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# Immunomodulatory Effects of Combined Hyssopus Officinalis and Teucrium Polium Extracts on Cytokine Production and Th17/Treg Balance in Human Peripheral Blood Mononuclear Cells

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**ABSTRACT**

**Purpose:** The balance between T regulatory (Treg) and T helper 17 (Th17) cells plays a major role in inflammatory and autoimmune diseases. Due to their immunomodulatory potential, medicinal plants are attractive therapeutic options. This study examined the effects of Hyssopus officinalis and Teucrium polium extracts, individually and in combination, on Treg/Th17 balance in activated human T lymphocytes in vitro.

**Methods:** eripheral blood mononuclear cells (PBMCs) were isolated from healthy donors and used exclusively for the isolation of untouched human Pan T cells by negative magnetic selection. Purified Pan T cells were activated and cultured under standard conditions. Experimental groups included untreated control, H. officinalis, T. polium, and combined extracts. Cell viability and non-toxic extract concentrations were assessed by MTT assay. Treg cells (CD4+CD25+CD127^low/-) and Th17 cells (CD4+IL-17A+) were analyzed by flow cytometry. IL-10, TGF-β, and IL-17A levels were measured by ELISA, and gene expression of FOXP3, RORγt, IL-10, TGF-β, and IL-17A was analyzed by quantitative real-time PCR.

**Results:** he MTT assay showed no significant reduction in cellular metabolic activity at the selected extract concentrations. Combined treatment significantly increased the frequency of Treg cells and reduced Th17 cells compared with controls ($p < 0.05$). ELISA revealed increased IL-10 and TGF-β secretion with decreased IL-17A production. Real-time PCR showed upregulation of FOXP3, IL-10, and TGF-β and downregulation of RORγt and IL-17A.

**Conclusion:** Combined extracts of Hyssopus officinalis and Teucrium polium modulated Treg/Th17-associated markers in activated human Pan T cells under in vitro conditions, suggesting potential immunomodulatory effects in inflammatory and immune-mediated conditions.

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## 1. Introduction

The immune response is a complicated biological reaction triggered by vascular tissues, cells of the immune system, and soluble mediators that are directed toward damaging stimuli like pathogens, toxins, or injury to tissue. Under physiological conditions, inflammation contributes to host defense, pathogen clearance, and tissue repair.[1] When inflammation is dysregulated, it becomes chronic and leads to multiple, long-lasting diseases, including autoimmune diseases, inflammatory bowel disease, metabolic diseases, allergies, and cancer. Immunomodulation has been studied extensively of late as a potential therapeutic means of controlling excessive inflammatory responses and re-establishing immune balance. Conventional anti-inflammatory medications, such as non-steroidal anti-inflammatory drugs (NSAIDs), are frequently used to treat chronic illnesses; however, prolonged use can produce various negative adverse effects such as gastrointestinal perforation, cardiovascular morbidity, nephrotoxicity, and hepatotoxicity.[2]

Several immune-cell populations regulate the initiation, magnitude, and resolution of inflammation. A major player in regulating the immune response is CD4+ T cells. A particularly important part of regulating immune responses is the balance between T helper 17 (Th17) cells and regulatory T (Treg) cells.[3] The differential regulation of these cell types is critically important to sustain immune tolerance and prevent pathological inflammation. Treg cells are marked by expression of the transcription factor FOXP3, and by producing the anti-inflammatory cytokines IL-10 and TGF-β. Treg cells regulate excessive immune activation, as well as maintain peripheral tolerance, by using numerous regulatory mechanisms.[4]
In contrast, Th17 cells express the transcription factor RORγt and produce the pro-inflammatory cytokine IL-17A, which is critical for host defense but is also associated with inflammatory pathologies. The differentiation and functional plasticity of Th17 and Treg cells is strongly influenced by the cytokine environment and signaling pathways. Evidence is accumulating that dysregulation of the Treg/Th17 balance contributes to the pathogenesis of autoimmune disease and chronic inflammatory diseases. Therefore, therapies that may be able to restore Treg/Th17 balance may have beneficial effects on the immune response.[5,6]

Medicinal plants yield many bioactive substances with antioxidant, anti-inflammatory, and immunoregulatory effects. Numerous phytochemicals are capable of modulating inflammatory signaling pathways, regulating cytokine release, decreasing oxidative stress, and/or affecting the developmental fate of immune cells (e.g., through regulating the expression of cell surface markers) through flavonoids and polyphenols, and terpenoids and phenolic compounds that are examples of phytochemicals that have been shown to have the ability to do this. Since ancient times, hyssop (Hyssopus officinalis), a Lamiaceae medicinal plant, has also been utilized in traditional medicine in the treatment of respiratory diseases (e.g., bronchitis, asthma), gastrointestinal diseases (e.g., indigestion, flatulence), and inflammatory diseases in humans. Previous studies have demonstrated that *H. officinalis* possesses antimicrobial, antiviral, antioxidant, and anti-inflammatory activities. Several studies demonstrate that certain components derived from hyssop affect cytokine production, modulate inflammatory signaling pathways, and therefore would impact immune responses.[7]

