https://doi.org/10.48048/tis.2025.9235

Peeling Back Immunity: Citrus Peel Extract as A Potential Natural Immunomodulator for Systemic Lupus Erythematosus

Hasan Shodiq Alaydrus[1,*] and Ali Akbar Alaydrus[2]

[1]Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
[2]Faculty of Manufacturing Engineering, The University of British Columbia, Kelowna, Canada

([*]Corresponding author's e-mail: hasanshodiq2005@gmail.com)

Received: 29 October 2024, Revised: 13 November 2024, Accepted: 20 November 2024, Published: 20 January 2025

Abstract

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by chronic inflammation and dysregulation of the immune system. Current treatments for SLE often involve general immunosuppressants and antimalarial medications, which can have significant side effects and may not be effective for all patients. This has led researchers to explore alternative therapeutic options, including natural compounds with anti-inflammatory and immunomodulatory properties. Citrus peel extract has emerged as a promising candidate for SLE treatment due to its rich content of bioactive compounds, mainly polyphenols. In various studies, various studies have shown that these compounds possess potent anti-inflammatory and immunomodulatory effects. This study investigated Citrus peel extract's anti-inflammatory and immunomodulatory effects on peripheral blood mononuclear cells (PBMCs) from SLE patients. PBMCs from ANA-positive SLE patients were treated with Citrus peel extract at 25, 50, and 100 μg/mL. Flow cytometry was used to analyze Th1 (CD4+IFNγ+), Th17 (CD4+IL17A+), and Treg (CD4+CD25+Foxp3+) cell populations. Citrus peel extract significantly decreased Th1 and Th17 cells while increasing Treg cells dose-dependently by inducing interleukin-10 anti-inflammatory cytokine. At 100 μg/mL, the extract reduced Th1 cells from 12.70 to 7.83 % and Th17 cells from 18.47 to 11.67 % while increasing Treg cells from 0.14 to 3.67 %. Phytochemical screening revealed the presence of flavonoids, which likely mediate these immunomodulatory effects through suppression of inflammatory cytokines and transcription factors. These results suggest Citrus peel extract can help restore immune balance in SLE by suppressing pathogenic Th1/Th17 responses and promoting regulatory T cells and IL-10. The extract shows promise as a potential natural immunomodulatory adjuvant therapy for autoimmune conditions like SLE. Further research is needed to elucidate the molecular mechanisms and evaluate clinical efficacy.

Keywords: Citrus peel extract, Immunomodulator, Anti-inflammation, SLE

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by autoantibody production and immune complex deposition in various tissues, leading to chronic inflammation and organ dysfunction [1]. The global incidence of SLE increased to 3.7 cases per 100,000 population annually in 2021, with a mortality rate of up to 67 % [2]. In Indonesia, 2,166 SLE-related hospitalizations were reported in 2021, with 550 resulting in fatalities. Prolonged inflammation and T-cell tolerance failure to self-antigens in SLE patients are primary factors contributing to organ failure and mortality [3,4]. Inflammatory lymphocyte autoactivation causes an imbalanced modulation of T helper 1 (Th1) and T helper 17 (Th17) cells, along with suppression of regulatory T cells (Treg). This imbalance leads to excessive proinflammatory cytokine secretion, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 (IL-1), and interferon-gamma (INF-γ). It suppresses cytokine anti-inflammatory such as interleukin-10 (IL-10), resulting in tissue damage in autoimmune disorders like SLE [5].

SLE manifests with diverse symptoms and variable disease progression, necessitating lifelong treatment and carrying a high mortality risk [6]. Current standard therapies include corticosteroids and immunosuppressants [7,8]. However, long-term use of these synthetic drugs remains controversial due to side effects such as neurological dysfunction, vascular dementia, gastrointestinal toxicity, and organ damage [9]. There is a pressing need to develop effective, targeted compounds that regulate inflammation with minimal side effects for lifelong SLE immunotherapy.

