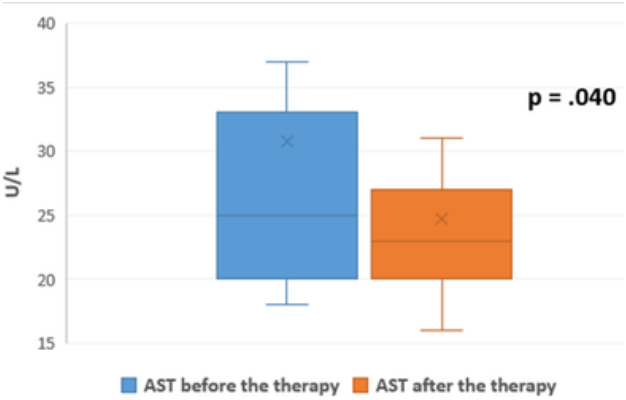

Figure 3. *Changes in AST (U/L) after three months of therapy with Thymus serpyllum essential oil.*
For comparison, the Wilcoxon signed-rank test was used.

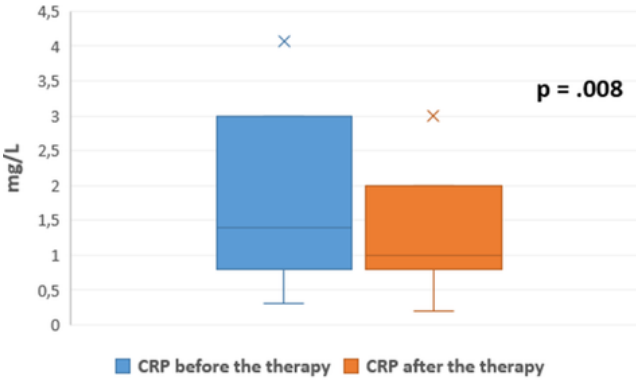

Figure 4. *Changes in CRP after three months of therapy with Thymus serpyllum essential oil.*
For comparison, the Wilcoxon signed-rank test was used.

considered markers of sympathetic activity (19,20). Furthermore, LFnu represents the relative contribution of LF activity, providing insight into autonomic balance with reduced sensitivity to total power variations; however, normalized units should be interpreted alongside absolute values (ms^2) to fully reflect the spectral distribution (21,22). Additionally, the LF/HF ratio, which showed a statistically significant increase after therapy in this study (Table 2), is commonly used to describe sympathovagal balance, with values above 2 typically indicating sympathetic predominance (19,20). HFnu (not shown here, as it is redundant—being derivable directly from 100% minus LFnu) and HF (ms^2) were both reduced following three months of therapy. HF, often referred to as the "respiratory band," is a key HRV marker of vagal (parasympathetic) activity (19) and is primarily modulated by respiration through respiratory sinus arrhythmia (19). It is also important to note that in short-term HRV recordings, especially during resting and supine conditions, the parasympathetic nervous system is the predominant contributor to HRV (19). Interestingly, these shifts in autonomic balance were not accompanied by significant changes in heart rate or blood pressure during the 5-minute recording period. Therefore, it may be inferred that, after three months of therapy, there was a shift towards sympathetic predominance with a concomitant reduction in parasympathetic tone in these patients. It is important to emphasize, as shown in Table 1, that nearly 85% of the study population had orthostatic hypotension as a key marker of sympathetic dysfunction prior to initiating therapy. Considering that these patients, who exhibited increased sympathetic tone after three months of treatment, are often managed conservatively (e.g., by avoiding provoking factors and using compression stockings) and, when necessary, with sympathomimetics such as midodrine, it would be reasonable to consider the potential use of *T. serpyllum* as an additional therapeutic option. However, further studies are required to clarify its role and confirm its efficacy in this specific patient population.

Regarding blood pressure, although values were higher after three months, no statistically significant changes were detected over the 24-hour period (Table 3). Animal studies have shown significant reductions in blood pressure and peripheral vascular resistance through a nitric oxide–independent mechanism, with rosmarinic acid identified as the main active compound using extracts of *T. serpyllum*. (14,15). Similar cardiovascular effects have also been reported in other species of the genus *Thymus*. In an experimental study, the essential oil of *Thymus* serrulatus produced dose-dependent reductions in arterial blood pressure, exhibiting vasodilatory and cardiac-depressant properties. These effects were attributed to α1-adrenergic antagonism and calcium channel blockade (23). These findings further support the potential cardiovascular activity of *Thymus* essential oils and suggest that members of this genus may influence vascular regulation through multiple mechanisms (23).