Further, Teucrium polium (commonly known as polecat or polium) belongs to the family Lamiaceae, which are known for treating many inflammatory diseases such as diabetes, gastrointestinal disorders, and rheumatism.[8] Studies performed in vitro in the past have shown that extracts from pulminaria (T. polium) have a significant effect on PBMCs through their ability to regulate the active state of T lymphocytes and also influence the number

of cytokines produced. However, the immunomodulatory effects of T. polium on human T-cell responses remain insufficiently characterized.[9,10]

Therefore, the present study aimed to investigate the immunomodulatory effects of H. officinalis and T. polium extracts, individually and in combination, on the balance between Treg and Th17 cells in activated human T lymphocytes in vitro. In particular, this study evaluated alterations in Treg and Th17 cell populations, cytokine secretion profiles, and expression levels of FOXP3, RORγt, IL-10, TGF-β, and IL-17A in order to clarify the potential regulatory effects of these medicinal plant extracts on inflammatory immune responses.

## 2. Materials and Methods

### *2.1 Materials*

RPMI-1640 medium, fetal bovine serum (FBS), penicillin–streptomycin solution, phosphate-buffered saline (PBS), Ficoll-Paque PLUS, phorbol 12-myristate 13-acetate (PMA), ionomycin, and brefeldin A were obtained from Sigma-Aldrich. Human Pan T cells were isolated using the Human Pan T Cell Isolation Kit with Catalog no. (130-096-535) provided by MACS Technologies. Human T-Activator CD3/CD28 was supplied by Thermo Fisher Scientific. Fluorochrome-conjugated monoclonal antibodies against human CD4, CD25, CD127, and IL-17A were provided by BioLegend. Human ELISA kits for IL-10, TGF-β, and IL-17A were obtained from MyBioSource. RNA extraction and cDNA synthesis kits were supplied by Yekta Tajhiz Azma and SYBR Green PCR Master Mix provided by Yekta Tajhiz Azma.

### *2.2 Blood Sampling*

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethics code: IR.TBZMED.REC.1403.763). Peripheral blood samples were collected from 30 healthy volunteers after obtaining written informed consent. Approximately 20 mL of venous blood was drawn into sterile sodium heparin-containing tubes under aseptic conditions**.**

### *2.3 Isolation of Peripheral Blood Mononuclear Cells (PBMCs)*

PBMCs were obtained from whole blood through Ficoll-Paque density centrifugation (Ficoll density of 1.077 g/mL) and then diluted 1:1 in sterile PBS and carefully layered over this solution in a centrifuge at 450g for 20 minutes without using the brake. After this procedure, the PBMC layer (buffy coat) was collected and washed twice with PBS. Cell viability was determined by using Trypan Blue exclusion, and subsequently, PBMCs from samples that met the cell viability criterion (> 95% viable) were used in subsequent experimental procedures. PBMCs served solely as the starting material for Pan T-cell isolation and were not used directly in any downstream functional assays.

### *2.4 Isolation and Activation of T Lymphocytes*

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using Ficoll-Paque PLUS density-gradient centrifugation. Untouched human Pan T cells were subsequently isolated from PBMCs by negative magnetic selection using the Human Pan T Cell Isolation Kit (Miltenyi Biotec, Germany), according to the manufacturer's instructions. Briefly, non-T cells were magnetically labeled with a cocktail of biotin-conjugated antibodies and anti-biotin microbeads, followed by magnetic separation using MACS columns. The unlabeled Pan T cells were collected and resuspended in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS)

and 1% penicillin–streptomycin. Cell purity was assessed by flow cytometry prior to downstream experiments. For T cell activation, isolated Pan T cells were cultured in complete RPMI-1640 medium and stimulated using Human T-Activator CD3/CD28 according to the manufacturer's protocol. Cells were incubated for 5 hours at 37°C in a humidified atmosphere containing 5% $CO_2$. For intracellular cytokine staining, phorbol 12-myristate 13-acetate (PMA), ionomycin, and brefeldin A were added during the final hours of culture.