Citrus peel, a by-product of citrus fruit consumption, must be utilized more [10,11]. In Indonesia, citrus fruits are the second most consumed after bananas, with an average consumption of 0.09 kg/day/person in 2021 [12,13]. Previous studies have reported that citrus peel contains essential oils (α-pinene, limonene, citronellal, linalool, terpinen-4-ol, mycene, α-terpineol, citral) and flavonoids with various biological activities, including anticancer, antioxidant, anti-inflammatory, and immunosuppressive properties [14]. Limonene, in particular, has demonstrated immunosuppressive effects by inhibiting inflammation through the induction of transforming growth factor-β (TGF-β) and IL-10 release [15-17]. This mechanism can potentially inhibit abnormal responses of proinflammatory effector cells, including Th1 and Th17 cells, ultimately inducing normal immune responses in SLE. In addition, other compounds in citrus extract, such as quercetin, demonstrate potent anti-inflammatory properties by inhibiting pro-inflammatory cytokine production, including TNF-α, IL-6, and IL-1β, while suppressing the activation of the NF-κB signaling pathway [18-20]. Ascorbic acid also modulates the Th1/Th2 balance and suppresses inflammatory mediator production, contributing to their overall immunomodulatory effects [21]. Limonin and nomilin activate the Nrf2 pathway, induced phase II detoxification enzymes, and exhibited anti-inflammatory properties [15,17,22]. Furthermore, limonoids have been shown to modulate immune cell differentiation, contributing to their immunomodulatory effects.

Additionally, flavonoids in citrus peel have been reported to decrease INF-γ levels, thereby inhibiting interleukin-17 (IL-17) secretion responsible for Th17 formation [23,24]. This inhibition suppresses inflammation in over-inflammatory conditions such as SLE [25]. The effects of citrus peel extract in modulating Th1, Th17, and Treg cell responses and increasing antiinflammation cytokine IL-10 in SLE patients remain unclear. Therefore, this study aims to investigate the effects of citrus peel extract on regulating Th1, Th17, Treg cells, and IL-10 in peripheral blood mononuclear cells (PBMCs) of SLE patients using an *in vitro* co-culture method. This study will also develop a simple formulation of citrus peel extract into a convenient and easy-to-use capsule dosage form.

Materials and methods

Ethics approval

The study was approved by the Ethics Committee of Sultan Agung Islamic University, Semarang, Indonesia, under No. 076/ I1/2024/Komisi Bioetik.

Citrus peel extraction

Citrus peel extraction was performed using the maceration method. Five hundred grams of citrus peel powder were soaked for 72 h at room temperature and protected from light. Ethanol solvent 70 % (5L) was added to the extraction chamber until it completely submerged the plant material. After extraction, the solution was carefully filtered using Whatman paper (Grade 3) to eliminate any remaining solid particles. The filtrate was then subjected to solvent removal using a rotary evaporator under reduced pressure [26,27]. This process continued until all the ethanol had evaporated, resulting in a concentrated crude extract. The obtained crude extract was stored in a refrigerator maintained at 4 °C for preservation and future analysis.

Phytochemical profile analysis

Phytochemical screening was conducted with modifications to identify flavonoids, alkaloids, phenolics, saponins, steroids, and triterpenoids. Flavonoids were identified using the Shinoda test with Mg powder and HCl. Alkaloids were detected using Wagner's reagent. Phenolics were determined by adding 1 % $FeCl_3$. Saponins were identified through the foam test. Terpenoids and steroids were detected using the Liebermann-Burchard reagent [28].

SLE patient blood collection

Blood samples from 3 SLE patients were obtained from the antecubital vein using a Vacutainer Eclipse Blood Collection Needle and Vacutainer Blood Collection tube (BD Science). Collected blood was stored in a freezer (−20 °C) until PBMC isolation.

Peripheral blood mononuclear cells (PBMC) isolation

Peripheral Blood Mononuclear Cells were isolated from whole blood using density gradient centrifugation with Ficoll-Paque in SepMate tubes. This was followed by washing with PBS containing 2 mM EDTA and cell counting using the trypan blue exclusion method. Dead cells, identified by blue staining, were excluded from the count. Furthermore, the PBMC was treated with different concentrations of citrus peel extract: treatment 1 used 25 µg/mL citrus peel extract, treatment 2 used 50 µg/mL citrus peel extract, and Treatment 3 used 100 µg/mL citrus peel extract [29].

Trans-well co-culture of PBMC and citrus peel extract

Counted PBMCs were placed in wells with 0.4 µm pore size to prevent direct contact with the citrus peel extract (**Figure 1**). The transwell culture media composition included RPMI (Gibco), 10 % Fetal Bovine Serum (FBS) (Gibco), 0.25 % amphotericin-B (Sigma-Aldrich), 2 % Penicillin Streptomycin (Thermo Fisher Scientific), and 1 % phytohaemagglutinin (PHA) (Sigma-Aldrich). Incubation occurred for 72 h at 37 °C, 5 % CO2, and 90 % humidity.

