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The impact of *Zataria multiflora* on oxidative stress biomarkers and ulcerative colitis symptoms: A multicenter, triple-blind, placebo-controlled clinical trial

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ABSTRACT

Background: Oxidative stress plays a crucial role in the pathogenesis of ulcerative colitis (UC), contributing to mucosal damage and inflammation. *Zataria multiflora* possesses antioxidant properties, yet clinical evidence regarding its effects in UC remains limited. This study aimed to evaluate the impact of *Z. multiflora* extract on oxidative stress markers and disease severity in UC patients.

Methods: In this triple-blind, randomized, placebo-controlled trial, 92 patients with mild-to-moderate UC were randomly assigned to receive either *Z. multiflora* extract (6 mg/kg/day) or a placebo for two months. Oxidative stress markers, including malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), and thiol groups (SH), were measured before and after treatment. Disease severity was assessed using the Partial Simple Clinical Colitis Activity Index (P-SCCAI).

Results: Z. multiflora supplementation significantly increased TAC ($p = 0.01$), SOD ($p = 0.02$), and SH ($p = 0.01$) levels, indicating enhanced antioxidant defenses. However, MDA levels did not significantly decrease ($p = 0.06$). Clinically, the *Z. multiflora* group exhibited significant improvements in bowel frequency ($p < 0.001$), urgency of defecation ($p < 0.001$), general well-being ($p < 0.001$), and final P-SCCAI scores ($p < 0.001$) compared to the placebo group.

Conclusion: Z. multiflora supplementation improved antioxidant markers and alleviated UC symptoms, though MDA levels remained unchanged. These findings suggest its potential as a complementary therapy for UC. Further large-scale, long-term studies are warranted to confirm its efficacy and optimize dosing.

Introduction

Zataria multiflora (*Z. multiflora*), commonly known as Shirazi thyme, is a medicinal plant from the Lamiaceae family native to Iran, Afghanistan, and Pakistan. Traditionally, it has been used to treat various ailments.[1,2] The therapeutic potential of *Z. multiflora* is attributed to its rich phytochemical composition, including thymol, carvacrol, linalool, and p-cymene, which exhibit strong antioxidant and anti-inflammatory properties. Given its widespread use in traditional medicine, *Z. multiflora* has gained increasing attention as a complementary approach for managing oxidative stress-related conditions, such as ulcerative colitis (UC).[3-5]

UC is a chronic inflammatory disorder of the colon, characterized by recurrent episodes of mucosal inflammation, leading to symptoms such as persistent diarrhea, abdominal cramping, rectal bleeding, and urgency to defecate.[6] The disease significantly impacts quality of life and has been rising in prevalence worldwide, including in Asian countries and Iran. By 2035, UC cases in Iran are projected to increase substantially, highlighting the need for effective and well-tolerated treatment options.[7]

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Although the exact etiology of UC remains unclear, oxidative stress plays a crucial role in its pathogenesis. Increased production of reactive oxygen species (ROS) and impaired antioxidant defense mechanisms contribute to mucosal damage, intestinal barrier dysfunction, and disease progression.[8,9] Elevated levels of oxidative stress biomarkers, such as malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD), thiol, and nitrite, have been observed in UC patients, indicating an imbalance between oxidative damage and antioxidant protection. Targeting oxidative stress may therefore present a promising therapeutic strategy for UC management.[8,10]

Several studies have demonstrated that bioactive compounds in *Z. multiflora*, particularly thymol and carvacrol, exhibit potent antioxidant effects by neutralizing ROS, reducing lipid peroxidation, and enhancing both enzymatic and non-enzymatic antioxidant defense systems.[1,11,12] Preclinical investigations have shown that *Z. multiflora* administration in experimental UC models reduces oxidative stress markers, improves mucosal integrity, and alleviates colonic damage. However, despite strong laboratory evidence, clinical studies evaluating the effects of *Z. multiflora* on oxidative stress in UC patients remain limited.[4,13]

Given its antioxidant potential and traditional use for gastrointestinal health, further clinical research is needed to clarify the role of *Z. multiflora* in UC treatment. This study aims to assess the effects of *Z. multiflora* hydroalcoholic extract on oxidative stress biomarkers and UC symptoms, providing insights into its potential as a complementary therapeutic option.

Materials and methods

Study design and participants

This multicenter, triple-blind, randomized, placebo-controlled clinical trial was conducted between October 2023 and May 2024 at the Alimentary Tract Research Center and two gastroenterology clinics affiliated with Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The study followed a parallel-group design with a 1:1 allocation ratio. Participants were diagnosed with ulcerative colitis (UC) based on medical history, physical examination, laboratory tests (blood and stool analysis), and endoscopic findings, following the Mayo grading scale and Rome III diagnostic criteria for functional gastrointestinal disorders.

Eligible participants were 18 to 65 years old, had a body mass index (BMI) between 18.5 and 30 kg/m^2, and had a confirmed UC diagnosis for six months to five years. The BMI range was chosen to exclude underweight individuals, who may experience acute inflammation due to malnutrition, and obese patients, as excessive adipose tissue may contribute to chronic low-grade inflammation, potentially confounding treatment effects.