As shown in Table 4, notable changes in biochemical parameters were also observed after three months of therapy. While decreases were observed in the mean values of creatinine, uric acid, AST, and C-reactive protein, only the changes in the latter two reached statistical significance. Previous studies have demonstrated the potential hepatoprotective effects of *T. serpyllum* extracts in animal models, particularly in cases of alcohol-induced liver injury (24). The same study confirmed increased antioxidant enzyme activity and reduced oxidative stress (24).

Additionally, Jalil et al. highlighted the anti-inflammatory and antimicrobial properties of *T. serpyllum* extracts (9). Similar findings have been reported for carvacrol, one of the principal constituents of *Thymus* essential oils. A recent review summarized multiple experimental studies demonstrating that carvacrol consistently reduces oxidative stress, lipid peroxidation, and pro-inflammatory cytokine production while enhancing endogenous antioxidant defense systems, including superoxide dismutase, catalase, and glutathione-related pathways, thereby providing antioxidant and anti-inflammatory protection in various disease models (25). Furthermore, thymol, one of the key components of *Thymus* essential oils, has demonstrated cardioprotective effects in experimental models of adrenaline-induced myocardial injury (26). These effects were accompanied by reductions in AST, creatine kinase, and lactate dehydrogenase levels, restoration of antioxidant defenses through increased glutathione levels and reduced lipid peroxidation, as well as suppression of inflammatory mediators including NF-κB and IL-1β (26). In addition, thymol attenuated myocardial apoptosis through decreased caspase-3 expression and increased Bcl-2 expression, further supporting its antioxidant and anti-inflammatory properties (27).

In diabetic rabbits, although not observed in our study, a single high dose of 500 mg/kg of the aqueous extract of *T. serpyllum* reduced serum cholesterol, triglycerides, and LDL levels without affecting HDL levels (27). The absence of such pronounced effects in our study population may be attributed to (1) the fact that the study participants were patients with syncope who did not have significant liver disease or hyperlipoproteinemia, and only two participants had diabetes mellitus; (2) differences in dosage and mode of administration; and (3) potential interspecies metabolic differences between animal models and humans.

Study Limitations

This study has several significant limitations that should be acknowledged. First, the relatively small sample size restricts the generalizability of the findings. To draw more robust and reliable conclusions, future research should include a larger number of participants and incorporate a placebo-controlled arm to better delineate the specific effects of the investigated intervention.

Second, the lack of inclusion of patients with relevant comorbidities or specific diseases of interest may have limited the scope of biomarker evaluation. Although this study focused exclusively on patients with syncope, enrolling individuals with additional comorbid conditions would allow for a more comprehensive assessment of the potential effects of *T. serpyllum* on broader physiological and biochemical parameters.

Third, the follow-up duration was limited to three months. While this time frame was sufficient to detect short-term changes in autonomic parameters and selected biochemical markers, it may not have captured the sustainability of these effects or any potential delayed adverse outcomes. Longer-term studies are warranted to clarify the durability and safety of *T. serpyllum* supplementation.

Finally, the single-center design may limit the external validity of the findings, as the results obtained in a specialized neurocardiology laboratory may not be fully generalizable to other clinical settings or patient populations.

In conclusion, dysautonomia, reflecting dysfunction of the autonomic nervous system, is a key feature in patients with syncope, particularly those with orthostatic hypotension. This study demonstrated that a three-month course of *Thymus serpyllum* L. essential oil in patients with syncope was associated with increased sympathetic tone and parasympathetic withdrawal, as evidenced by beat-to-beat analysis, without significant changes in 24-hour blood pressure. Additionally, reductions in AST and C-reactive protein levels suggest potential hepatoprotective and anti-inflammatory effects of *T. serpyllum* in this patient population.

Based on these findings, *T. serpyllum* essential oil may be considered a potential adjunctive therapeutic option for patients with syncope, particularly those with orthostatic hypotension. Nevertheless, further well-designed studies with larger sample sizes and longer follow-up periods are warranted to confirm its efficacy and safety in this clinical context.

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Author Contributions

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Statement of Ethics

This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Institute for Cardiovascular Diseases "Dedinje" (protocol code 6472, approval date: 11 December 2024).

Statement of Competing Interest

The authors declare no relevant conflicts of interest.

Statement of Data Availability

Available upon reasonable request from the authors.

Statement of Generative AI Use

No generative AI was used.

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