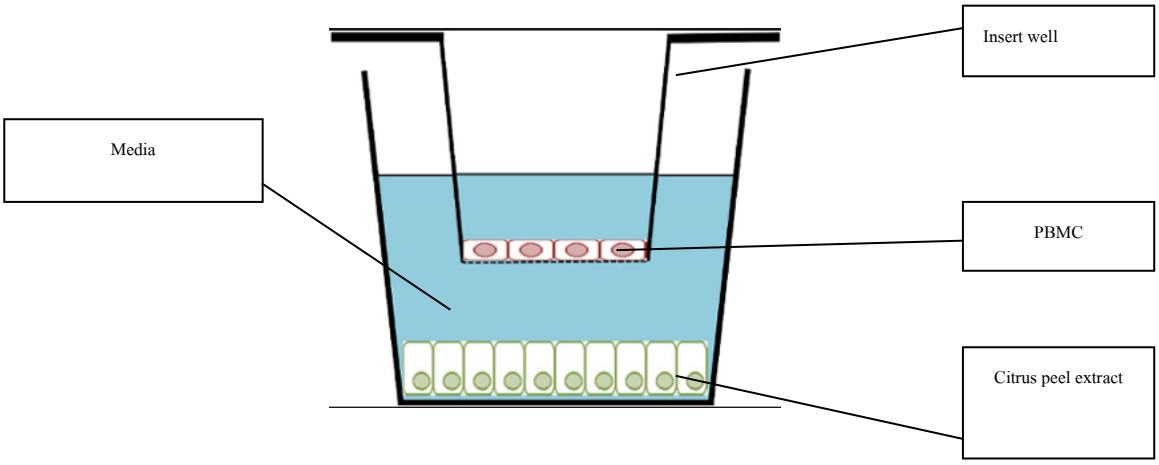

Figure 1 Trans well culture.

Medium and PBMC collection

After 72 h of incubation, PBMCs were collected from the wells and centrifuged at 1000xG for 10 min. The supernatant was carefully discarded to avoid PBMC loss. Cells were washed with 1 mL of phosphate-buffered saline (PBS, Gibco) and stored at 4 - 8 °C until analysis. Culture media from the transwell were transferred to vials and stored at −80 °C until analysis.

Flow cytometry analysis

Th1, Th17, and Treg cell quantities were analyzed using the Cytometric Bead Array (CBA) (BD Biosciences) method with a Flow cytometer (BD Accuri 6 plus). Peripheral blood mononuclear cells (PBMCs) were isolated using density gradient centrifugation, stimulated with PMA and ionomycin in the presence of Brefeldin A, then stained for surface markers (CD3, CD4) (Santa Cruz Biotechnology, USA) and intracellular cytokines (IFN-γ for Th1, IL-17A for Th17, and FOXP3 for Treg), followed by analysis using a CBA assay (BD Biosciences) to quantify the respective T cell population. Gating strategies on lymphocytes based on FSC/SSC properties based on cell population identification, Th1 used CD3+CD4+IFN-γ+, Th17 used CD3+CD4+IL-17A+, and Treg used CD4+CD25+CD127low/−FOXP3+. Furthermore, the cytokine levels are quantified using standard curves generated from cytokine standards.

IL-10 gene expression analysis under qRT-PCR

Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) using a commercial RNA isolation kit (Qiagen), followed by reverse transcription to synthesize cDNA, and quantitative reverse transcription PCR (qRT-PCR) was performed to analyze IL-10 gene expression using specific primers (Qiagen) (**Table 1**), with GAPDH (Qiagen) as a housekeeping gene for normalization. For gene expression analysis, relative quantification was performed using the ΔΔCt method, with GAPDH as the reference gene.

Tabel 1 Primer sequence of IL-10.

Primer	Sequence
IL-10	Forward:5'-CCCAGACATCAAGGCGCATGTG-3'
	Reverse: 5'-GTAGATGCCTTTCTCTTGGAGC-3'
GAPDH	Forward:5'-CCATGAGAAGTATGACAAC-3'
	Reverse: 5'-GAGTCCTTCCACGATACC-3'

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 24.0. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was checked with Levene's test. One-way ANOVA with Tukey's post-hoc test was used to compare groups. A p-value < 0.05 was considered statistically significant.