Exclusion criteria included individuals in the acute phase of UC, those with autoimmune diseases, inflammatory conditions (renal, hepatic, cardiovascular diseases, cancer, and HIV), thyroid disorders, diabetes, or a history of gastrointestinal surgery. Pregnant or lactating women and individuals with a known allergy to *Z. multiflora* were also excluded. Participants were removed from the study if they developed an acute disease episode, changed medications, started taking anti-inflammatory or antioxidant supplements, or demonstrated poor compliance (<80 %) with the intervention.

Preparation of Z. multiflora Elixir and Placebo

The hydroalcoholic extract of *Z. multiflora* was supplied by Giah Essence Phytopharmaceutical Company (Gorgan, Iran) and approved by the Food and Drug Organization of Iran (FDO). The extract, containing 20 % alcohol, was analyzed at the Central Laboratory of Shahid Chamran University of Ahvaz for its composition. Carvacrol content was determined using gas chromatography (GC) with an Agilent 7890B-597A GC-MS system, following a temperature gradient protocol (67°C to 270°C). The chromatographic profile confirmed a distinct carvacrol peak at 21.93 minutes.

The *Z. multiflora* elixir was prepared at the Center for Pharmaceutical Technology Innovation, Ahvaz Jundishapur University of Medical Sciences, by dissolving the extract in a simple syrup of sucrose and purified water (28 mg/mL). The alcohol content was reduced to less than 5 % in the final preparation. The placebo solution contained 5 % alcohol in simple syrup (80 % w/v sucrose), with FD&C Yellow #6 added to match the color of the *Z. multiflora* elixir. Additionally, a trace amount of thymol was included in the placebo to mask the distinct herbal scent of thymol and carvacrol. The formulation was based on previous research.[14]

Randomization and blinding

Participants who met the eligibility criteria were randomly assigned to either the *Z. multiflora* or placebo group using block randomization (blocks of 4) via www.sealedenvelope.com. The allocation sequence (e.g., BABA, BBAA) was generated by an independent statistician. Opaque, sequentially numbered, sealed envelopes ensured allocation concealment. To balance disease severity across groups, stratified randomization was performed using the Partial Mayo Score (pMayo Score).

This study was triple-blind, meaning that participants, investigators, and data analysts were unaware of treatment assignments. To maintain blinding, a pharmacology consultant coded the elixirs and placebos, and the researcher administering the intervention followed the predetermined patient enrollment order without knowledge of group assignments.

Intervention protocol

Participants received *Z. multiflora* extract at 6 mg/kg/day, divided into three daily doses for two months. A dropper was provided for precise dosing. This dosage was based on previous trials in respiratory diseases, where 3–10 mg/kg/day significantly reduced inflammatory markers while maintaining safety.[14,15] The two-month duration was chosen to allow the extract's antioxidant effects to manifest, aligning with chronic disease evaluation guidelines.[14,15] Both *Z. multiflora* extract and placebo were packaged in dark-colored bottles to maintain blinding. Follow-up was conducted every 10 days via phone calls and interviews. Patients with <80 % adherence were excluded.

Adverse events

One patient in the *Z. multiflora* group withdrew due to worsening constipation after two weeks of intervention. No adverse effects were reported in the placebo group.

Demographics, diet, and physical activity data

At baseline, all participants completed a demographic and medical history questionnaire, which included age, gender, marital status, education, employment, smoking habits, family history of UC, and medication use. Dietary intake was assessed using a 24-hour dietary recall (one weekday and one weekend day) and analyzed using Nutritionist IV software. Physical activity was evaluated using the International Physical Activity Questionnaire (IPAQ) short form to account for variations between groups. Participants were instructed to maintain their usual diet, physical activity, and lifestyle habits throughout the study to minimize confounding effects.

Anthropometric measurements

Anthropometric measurements followed standardized protocols. Height was recorded using a standalone stadiometer (Seca, Hamburg,

Germany) with participants standing upright, barefoot, and aligned with the Frankfurt horizontal plane. Weight was measured using a calibrated digital scale (Seca, Hamburg, Germany), with participants wearing minimal clothing and no shoes. BMI was calculated as: Weight (kg)/ Height (m)[2]

Assessment of oxidative stress markers

Participants fasted overnight before blood sample collection. A total of 15 mL of venous blood was drawn from each patient both before and after the intervention. Samples were immediately centrifuged at 3,000 rpm for 10 minutes to separate plasma, which was stored at -80°C until analysis. Oxidative stress biomarkers, including malondialdehyde (MDA), total antioxidant capacity (TAC), superoxide dismutase (SOD), and total thiol groups, were assessed using commercially available assay kits manufactured by KiaZist.

MDA, a key marker of lipid peroxidation, was quantified using the thiobarbituric acid reactive substances (TBARS) assay. This method relies on the reaction of MDA with thiobarbituric acid (TBA) under acidic and high-temperature conditions, resulting in the formation of a pink-colored complex. The absorbance of this complex was measured at 532 nm using a spectrophotometer. The MDA concentration was expressed in μmol/L. All measurements were performed using the KiaZist MDA assay kit (Kiazist, Iran).