### *2.5 Preparation of Plant Extracts*

In this study, based on our previous investigation, two polyherbal formulations were prepared via steam distillation from either the aerial parts (leaves, flowers, and terminal branches) or the whole plant material.[11] The first formulation, designated POLIUM, comprised Teucrium polium L., Cuscuta epithymum Murr, and Cichorium intybus L. in a 3:1:2 ratios (production license no. 5/80245-733; issued 10 March 2018 by the Deputy of Food and Drugs, Iran). The second formulation, designated HYSSOP, consisted of Hyssopus officinalis L., Echium amoenum Fisch & C.A. Mey, and Glycyrrhiza glabra L. in a 2:1:1 ratio (same license number and date). Both preparations were standardized based on their total phenolic content and antioxidant capacity.

### *2.6 Flow Cytometric Analysis of Th17 and Treg Cells*

Flow cytometric analysis was performed on $1 \times 10^6$ Pan T cells following staining with fluorochrome-conjugated monoclonal antibodies. The panel comprised FITC-conjugated anti-CD4, PE-conjugated anti-IL-17A (to detect Th17 cells), and PE-conjugated anti-CD25 along with APC-conjugated anti-CD127 (to identify Tregs). Intracellular cytokine staining was performed following cell stimulation for 5 h with 50 ng/mL phorbol 12-myristate 13-acetate (PMA) and 1 μg/mL ionomycin in the presence of brefeldin A (Biolegend, San Diego, CA, USA). After surface antigen staining, cells were fixed and permeabilized with the Fixation/Permeabilization Solution Kit (Biolegend, San Diego, CA, USA) as per the manufacturer's specifications. Flow cytometric acquisition was carried out on a BD FACSCalibur (BD Biosciences), collecting at least 50,000 events within the lymphocyte scatter gate. Compensation was applied using single-color stained controls and BD CompBeads.

### *2.7 Cytokine Quantification by ELISA*

Human IL-10 (Catalog No. MBS764410), TGF-β (Catalog No. MBS266143), and IL-17A (Catalog No. MBS2127177) ELISA kits were obtained from MyBioSource. The detection ranges and analytical sensitivities of the kits were 7.813–500 pg/mL and 4.688 pg/mL for IL-10, 15.6–1000 pg/mL and up to 5 pg/mL for TGF-β, and 7.8–500 pg/mL and <3.1 pg/mL for IL-17A, respectively. In brief, Activated Pan T-cell culture supernatants were incubated in 96-well micro-plates coated with capture antibodies and the appropriate amount of cell culture supernatant for 2 hours at room temperature. After washing, the biotinylated detection antibody and the streptavidin-horseradish peroxidase were added sequentially. The development of the reaction was completed with TMB substrate solution and stopped with sulphuric acid. Absorbance was measured at 450 nm using an ELISA micro plate reader (LabSystems, Helsinki, Finland). The determination of cytokine concentrations was made on the basis of standard curves for each cytokine generated from the ELISA assay performed in triplicate.

### *2.8 Quantitative Real-Time PCR*

Total RNA was extracted from treated activated Pan T cells using a commercially available RNA extraction kit according to the manufacturer's instructions (Yekta Tajhiz Azma). The concentration and purity of the isolated RNA were assessed with the NanoDrop spectrophotometer. One microgram (1 μg) of the total extracted RNA was utilized in the reverse transcription to produce complementary DNA (cDNA) using a reverse transcription kit (Yekta Tajhiz Azma). The PCR amplification protocol consisted of an initial denaturation step of 95°C for ten minutes, followed by forty cycles of denaturation (15 seconds at 95°C), followed by annealing and extension (60 seconds at 60°C). The relative expression of FOXP3, RORγt, IL-10, TGF-β, and IL-17A were normalized against the expression of β-actin, and the expression levels of each gene were calculated using the 2^-ΔΔCt method. The primer sequences used in this study are detailed in Table 1.

**Table1.** Forward and Reverse Primers

| Gene | Forward (5'→3') | Reverse (5'→3') | Reference |
|---|---|---|---|
| **IL-10** | TCTCCGAGATGCCTTCAGCAGA | TCAGACAAGGCTTGGCAACCCA | Origene (Human IL10 qPCR primer pair) |
| **TGF-β** | TACCTGAACCCGTGTTGCTCTC | GTTGCTGAGGTATCGCCAGGAA | Origene (Human TGF-β qPCR primer pair). |
| **IL-17A** | CGGACTGTGATGGTCAACCTGA | GCACTTTGCCTCCCAGATCACA | Origene (Human IL-17A qPCR primer pair). |
| **FOXP3** | GGCACAATGTCTCCTCCAGAGA | CAGATGAAGCCTTGGTCAGTGC | Origene — FOXP3 Human qPCR Primer Pair. |
| **RORC (RORγt)** | GAGGAAGTGACTGGCTACCAGA | GCACAATCTGGTCATTCTGGCAG | Origene — RORC (RORγ) Human qPCR Primer Pair. |
| **β-actin** | TCCCTGGAGAAGAGCTACG | GTAGTTTCGTGGATGCCACA | Origene (Human β-actin qPCR primer pair) |

### *2.9 Statistical Analysis*

All assays were repeated three times to confirm experimental reproducibility. Data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using GraphPad Prism version 8.0. Differences between groups were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A p-value less than 0.05 was considered statistically significant.