Results

Extraction and characterization of citrus peel extract

The citrus peel extract was obtained through maceration using 70 % ethanol, yielding an extract yield of 4.68 %. Phytochemical screening revealed that the citrus peel extract contained alkaloids, saponins, tannins, flavonoids, and terpenoids. Flavonoid compounds in citrus peel, such as anthocyanins, kaempferol, hesperidin, hesperetin, naringenin, nobiletin, and quercetin [30], have been shown to possess antioxidant and immunoregulatory activities [31].

Effect of citrus peel extract on Th1 cell population in PBMCs from SLE patients

Targeting Th1, Th17, and Treg cells can be considered a rational alternative for treating SLE patients. Th1 and Th17 cells were CD4+IFNγ+ and CD4+IL17A+, respectively (**Figure 2**). Th1 cells are characterized as CD4+IFNγ+ because autoreactive CD4+ T cell effectors have been linked to the pathogenesis of several autoimmune disorders. Additionally, Th1 cells have been shown to produce IFN-γ, which contributes to eliminating intracellular pathogens and is involved in delayed-type hypersensitivity responses [32-34]. The Th17 cell response significantly increases in IFN-γ-deficient mice [35,36]. This study explored the effects of citrus peel extract on the numbers of Th1, Th17, and Treg cells in PBMCs from SLE patients.