TAC was determined using the ferric reducing antioxidant power (FRAP) assay, which evaluates the ability of plasma antioxidants to reduce ferric (Fe^{3+}) to ferrous (Fe^{2+}) ions. In this assay, the reduction of Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) generates a blue-colored ferrous-TPTZ complex, whose absorbance was recorded at 593 nm. The results were expressed as mmol Fe^{2+}/L plasma. The analysis was conducted using the KiaZist TAC assay kit (Kiazist, Iran).

SOD activity was evaluated based on its ability to inhibit the reaction between superoxide anions and a chromogenic substrate. Superoxide radicals, generated by the xanthine-xanthine oxidase system, interact with a tetrazolium salt, leading to the formation of a colored formazan dye, which is detected at 450 nm. The level of SOD activity was determined by measuring the degree of inhibition, and the results were expressed as U/mL plasma. The KiaZist SOD assay kit (Kiazist, Iran) was used for this analysis.

Total thiol groups, which serve as indicators of protein oxidation, were quantified using Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid), DTNB). In this method, thiol groups react with DTNB to produce a yellow-colored 2-nitro-5-thiobenzoate (TNB) anion, which absorbs at 412 nm. The thiol concentration was calculated using a standard curve and was expressed in μmol/L plasma. The analysis was performed using the KiaZist total thiol assay kit (Kiazist, Iran).

Clinical assessments

The severity of ulcerative colitis was evaluated using the Patient Simple Clinical Colitis Activity Index (P-SCCAI), a validated tool designed to assess disease activity based on key clinical symptoms. The index consists of six parameters: bowel frequency (day and night), urgency of defecation, rectal bleeding, general well-being, and extracolonic manifestations. Each parameter is assigned a score based on severity, with the total P-SCCAI score ranging from 0 to 20, where higher scores indicate greater disease activity.[16]

Sample size estimation and statistical analysis

Based on the findings of Ghorani et al. (2020), the sample size was estimated using MDA as the primary outcome variable.[14] The calculation was performed considering the MDA levels in participants receiving 6 mg/kg of *Z. multiflora* extract, treating pre- and post-intervention values as independent groups. The anticipated effect size (Cohen's d) was determined to be 0.89, based on the difference in means and standard deviations from the referenced study. To ensure a 95 % statistical power with a 0.05 significance level (α) under a two-tailed hypothesis test, the minimum required sample size was estimated to be 34 subjects per group. The G*Power (version 3.1) software was utilized for this calculation. To account for potential participant dropout, a maximum dropout rate of 20 % was considered. Given the initial requirement of 34 participants per group, an adjusted sample size was calculated to ensure statistical power was maintained. Applying this dropout rate, the final recommended sample size per group was 42 participants, leading to a total of 84 participants in the study. However, 92 participants were enrolled to maintain statistical power. During the study, 11 participants were lost to follow-up due to withdrawal, non-compliance, or adverse events. Despite these losses, all 92 participants were included in the intention-to-treat (ITT) analysis to ensure methodological rigor.

For data presentation, descriptive statistics and frequency distribution tables were utilized. Statistical analyses were conducted using Statistical Package for the Social Sciences (version 25.0, SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was applied to assess the normality of quantitative data, which were expressed as mean ± standard deviation (SD). Changes in continuous variables before and after the intervention were analyzed using the paired t-test, while non-parametric alternatives (Wilcoxon signed-rank test and Mann-Whitney U test) were used for variables violating normality assumptions or having small sample sizes. The choice of non-parametric methods ensured statistical validity without reliance on normal distribution assumptions. Qualitative data were analyzed using the Chi-square test.

To assess the intervention's effect within groups, the paired t-test or Wilcoxon signed-rank test was applied, while comparisons between groups were performed using the independent t-test or Mann-Whitney U test. A change analysis was conducted to evaluate the significance of intervention effects by comparing pre- and post-treatment values between groups using a t-test. An intention-to-treat (ITT) analysis was performed, maintaining participants in their original assigned groups regardless of completion status. To handle missing data, the last observation carried forward (LOCF) method was used to impute post-treatment values based on each participant's last available measurement. A sensitivity analysis was conducted to compare ITT results (using LOCF) with a complete-case analysis, excluding participants with missing data. The findings from both approaches were consistent, confirming the robustness of the results. A p-value < 0.05 was considered statistically significant.

Results

Fig. 1 presents the participant flowchart following the Consolidated Standards of Reporting Trials (CONSORT) guidelines. Initially, 547 individuals were screened for eligibility, with 455 being excluded. Consequently, 92 participants were randomly assigned to either the *Z. multiflora* intervention group (n=46) or the control group (n=46). In the *Z. multiflora* group, two participants were lost to follow-up due to unsuccessful contact, while three withdrew due to constipation, medication changes, or noncompliance. Similarly, in the control group, two participants were lost to follow-up due to contact failure, and four discontinued participation because of medication changes or noncompliance. Despite these losses, all 92 participants were included in the intention-to-treat (ITT) analysis.