### *2.10 Experimental Groups*

Four experimental groups were included in the study: (1) untreated T cells, (2) T cells treated with *Hyssopus officinalis* extract (40 µg/mL), (3) T cells treated with *Teucrium polium* extract (40 µg/mL), and (4) T cells treated with the combination of both extracts at a 1:1 ratio (20 µg/mL H. officinalis + 20 µg/mL T. polium; total extract concentration: 40 µg/mL).

## 3. Results

### *3.1 Effect of extract concentration on cellular metabolic activity assessed by MTT assay*

MTT assay was performed to determine the non-cytotoxic concentrations of H. officinalis, T. polium, and their combined extracts for subsequent experiments. Cells were exposed to increasing concentrations of the extracts (10–160 µg/mL) for 48 h. Cellular metabolic activity remained above 90% at concentrations up to 40 µg/mL total extract concentration, whereas higher concentrations resulted in a progressive reduction in cellular metabolic activity (Figure 1). Based on these findings, 40 µg/mL was selected as the working concentration for individual extracts, while the combined treatment was performed using a 1:1 ratio of H. officinalis and T. polium extracts (20 µg/mL + 20 µg/mL; total concentration 40 µg/mL).

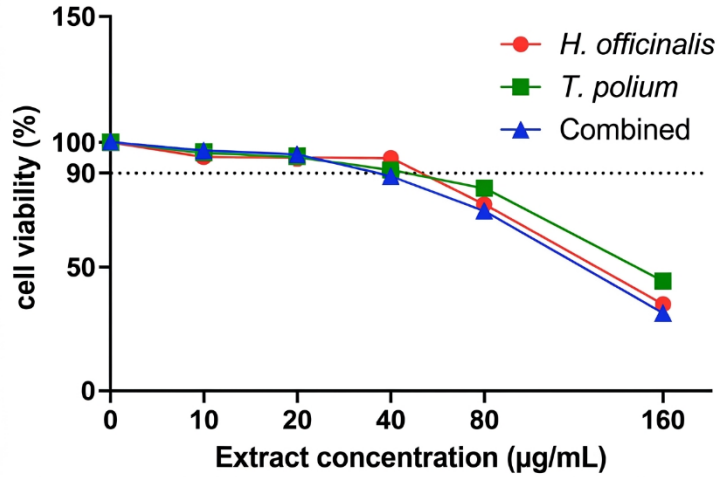



**Figure 1.** Effect of *H. officinalis*, *T. polium*, and their combined extracts on the metabolic activity of activated human Pan T cells. Activated human Pan T cells isolated from healthy donors were treated with different concentrations (10–160 µg/mL) of *H. officinalis*, *T. polium*, or their combined extracts for 48 h. Cellular metabolic activity was assessed using the MTT assay. Cell metabolic activity remained above 90% at concentrations up to 40 µg/mL and decreased at higher concentrations. Data are presented as mean ± SD. Experiments were performed using cells from healthy donors, and each assay was repeated three times to confirm reproducibility. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. $p < 0.05$ versus untreated control.

### *3.2 Effect of Combined Plant Extracts on Cytokine Production in Activated Human Pan T Cells*

ELISA analysis was utilized to assess levels of IL-10, TGF-β, and IL-17A in the culture supernatants at 48 h post-treatment (Figures 2A-C). Neither *H. officinalis* nor *T. polium* alone significantly changed TGF-β levels compared to PBS control. The combination of both extracts significantly increased TGF-β levels (p=0.021). *H. officinalis* alone significantly increased IL-10 levels compared to PBS control (p=0.0015). The combination of both extracts produced a highly significant increase in IL-10 levels (p<0.0001).

In contrast, IL-17A levels were not significantly changed by either extract alone, but the combined treatment significantly decreased IL-17A compared to control ($p<0.0001$).