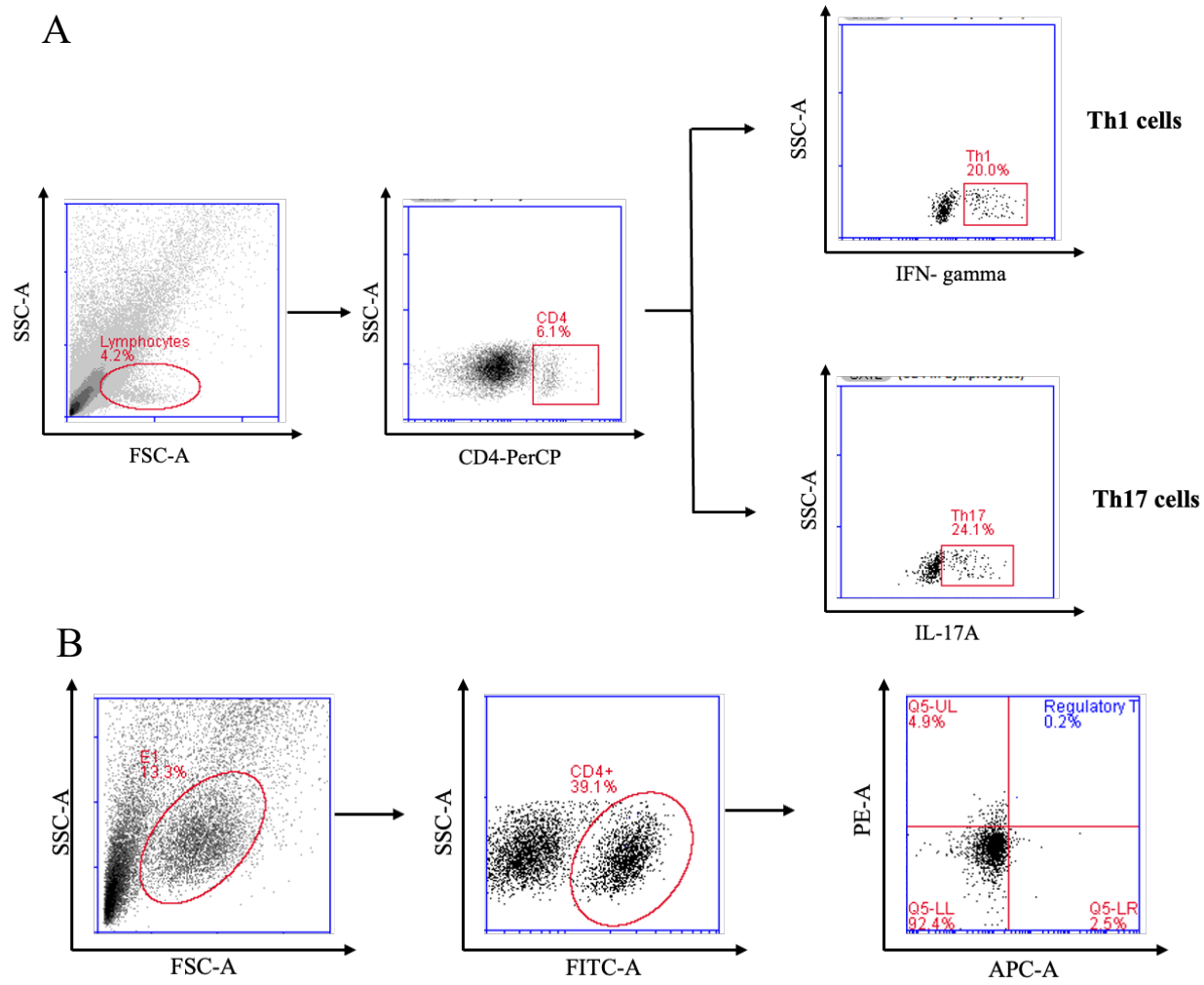

Figure 2 (A) Gating strategy for Th1 and Th17 cell populations from CD4+ cells. (B) Gating strategy for Treg cell population. FSC: Forward scatter; SSC: Side scatter; FITC: Fluorescein isothiocyanate; PE: Phycoerythrin; APC: Allophycocyanin; CD4: Cluster of differentiation 4; INFγ: Interferon-gamma; IL17A: Interleukin 17A.

In this study, the control PBMC group without treatment showed many Th1 cells (12.70 ± 1.20). Administration of citrus peel extract was shown to decrease the number of Th1 cells in a dose-dependent manner. The 25 µg/mL citrus peel extract group showed a non-significant decrease in Th1 cells (11.57 ± 0.55) compared to the control. However, the 50 µg/mL (8.63 ± 0.70) and 100 µg/mL (7.83 ± 0.35) citrus peel extract groups exhibited significant decreases in Th1 cell numbers (**Figure 3**).

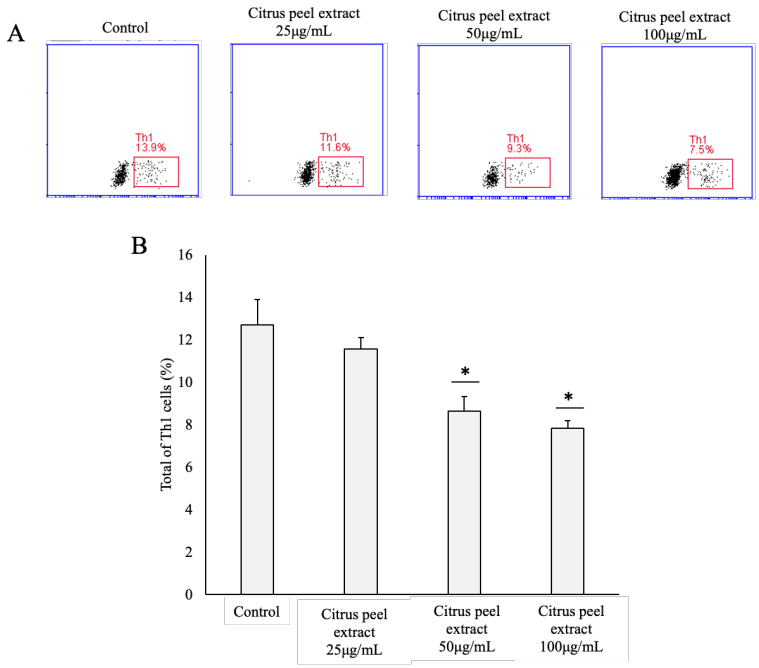

Figure 3 (A) Flow cytometry analysis of Th1 cells in PBMCs from SLE patients after treatment with citrus peel extract. (B) Quantification of Th1 cell numbers. Data from this study consist of 3 replicates ($^{*}p < 0.05$) indicating significant differences.

Effect of citrus peel extract on Th17 cell population in PBMCs from SLE patients

In this study, the control PBMC group without treatment showed many Th17 cells (18.47 ± 1.11). The 25 µg/mL citrus peel extract group experienced a non-significant increase in Th17 cells (20.67 ± 1.91) compared to the control. However, the 50 µg/mL (14.17 ± 0.50) and 100 µg/mL (11.67 ± 2.41) citrus peel extract groups showed a significant decrease in Th17 cell numbers (**Figure 4**). This indicates that the flavonoid compounds in citrus peel can have an immunosuppressive effect by suppressing the number of Th1 and Th17 cells.