Table 1 presents the baseline characteristics of participants in the *Z. multiflora* and control groups, demonstrating no significant differences between them. Both groups were comparable in age ($p = 0.52$), BMI ($p = 0.07$), disease duration ($p = 0.89$), and physical activity levels ($p = 0.45$). Additionally, no significant differences were observed in sex distribution ($p = 0.20$), colitis type ($p = 0.68$), or medication use, with aminosalicylates being the most commonly prescribed (~88 %). These findings confirm that the two groups were well-matched at baseline.

Table 2 presents oxidative stress markers before and after the

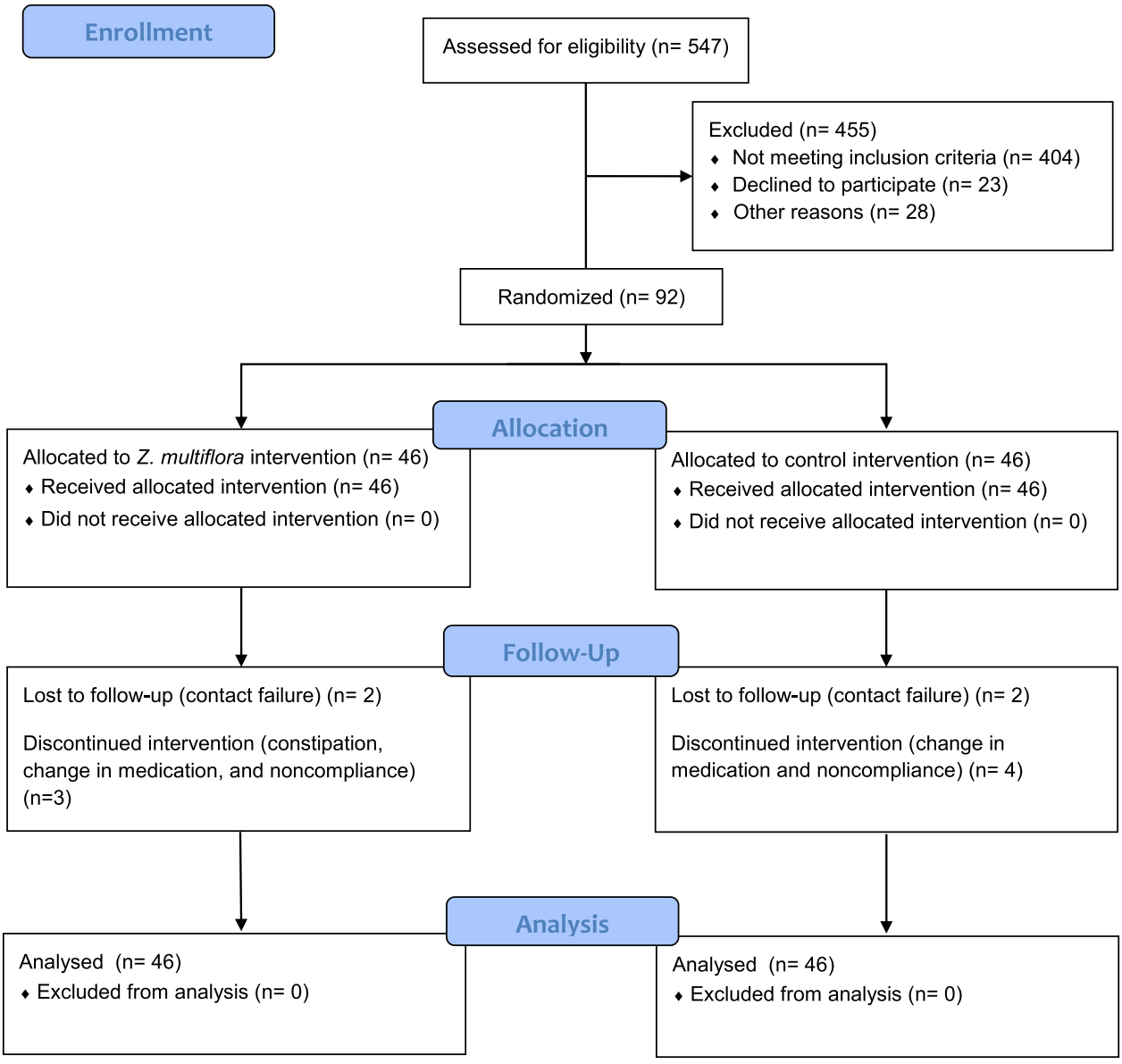

Fig. 1. CONSORT Flow Diagram.

intervention. The *Z. multiflora* group exhibited a slight but non-significant reduction in MDA levels ($p = 0.06$), while the control group showed a non-significant increase ($p = 0.23$). However, these changes were not statistically significant between groups.

Conversely, TAC levels significantly increased in the *Z. multiflora* group after two months ($p = 0.01$), whereas no notable change was observed in the control group ($p = 0.59$). Similarly, SOD activity significantly improved in the *Z. multiflora* group ($p = 0.02$), with no meaningful alteration in the control group ($p = 0.48$). Furthermore, thiol group (SH) concentrations significantly increased following *Z. multiflora* supplementation ($p = 0.01$), while no substantial change occurred in the control group ($p = 0.67$). These findings suggest that *Z. multiflora* supplementation enhances antioxidant defenses by increasing TAC, SOD, and SH levels.