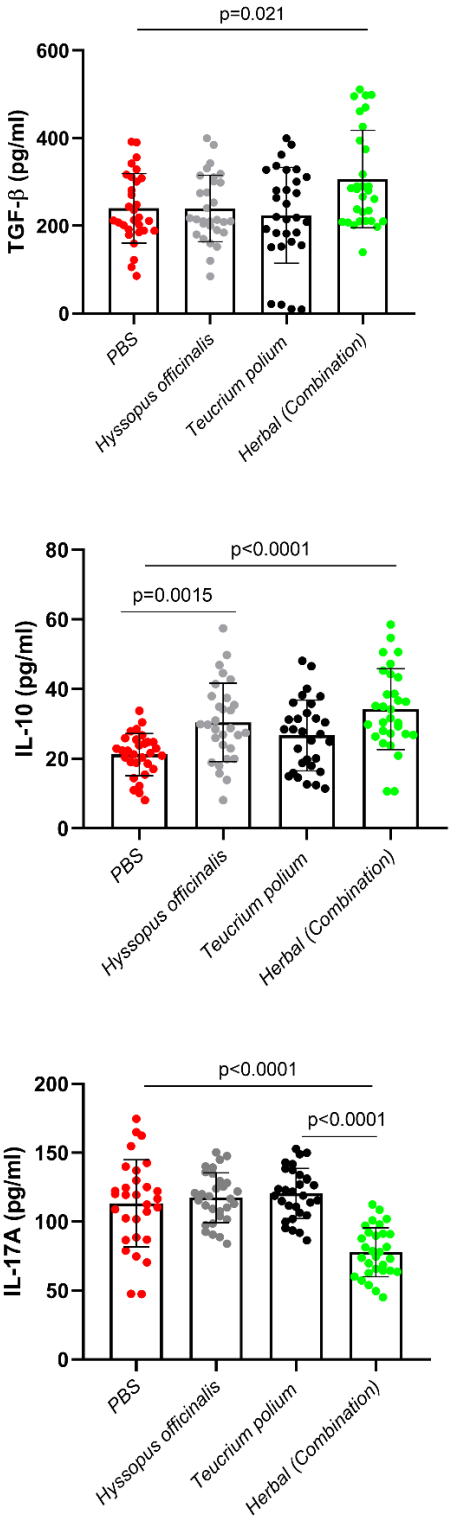



**Figure 2.** Effect of *H. officinalis*, *T. polium*, and their combined extracts on cytokine production by activated human Pan T cells. Activated human Pan T cells were treated with *H. officinalis*, *T. polium*, or their combined extracts (20 + 20 µg/mL; total concentration 40 µg/mL) for 48 h. IL-10, TGF-β, and IL-17A concentrations in culture supernatants were determined by ELISA. Data are presented as mean ± SD. Experiments were performed using cells from healthy donors, and each assay was repeated three times. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. $p < 0.05$ versus untreated control.

### *3.3 Effect of Combined Plant Extracts on Inflammatory and Regulatory Gene Expression*

The expression levels of FOXP3, RORγt, IL-10, TGF-β, and IL-17A were evaluated by quantitative real-time PCR. When compared to the untreated control there was no significant difference in expression levels of these genes in either H. officinalis or T. polium treated only cells ($p > 0.05$). However, the use of both extracts together did significantly increase FOXP3 ($p=0.0009$), IL-10 and TGF-β ($p<0.0001$) expression, whereas RORγt and IL-17A expression were significantly decreased ($p < 0.0001$). Additionally, the combined use of the extracts showed a significant difference compared to the separate use of each extract. Consequently, it appears that only combined treatment resulted in significantly altered transcriptional pathways regulating Treg/Th17 balance (Figure 3).