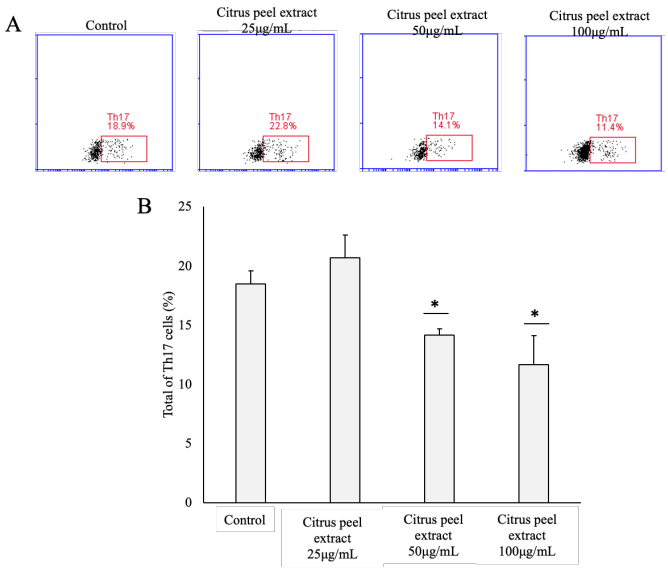

Figure 4 (A) Flow cytometry analysis of Th17 cells in PBMCs from SLE patients after treatment with citrus peel extract. (B) Quantification of Th17 cell numbers. Data from this study consist of 3 replicates ($^{*}p < 0.05$) indicating significant differences.

Effect of citrus peel extract on Treg cell population in PBMCs from SLE patients

This study also obtained results supporting previous research that citrus peel extract can increase the number of Treg cells. Treg cells are characterized by APC-A positive and PE-A positive (**Figure 2(B)**).

The control PBMC group without treatment showed low Treg cells (0.14 ± 0.05) in this study. The 25 µg/mL citrus peel extract group experienced a non-significant increase in Treg cells (0.25 ± 0.05) compared to the control. However, the 50 µg/mL (2.07 ± 0.57) and 100 µg/mL (3.67 ± 0.51) citrus peel extract groups showed a significant increase in Treg cell numbers (**Figure 5**).

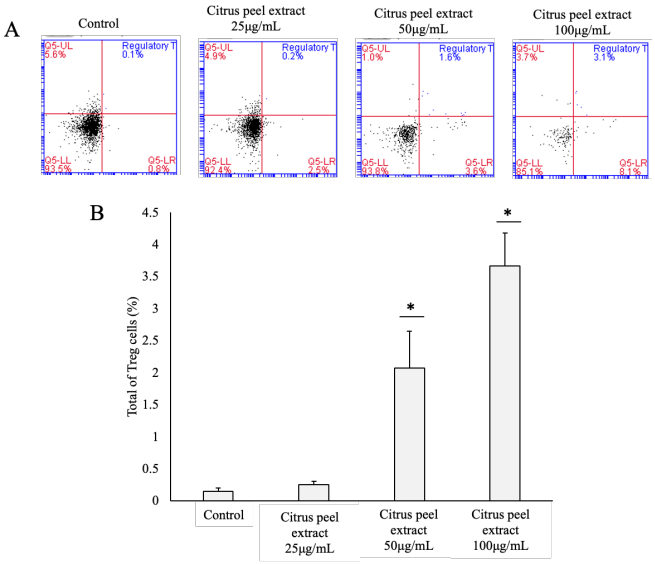

Figure 5 (A) Flow cytometry analysis of Treg cells in PBMCs from SLE patients after treatment with citrus peel extract. (B) Quantification of Treg cell numbers. Data from this study consist of 3 replicates ($^{*}p < 0.05$) indicating significant differences.

Citrus peels are rich in flavonoids, particularly hesperidin and polymethoxylated flavones (PMFs), which have potent anti-inflammatory and immunomodulatory properties. This study showed that citrus peel extract increased the IL-10 gene expression dose-dependently (**Figure 6**).

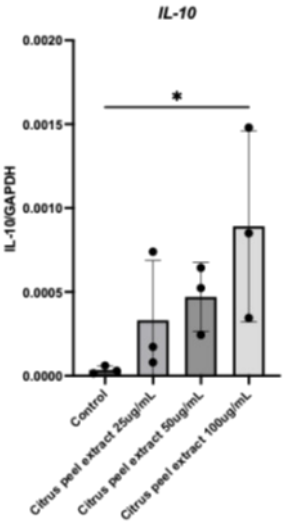

Figure 6 Ratio gene expression of IL-10 after 24 h treatment of several concentrations citrus peel extract on PMBC culture *f* of SLE patient. Data from this study consist 3 replicates ($^{*}p < 0.05$) indicating significant differences.