Fig. 2 illustrates the mean differences in P-SCCAI index components following the intervention. In the *Z. multiflora* group, significant reductions were observed in daytime bowel frequency ($p < 0.001$), nighttime bowel frequency ($p = 0.014$), and urgency of defecation ($p < 0.001$), with negative mean differences indicating symptom improvement. Additionally, general well-being significantly improved ($p < 0.001$), showing a positive mean difference.

However, blood in stool ($p = 0.180$) and extracolonic features ($p = 0.317$) did not change significantly. The final P-SCCAI score showed a mean reduction of -2.6522 (SD: 1.88818, $p < 0.001$), reflecting a meaningful improvement in disease severity. In contrast, the placebo group did not show significant improvements in any of the assessed symptoms, with p-values ranging from 0.180 to 1.000. The final P-SCCAI score remained almost unchanged (mean difference: -0.0217, SD: 0.61424, $p = 0.808$).

A between-group comparison confirmed that the *Z. multiflora* group experienced significantly greater improvements in daytime bowel frequency ($p < 0.001$), nighttime bowel frequency ($p = 0.012$), urgency of defecation ($p < 0.001$), general well-being ($p < 0.001$), and the final P-SCCAI score ($p < 0.001$). However, differences in blood in stool ($p = 0.405$) and extracolonic features ($p = 0.986$) were not statistically significant between groups.

Discussion

The present study evaluated the effects of *Z. multiflora*

Table 1
Baseline characteristics of participants.

Variable			ZT (n=46)	Control (n=46)	p-value
Age, years			38.0 ± 8.6	36.6 ± 11.6	0.52*
BMI, kg/m²			27.3 ± 4.1	25.9 ± 3.3	0.07*
Disease duration, months			32.7 ±16.8	33.9 ± 17.0	0.89*
Physical activity, (MET-minute/week)			1126± 217	1317 ± 405	0.45*
Sex	**Male, n (%)**		18 (39.1)	24 (52.2)	0.20**
	Female, n (%)		28 (60.9)	22 (47.8)	
Medical center	**Alimentary tract research center, n (%)**		11 (23.9)	9 (19.6)	0.49**
	Gastroenterology clinic 1, n (%)		10 (21.7)	15 (32.6)	
	Gastroenterology clinic 2, n (%)		25 (54.3)	22 (47.8)	
Type of colitis	**Ulcerative proctitis, n (%)**		14 (30.4)	18 (39.1)	0.68**
	Left sided ulcerative colitis, n (%)		15 (32.6)	13 (28.3)	
	Extensive ulcerative colitis, n (%)		17 (37.0)	15 (32.6)	
Medications	**Aminosalicylates, n (%)**	**Yes, n (%)**	41 (89.1)	40 (87.0)	0.74**
		No, n (%)	5 (10.9)	6 (13.0)	
	Corticosteroids, n (%)	**Yes, n (%)**	13 (28.3)	8 (17.4)	0.21**
		No, n (%)	33 (71.7)	38 (82.6)	
	Immunomodulators, n (%)	**Yes, n (%)**	9 (19.6)	11 (23.9)	0.61**
		No, n (%)	37 (80.4)	35 (76.1)	

Data are presented as mean and standard deviation (SD) or median for quantitative variables, and as absolute (N) and relative frequencies (%) for qualitative variables.

* Independent sample t-test (parametric) or Mann-Whitney U test (nonparametric) was used for the comparison of quantitative variables between the two treatment groups.

** The chi-square test was used to compare qualitative variables between the two treatment groups.

Abbreviations: *Zataria multiflora* (ZT), Body mass index (BMI).

supplementation on oxidative stress markers and clinical symptoms in patients with ulcerative colitis (UC). Our findings demonstrate that *Z. multiflora* supplementation significantly improved total antioxidant capacity (TAC), superoxide dismutase (SOD), and sulfhydryl (SH) levels while reducing markers of disease activity, particularly bowel frequency, urgency of defecation, and overall disease severity, as reflected in the P-SCCAI score.

Effects on oxidative stress markers

Oxidative stress plays a critical role in the pathogenesis and progression of UC. Our results indicate that TAC, SOD, and SH levels significantly increased following two months of *Z. multiflora* supplementation, suggesting a potential antioxidative effect. Conversely, malondialdehyde (MDA), a marker of lipid peroxidation, showed a non-significant reduction in the *Z. multiflora* group. Due to the limited availability of clinical trials investigating the effects of *Z. multiflora* in

Table 2
Oxidative stress markers before and after the intervention.