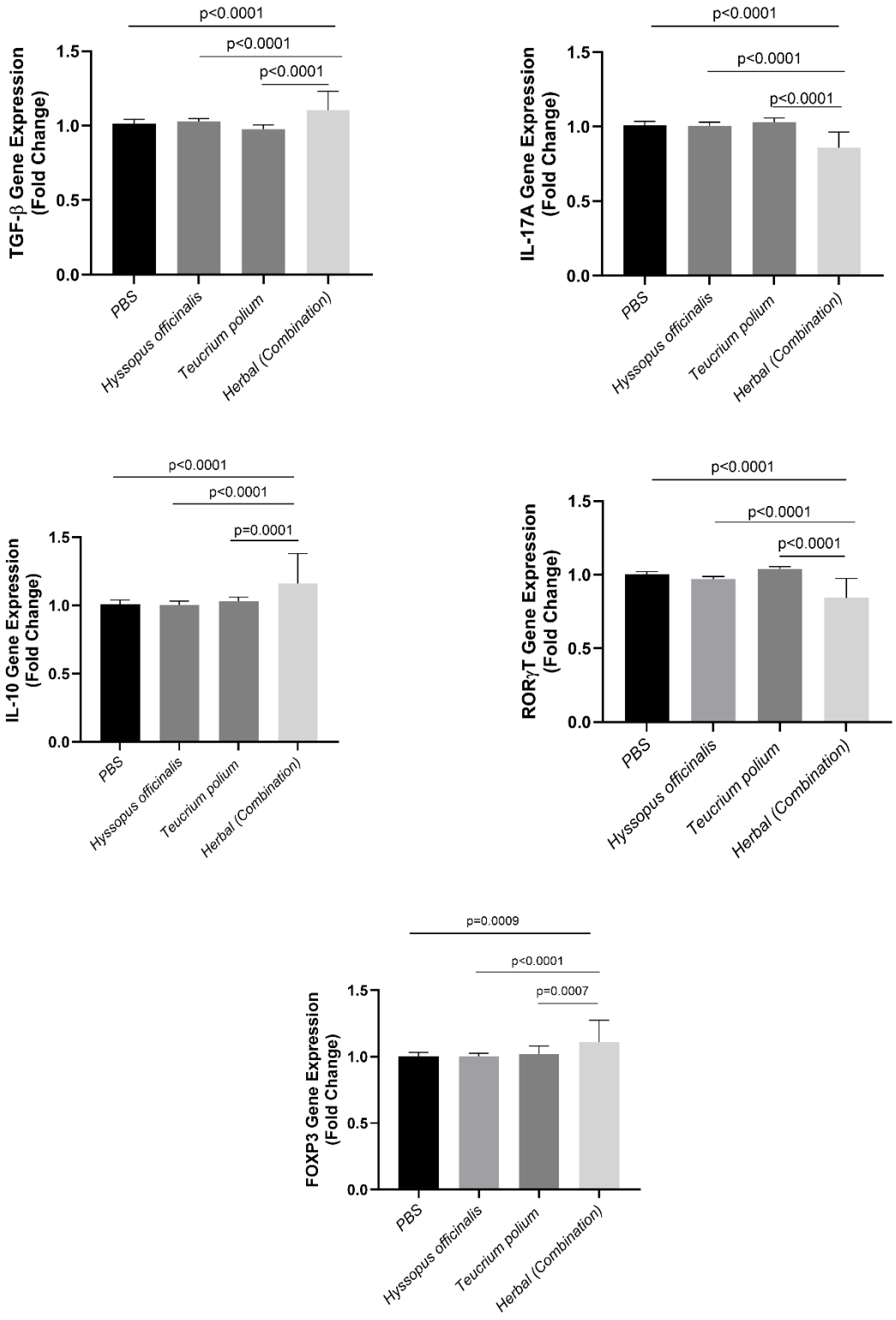



**Figure 3.** Effect of *H. officinalis*, *T. polium*, and their combined extracts on gene expression in activated human Pan T cells. Activated human Pan T cells were treated with *H. officinalis*, *T. polium*, or their combined extracts (20 + 20 µg/mL; total concentration 40 µg/mL) for 48 h. Relative mRNA expression levels of FOXP3, RORγt, IL-10, TGF-β, and IL-17A were determined by quantitative real-time PCR and normalized to β-actin. Data are presented as mean ± SD. Experiments were performed using cells from healthy donors, and each assay was repeated three times. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. $p < 0.05$ versus untreated control.

### *3.4 Effect of Combined Plant Extracts on Th17 and Treg Cell Populations*

The effects of H. officinalis and T. polium herbal extracts on the frequency of $CD4^+CD25^+CD127^{low/-}$ Treg cells and $CD4^+IL\text{-}17A^+$ Th17 cells populations were assessed using flow cytometry. After performing a gating strategy that involved sequential gating of live lymphocytes based on their FSC/SSC characteristics (forward scatter, side scatter), excluding doublets (by selecting singlets), and finally identifying $CD4^+$ T cells, the Treg population was identified as $CD4^+CD25^+CD127^{low/-}$ cells, while the Th17 population was identified to be composed of $CD4^+IL\text{-}17A^+$ cells (Figure 4A). In the results, the combination of *H. officinalis* and *T. polium* significantly reduced Th17 cell frequency compared to all other groups ($p<0.0001$). Also, the combination of the extracts demonstrated significant differences with *H. officinalis* ($p<0.0001$) and *T. polium* ($p<0.0001$). In contrast, Treg cell frequency was significantly increased in the combination group compared to PBS control ($p=0.0011$). Moreover, the combination of the extracts showed a significantly differences with *H. officinalis* ($p<0.004$) and *T. polium* ($p<0.009$). This finding indicates an enhanced immunoregulatory profile (Figure 4B).