Discussion

Immune system abnormalities in autoimmune patients, such as those with SLE, are characterized by increased Th1 and Th17 cells and decreased Treg cells [37]. This study utilized PBMCs from SLE patients, identified by positive anti-nuclear antibody (ANA) tests. The study comprised 4 groups: 3 treatment groups and one control group. The treatment groups were divided based on citrus peel extract concentrations: 25, 50, and 100 μg/mL. These dosages were selected based on the effective use of herbal compounds *in vitro* tests, where herbal compounds show potential activity at treatment doses < 100 μg/mL [38]. Th1 cells are a subset of effector memory T cells whose development involves signals sent by antigen-presenting cells and various cytokines secreted in response to pathogens, including interferon (IFN)-γ, interleukin-12 (IL-12), and IL-18 [39]. Th17 cells are another subset of effector memory T cells that are crucial in inducing inflammation characteristics of many autoimmune and inflammatory diseases [40,41]. Increased Th1 and Th17 cells are associated with decreased Treg cells, leading to immune imbalance and increased pathogenicity in autoimmune disorders such as SLE [42].

In this study, the control PBMC group without treatment showed a high number of Th1 cells, and administration of citrus peel extract was shown to decrease the number of Th1 cells dose-dependent. Previous research has also reported that flavonoid compounds inhibit the differentiation and proliferation of Th1 and Th17 cells in a mouse model of autoimmune encephalomyelitis [43]. Other studies have reported that the anti-inflammatory effects of flavonoids are due to their ability to decrease the expression of cytokines IL-6, IL-17, and IL-1β and suppress various nuclear factor kappa B (NF-κB) transcription factor pathways, leading to inhibition of Th17 cell differentiation and increased Treg cells [44]. The increased number of Th17 cells, a subset of CD4+ Th cells in various organs, is associated with autoimmune disease activity mediating organ damage [45,46]. A high Th17/Treg ratio as a marker of cytokine imbalance can induce flares in SLE patients and is characteristic of SLE disease severity [46]. Although Th17 cells are essential in SLE flares, Th1 cells also exacerbate this autoimmune process. Th1 cells emerge as an effector response under IL-17 deficiency to continue driving the clinical manifestations of autoimmune disease and vice versa [36]. Therefore, controlling Th17 and Th1 using natural compounds containing flavonoids is necessary to inhibit SLE flares because flavonoids have immunosuppressive abilities on autoreactive lymphocytes by inducing Tregs and releasing anti-inflammatory cytokines [46].

In this study, citrus peel extract groups significantly decreased Th17 cell numbers. This indicates that the flavonoid compounds in citrus peel can have an immunosuppressive effect by suppressing the number of Th1 and Th17 cells. This study supports previous research that flavonoid compounds inhibit the expression of various cytokines INF-γ, IL-4, and IL-17, which prevent the proliferation of Th1 and Th17 cells. Quercetin inhibits Th17 cell differentiation by inhibiting the TLR4/MyD88/NF-κB signaling pathway. Naringenin, abundantly found in citrus peel, has also been shown to inhibit Th1 and Th17 cell differentiation by downregulating T-bet and RORγt expression [47]. Treg cells are critical immune cells and negative lymphocyte regulators that suppress harmful autoimmune T cells in the immune response. The imbalance between Th and Treg cells exacerbates autoimmune diseases [29]. Quercetin increases Foxp3 expression, which targets T cell immunoglobulin and mucin domain-containing protein 3 (Tim-3), a factor that can induce Treg cell differentiation [47]. IL-10 is crucial for suppressing excessive immune responses and promoting immune tolerance. Citrus peel extract can modulate T cell populations by affecting regulatory T cells (Tregs). Tregs are essential for maintaining immune homeostasis and preventing autoimmune reactions. Previous studies have demonstrated that citrus peel extract can decrease levels of pro-inflammatory cytokines like TNF-α and IFN-γ. This shift in the cytokine profile helps to resolve inflammation.