Variable	Group	Baseline	After two months	p-value	Difference	p-value¥
MDA, µmol/L	**ZT**	3.62 ± 1.23	3.46 ± 1.17	0.06**	-0.16 ± 0.57	0.21
	Control	3.38 ± 1.08	3.49 ± 1.04	0.23**	0.11 ± 0.56	
TAC, mmol Fe^{2+}/L	**ZT**	0.55 ± 0.20	0.72 ± 0.28	**0.01****	0.17 ± 0.20	**0.01**
	Control	0.53 ± 0.22	0.54 ± 0.25	0.59**	0.01 ± 0.12	
SOD, U/mL	**ZT**	2.15 ± 0.85	2.89 ± 0.91	**0.02****	0.74 ± 0.65	**0.03**
	Control	2.09 ± 0.78	2.15 ± 0.81	0.48**	0.06 ± 0.30	
SH, µmol/L	**ZT**	380.6 ± 52.3	420.8 ± 60.2	**0.01***	40.2 ± 35.1	**0.01**
	Control	375.2 ± 49.8	377.5 ± 50.6	0.67*	2.3 ± 20.5	

Data are presented as mean and standard deviation (SD).

* The Wilcoxon signed-rank test was used to compare non-parametric variables before and after treatment.

** Paired sample t-test was used to compare parametric variables before and after treatment.

¥ Mann-Whitney U test was used to compare variables between two treatment groups.

Abbreviations: *Zataria multiflora* (ZT), Malondialdehyde (MDA), Total Antioxidant Capacity (TAC), Superoxide Dismutase (SOD), Thiol Groups (SH).

UC, we compared our findings with studies conducted on other conditions.

Ghanbari-Niaki et al. investigated the effects of *Z. multiflora* supplementation combined with resistance training in postmenopausal women and observed a significant increase in irisin levels following supplementation and exercise. While MDA levels were significantly reduced in the resistance training and combination groups, they remained unchanged in the *Z. multiflora*-only group. Additionally, glutathione (GSH) levels increased in both the supplementation and exercise groups, whereas TAC remained unchanged. Their findings suggest that *Z. multiflora* may enhance antioxidant responses when combined with physical activity, but its independent effects on oxidative stress markers such as MDA may be limited. This aligns with our results, where *Z. multiflora* did not significantly reduce MDA levels in UC patients. However, the increase in GSH levels in their study highlights the potential of *Z. multiflora* in modulating cellular antioxidant defenses, which may have contributed to the clinical benefits observed in our study.[17]

In a study on asthmatic patients, Alavinezhad et al. reported a significant decrease in serum MDA levels in the treatment groups. Serum nitrite concentrations also decreased significantly in patients receiving higher doses of *Z. multiflora*. Moreover, serum thiol concentrations increased following treatment, suggesting an improvement in the antioxidant defense system. While SOD and catalase (CAT) activities did not change significantly, the percent changes in these markers were higher in the treated groups compared to placebo. These results suggest that *Z. multiflora* may enhance antioxidant capacity in asthmatic patients. In contrast, our study did not observe a significant reduction in MDA levels, which may be attributed to differences in disease pathology, oxidative stress burden, and study population. However, the increase in thiol levels in both studies suggests a potential shared mechanism in improving redox balance.[18]

Ghorani et al. studied the effects of *Z. multiflora* in chronic obstructive pulmonary disease (COPD) patients and found significant reductions in MDA and nitrite levels after two months of treatment. Additionally, thiol content, as well as SOD and CAT activities, significantly increased in the treatment groups. These findings contrast with

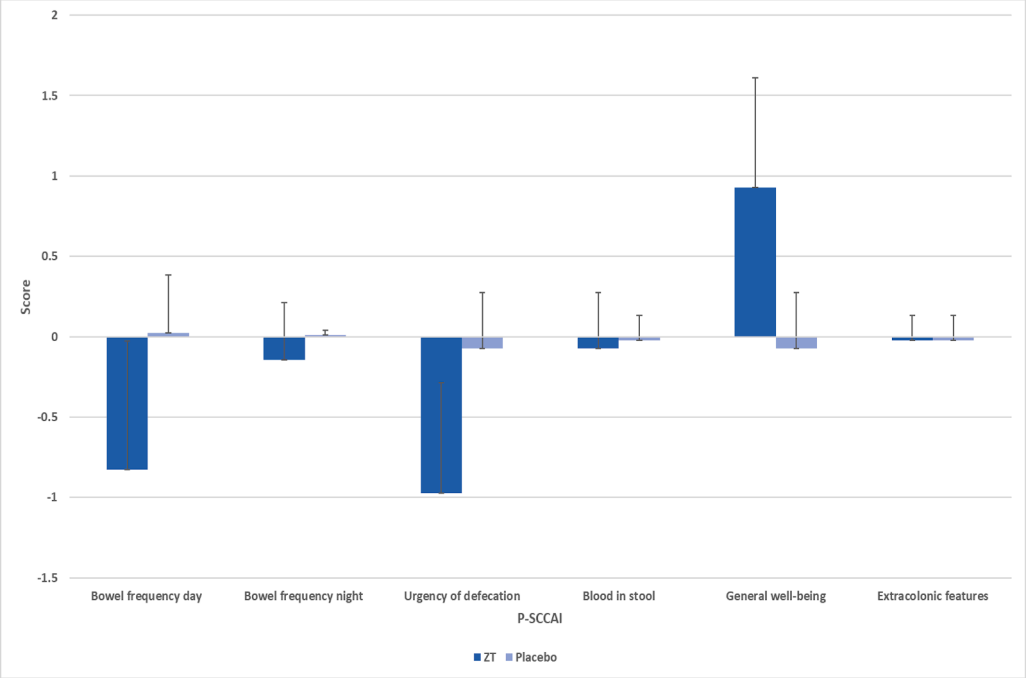

Fig. 2. Mean differences in Partial Simple Clinical Colitis Activity Index (P-SCCAI) components between the *Zataria multiflora* and placebo groups. Statistical comparisons were conducted using the Independent sample t-test and Mann-Whitney U test to evaluate variables between the two treatment groups.