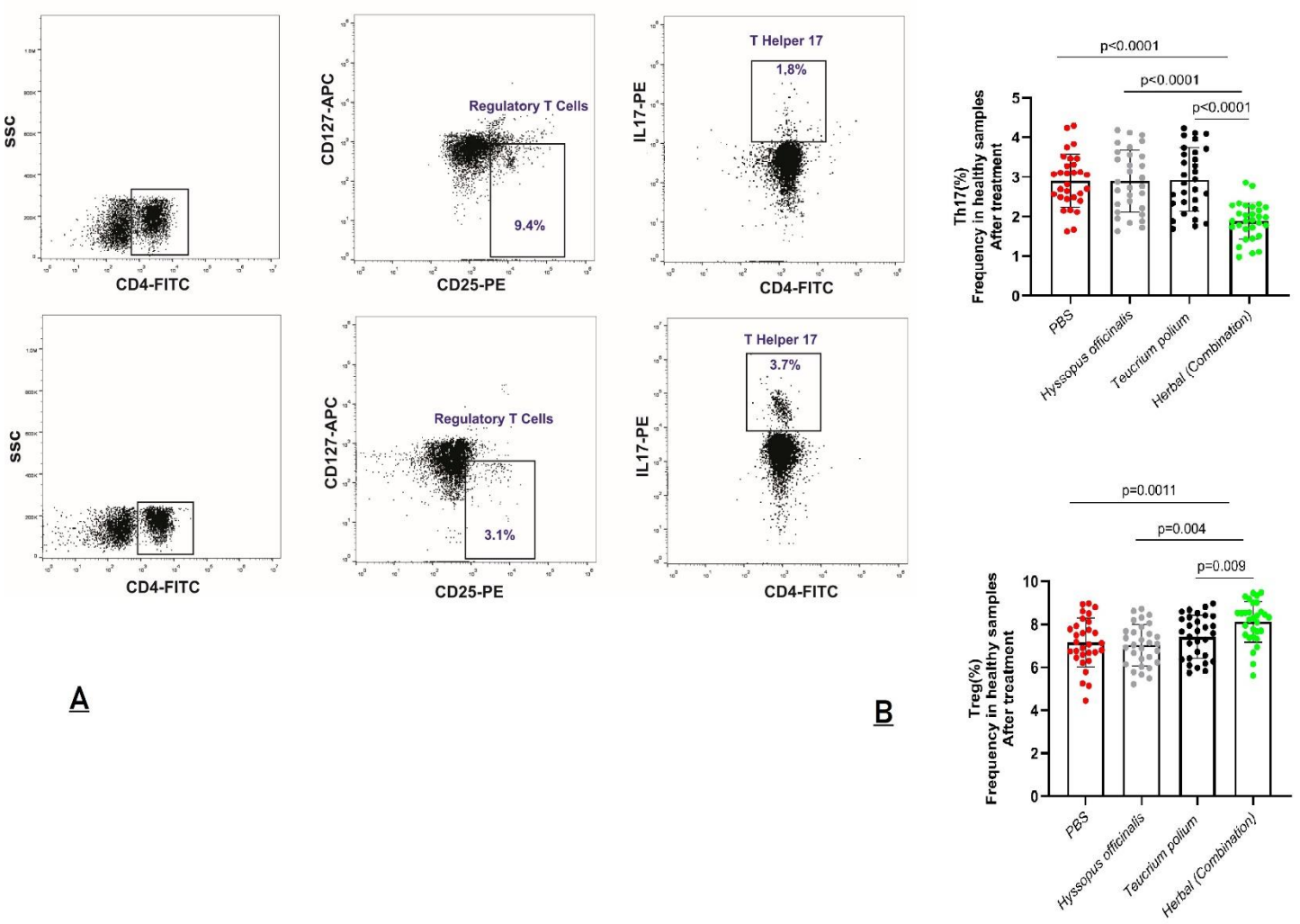



**Figure 4.** Effect of *H. officinalis*, *T. polium*, and their combined extracts on Th17 and Treg cell populations. Activated human Pan T cells isolated from healthy donors were treated with the indicated conditions for 48 h and analyzed by flow cytometry. Th17 cells were identified as $CD4^+IL\text{-}17A^+$ cells, and Treg cells were defined as $CD4^+CD25^+CD127^{low/-}$ cells. Data are presented as mean ± SD. Experiments were performed using cells from healthy donors, and each assay was repeated three times. Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple-comparison test. $p < 0.05$ versus untreated control.