our study, where MDA did not show a significant decrease, although improvements in thiol levels were consistent between both studies. The discrepancy in MDA response may be due to differences in oxidative stress dynamics in UC compared to COPD, where chronic inflammation and environmental stressors play a more prominent role.[14]

Khazdair et al. conducted a study on sulfur mustard-exposed veterans and reported a significant increase in thiol, SOD, and CAT levels in the *Z. multiflora*-treated groups, while MDA levels significantly decreased. This study further supports the antioxidant potential of *Z. multiflora*, particularly in conditions characterized by severe oxidative damage. Although our study did not find a significant reduction in MDA, the observed increase in thiol levels aligns with these findings, indicating a possible role of *Z. multiflora* in enhancing the antioxidant defense system. Differences in MDA response across studies could be related to variations in disease etiology, baseline oxidative stress levels, and treatment duration.[19]

Oxidative stress plays a central role in the pathogenesis of UC, where excessive production of reactive oxygen species (ROS) leads to lipid peroxidation, protein oxidation, and DNA damage.[20] The body's antioxidant defense system, consisting of enzymatic and non-enzymatic components, attempts to counteract these effects, but in UC, this balance is often disrupted.[21] *Z. multiflora* exerts its antioxidant effects through multiple mechanisms, contributing to the reduction of oxidative damage and the restoration of redox homeostasis.[4]

The polyphenolic compounds in *Z. multiflora*, such as flavonoids, carvacrol, and thymol, act as potent free radical scavengers. These compounds donate hydrogen atoms to unstable ROS, neutralizing them before they can cause cellular damage. Flavonoids, in particular, can directly scavenge hydroxyl radicals, superoxide anions, and peroxynitrite, reducing oxidative burden at the cellular level.[5,22] Additionally, the presence of phenolic compounds enhances the electron-donating capacity of *Z. multiflora*, further strengthening its antioxidant potential.[11]

Superoxide dismutase (SOD) and catalase (CAT) play essential roles in neutralizing ROS. SOD catalyzes the dismutation of superoxide anions into hydrogen peroxide, which is subsequently broken down by CAT into water and oxygen.[23,24] Studies have shown that *Z. multiflora* can upregulate the expression and activity of these enzymes, thereby enhancing the body's natural ability to detoxify ROS. By improving SOD and CAT activity, *Z. multiflora* helps mitigate oxidative stress, preventing further tissue damage in inflammatory conditions such as UC.[9,25]

Thiol-containing compounds, including glutathione (GSH) and protein-bound thiols, are crucial for maintaining cellular redox homeostasis. GSH serves as a major intracellular antioxidant, directly scavenging ROS and acting as a cofactor for glutathione peroxidase, which detoxifies lipid peroxides.[26] *Z. multiflora* has been shown to increase thiol group availability, thereby protecting cellular proteins from oxidative modification. This effect is particularly beneficial in UC, where oxidative stress leads to thiol oxidation and depletion of GSH reserves.[12]

Myeloperoxidase (MPO) is an enzyme released by activated neutrophils during inflammation and is a major contributor to oxidative damage in UC. MPO catalyzes the production of hypochlorous acid (HOCl), a highly reactive oxidant that exacerbates tissue injury.[27] *Z. multiflora* has been shown to inhibit MPO activity, reducing neutrophil-mediated oxidative stress and limiting damage to the colonic mucosa. By modulating MPO activity, *Z. multiflora* may help control inflammation-driven oxidative stress.[4]

Transition metals such as iron and copper play a critical role in ROS generation through the Fenton reaction, which produces highly reactive hydroxyl radicals.[28] Some polyphenols in *Z. multiflora* exhibit metal-chelating properties, reducing the availability of free iron and copper ions required for this reaction. By limiting Fenton chemistry, *Z. multiflora* may further decrease oxidative stress and lipid peroxidation in UC.[4,29]

Effects on clinical symptoms and disease activity

The clinical efficacy of *Z. multiflora* supplementation was evident in its ability to improve key PSCCAI index components. Patients receiving *Z. multiflora* experienced significant reductions in daytime bowel frequency, nighttime bowel frequency, and urgency of defecation, all of which are critical factors contributing to the disease burden. A combination of *Zataria multiflora* and *Trachyspermum copticum* has been shown to effectively reduce symptoms of irritable bowel syndrome, including

bloating, constipation, and abdominal pain.[30] Similarly, research on ICU nurses who received *Z. multiflora* for one month reported a decline in gastrointestinal discomfort; however, by the end of the trial, no significant difference was observed between those receiving the treatment and those in the placebo group.[31] Several experimental studies suggest that *Z. multiflora* and its major constituents, carvacrol and thymol, exert beneficial effects on ulcerative colitis through multiple mechanisms. These compounds contribute to reducing oxidative stress, downregulating inflammatory cytokines, and exhibiting antimicrobial properties against colitis-associated pathogens. Investigations in colitis animal models further demonstrate that carvacrol and thymol help preserve the intestinal mucosa, lower inflammatory markers such as myeloperoxidase (MPO) and cytokines, and alleviate visceral pain. Given these effects, *Z. multiflora* and its active components may serve as promising natural therapies for ulcerative colitis, potentially offering an alternative or complementary approach to conventional treatments.[4,13,32,33]

The increasing global interest in complementary and alternative medicine (CAM), particularly herbal therapies, reflects a shift toward integrative healthcare approaches aimed at improving overall health outcomes. Herbal remedies, including *Zataria multiflora*, have gained popularity due to their perceived safety, affordability, and potential therapeutic effects. Studies indicate that medicinal plants are widely used for managing chronic conditions such as dyslipidemia, noncommunicable diseases, and pregnancy-related complications, highlighting their integration into routine healthcare practices.[34-36] Given the burden of ulcerative colitis and the limitations of conventional treatments, herbal interventions like *Z. multiflora* offer a promising complementary strategy for symptom relief and oxidative stress modulation. However, further large-scale studies are needed to validate their long-term safety and efficacy in clinical settings.

Strengths and limitations of the study

One of the major strengths of this study is its triple-blind, randomized controlled design, which minimizes bias at multiple levels, ensuring more reliable and objective findings. The study also utilized validated clinical assessment tools, such as the Partial Simple Clinical Colitis Activity Index (P-SCCAI), to accurately evaluate symptom severity. Additionally, the comprehensive assessment of oxidative stress markers, including MDA, TAC, SOD, and thiol groups, provides valuable insights into the potential antioxidant effects of *Z. multiflora* in ulcerative colitis. The use of an intention-to-treat (ITT) analysis further strengthens the study by accounting for all participants, reducing the impact of dropouts on the final results.

However, this study has several limitations. The relatively small sample size may have limited the ability to detect significant changes in certain oxidative stress markers. The short duration of intervention also restricts conclusions about the long-term effects of *Z. multiflora* on disease progression. Additionally, inflammatory cytokines and gut microbiota composition were not assessed, which could have provided further mechanistic insights. Lastly, the lack of dose-response analysis makes it unclear whether different dosages would yield varying therapeutic effects.

Future directions

Future research should focus on conducting larger, multicenter randomized controlled trials with longer follow-up periods to evaluate the sustained effects of *Z. multiflora* on oxidative stress and disease progression. Investigating different dosages in a dose-response study could help determine the optimal therapeutic concentration. Additionally, examining inflammatory markers (e.g., TNF-α, IL-6, IL-10) and gut microbiota composition would provide a deeper understanding of its mechanisms of action. Comparative studies assessing *Z. multiflora* alongside conventional UC treatments could establish its potential as an adjunct or alternative therapy. Furthermore, in vitro and in vivo mechanistic studies could clarify its interaction with oxidative stress and inflammatory pathways at the molecular level. Addressing these areas would provide stronger evidence for the clinical application of *Z. multiflora* in ulcerative colitis management.

Conclusion

This study suggests that *Z. multiflora* supplementation enhances antioxidant defenses and improves clinical symptoms in ulcerative colitis. While MDA levels did not significantly decrease, increases in TAC, SOD activity, and thiol levels indicate its role in reducing oxidative stress. Symptom relief, including reduced bowel frequency and urgency, supports its potential as a complementary therapy. Further research is needed to confirm its long-term efficacy, determine optimal dosages, and explore its mechanisms of action in greater detail.

Ethics approval and consent to participate

The present study was approved by the Medical Ethics Committee of Iran University of Medical Sciences *(IR.IUMS.REC.1402.207)*, ensuring compliance with ethical standards set forth in the Declaration of Helsinki. Furthermore, the study was registered in the Iranian Registry of Clinical Trials *(IRCT20120415009472N27)*, a registry that promotes transparency and accountability in clinical research. This process involved explaining the purpose, procedures, potential risks, and benefits of the study to the participants, ensuring that they understood the information and voluntarily agreed to participate. All patients involved in this study signed and filled informed consent forms, ensuring their voluntary participation and understanding of the research process.

Consent for publication

Not applicable in the declarations section.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

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CRediT authorship contribution statement

Mehrnaz Morvaridi: Writing – review & editing, Writing – original draft, Visualization, Resources, Project administration, Methodology, Investigation, Data curation. **Naheed Aryaeian:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Pezhman Alavinejad:** Validation, Resources, Methodology, Conceptualization. **Seyed Saeed Seyedian:** Validation, Resources, Methodology, Conceptualization. **Mehri Ghafourian:** Validation, Resources, Methodology, Conceptualization. **Nima Bakhtiari:** Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Maryam Seyedtabib:** Validation, Software, Formal analysis.

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