## 4. **Discussion**

The immunomodulatory effects of H. officinalis and T. polium extracts were investigated in an in vitro model using activated human Pan T cells derived from healthy donors. This study demonstrated that treatment with H. officinalis and T. polium extracts significantly modulated the cytokine expression of certain Th17/Treg axis related cytokines (i.e. IL-10, TGF-β, and IL-17A) involved in regulating cell responses.[12,13] After treatment with these extract solutions, increased IL-10 and TGF-β levels were observed, which are cytokines commonly associated with regulatory immune profiles. These changes may indicate modulation of regulatory-associated immune responses under the tested in vitro conditions. Conversely, it was shown that the IL-17A expression level in treated activated human Pan T cells was significantly lower than the IL-17A expression level in untreated activated human Pan T cells. IL-17A is a pro-inflammatory cytokine produced primarily by Th17 cells, which plays an important role in inducing inflammatory reactions, causing tissue damage and contributing to the development of autoimmune and chronic inflammatory diseases. The down-regulation of IL-17A through the use of H. officinalis and T. polium extracts suggesting that these extracts may modulate Th17-associated inflammatory responses under the tested in vitro conditions.[14] To help explain how these changes occur, transcription factors specific to the lineage were assessed for expression levels as well. The analyses showed significantly increased levels of FOXP3 gene expression after treatment of the activated human Pan T cells with the plant extracts compared to control. FOXP3 is a key transcription factor associated with Treg lineage stability and regulatory immune functions.[15,16] At the same time, the expression of RORγt was decreased significantly in the treated cells. RORγt also is the primary transcription factor responsible for differentiating naïve $CD4^+$ T cells into Th17 cells and regulating the production of IL-17A by inducing IL-17A transcription. The reciprocal changes in FOXP3 and RORγt expression suggest modulation of transcriptional profiles associated with regulatory and Th17-related phenotypes in $CD4^+$ T cells.[17]

In support of the transcriptional shift, there also is a concurrent change of cytokine expression. The increase in IL-10 and TGF-β expression supports the increased level of FOXP3-mediated regulatory T cell activity, while the decrease in IL-17A reflects decreased RORγt-mediated Th17 differentiation. Collectively, these data suggest that the extracts influence cytokine profiles and transcriptional markers transcriptional markers related to T-cell regulation. Similarly, the increase in TGF-β will also likely aid in inducing and maintaining regulatory T cells and contribute to the creation of an anti-inflammatory environment for the immune system. Conversely, reduced IL-17A levels were consistent with decreased expression of a Th17-associated inflammatory marker.[18,19]

The coordinated regulation of FOXP3 and RORγt expression suggests that the extracts may influence the differentiation fate of $CD4^+$ T cells. Increased FOXP3 expression and decreased RORγt expression indicate changes in transcriptional markers associated with regulatory and Th17 phenotypes, respectively. This reciprocal regulation is a critical determinant of immune homeostasis, as the balance between Treg and Th17 cells plays a central role in controlling inflammatory responses. A shift toward FOXP3 dominance over RORγt is generally associated with reduced inflammation and improved immune regulation. Therefore, these findings suggest that H. officinalis and T. polium extracts may influence the Treg/Th17-associated transcriptional profile under the tested culture conditions.[20-22]

The findings of this study suggest that H. officinalis and T. polium extracts both consistently alter the immunological status of activated human Pan T cells, indicating modulation of activated human Pan T-cell function through modification of several cytokines (IL-10; TGF-β) and transcription factor (FOXP3; RORγt)

expression levels. The observed changes in IL-10, TGF-β, FOXP3, RORγt, and IL-17A suggest that the extracts were associated with modulation of regulatory and inflammatory immune markers. However, these findings represent changes observed under in vitro culture conditions and do not directly demonstrate restoration of immune tolerance or therapeutic anti-inflammatory effects. Although the MTT assay is widely used for preliminary cytotoxicity assessment, it primarily reflects cellular metabolic activity rather than direct cell viability. Therefore, further studies using complementary approaches, such as membrane integrity assays or absolute cell counting, are required to confirm the effects of these extracts on T-cell survival.

## 5. Conclusion

Briefly, the present study indicates that combined exposure to Hyssopus officinalis and Teucrium polium extracts modulated Th17/Treg-associated immune markers in activated human Pan T cells in vitro. Treatment with these extracts was associated with a relative increase in regulatory markers and reduction of Th17 markers. These findings suggest that bioactive compounds present in these plants may contribute to modulation of inflammatory cytokine profiles and T-cell-associated regulatory markers. Although further mechanistic and in vivo studies are required, the observed immunoregulatory properties support further investigation of these extracts through mechanistic studies, standardized preparations, and disease-relevant models.

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## Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Acknowledgments

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## Ethical Approval

The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (Ethics code: IR.TBZMED.REC.1403.763). Written informed consent was obtained from all participants before blood collection.

## Author contributions

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Writing–original draft: Javad Ahmadian Heris and Mehdi Yousefi
Writing – review & editing: Javad Ahmadian Heris and Mehdi Yousefi
All authors have read and approved the final version of the manuscript.

**Consent for Publication**

All authors have contributed to the research and approved its final version for submission.

**Declaration of generative AI and AI-assisted technologies in the writing process:**

During the preparation of this work, the authors used ChatGPT by OpenAI to improve paper readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

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