Citrus peel extract shows promise for development as a natural adjuvant immunoregulatory therapy in autoimmune patients. The effect of citrus peel extract in suppressing Th1 and Th17 cell numbers is due to the ability of flavonoids to regulate IFNγ release [43]. This study has several limitations, such as not examining many aspects of Th1 and Th17 deregulation in SLE conditions and not examining the molecular pathways

mediating the ability of citrus peel extract to increase Treg numbers.

Conclusions

Overall, the results of this study demonstrate the effectiveness of citrus peel extract containing various flavonoid compounds in regulating immunity in PBMCs from SLE patients.

Acknowledgments

We sincerely thank the chemical laboratory, UNNES, Indonesia for the invaluable support that made this research possible.

References

[1] VR Moulton, A Suarez-Fueyo, E Meidan, H Li, M Mizui and GC Tsokos. Pathogenesis of human systemic lupus erythematosus: A cellular perspective. *Trends in Molecular Medicine* 2017; **23(7)**, 615-635.

[2] MRW Barber, C Drenkard, T Falasinnu, A Hoi, A Mak, NY Kow, E Svenungsson, J Peterson, AE Clarke and R Ramsey-Goldman. Global epidemiology of systemic lupus erythematosus. *Nature Review Rheumatology* 2021; **17(9)**, 515-532.

[3] V Ocampo-Piraquive, I Nieto-Aristizabal, CA Canas and GJ Tobon. Mortality in systemic lupus erythematosus: Causes, predictors and interventions. *Expert Review of Clinical Immunology* 2018; **14(12)**, 1043-1053.

[4] J Bakshi, BT Segura, C Wincup and A Rahman. Unmet needs in the pathogenesis and treatment of systemic lupus erythematosus. *Clinical Reviews in Allergy & Immunology* 2018; **55(3)**, 352-367.

[5] AR Kustiyah, A Putra, T Nasihun and R Ramasamy. The normal ratio of Th17 and Th1 post-mesenchymal stem cells coculture with PBMCs of systemic lupus erythematosus patients. *Open Access Macedonian Journal of Medical Sciences* 2021; **9**, 169-176.

[6] DM Darlan, D Munir, A Putra, I Alif, ND Amalina, NK Jusuf and IB Putra. Revealing the decrease of indoleamine 2,3-dioxygenase as a major constituent for B cells survival post-mesenchymal stem cells co-cultured with peripheral blood mononuclear cell (PBMC) of systemic lupus erythematosus (SLE) patients. *Medicinski Glasnik* 2022; **19(1)**, 0.

[7] DM Darlan, D Munir, NK Jusuf, A Putra, R Ikhsan and I Alif. *In vitro* regulation of IL-6 and TGF-ß by mesenchymal stem cells in systemic lupus erythematosus patients. *Medicinski Glasnik* 2020; **17(2)**, 408-413.

[8] A Tsanaktsi, EE Solomou and SNC Liossis. Th1/17 cells, a subset of Th17 cells, are expanded in patients with active systemic lupus erythematosus. *Clinical Immunology* 2018; **195**, 101-106.

[9] JS Kornienko, IS Smirnova, NA Pugovkina, JS Ivanova, MA Shilina, TM Grinchuk, AN Shatrova, ND Aksenov, VV Zenin, NN Nikolsky and OG Lyublinskaya. High doses of synthetic antioxidants induce premature senescence in cultivated mesenchymal stem cells. *Scientific Reports* 2019; **9**, 1296.

[10] N Koolaji, B Shammugasamy, A Schindeler, Q Dong, F Dehghani and P Valtchev. Citrus peel flavonoids as potential cancer prevention agents. *Current Developments in Nutrition* 2020; **4(5)**, nzaa025.

[11] DDP Putri, GG Maran, Y Kusumastuti, RA Susidarti, E Meiyanto and M Ikawati. Acute toxicity evaluation and immunomodulatory potential of hydrodynamic cavitation extract of citrus peels. *Journal of Applied Pharmaceutical Science* 2022; **12(4)**, 136-145.

[12] SS Liew, WY Ho, SK Yeap and SAB Sharifudin. Phytochemical composition and *in vitro* antioxidant activities of *citrus sinensis* peel extracts. *PeerJ* 2018; **6**, e5331.

[13] ND Amalina, S Wahyuni and Harjito. Cytotoxic effects of the synthesized *citrus aurantium* peels extract nanoparticles against MDA-MB-231 breast cancer cells. *Journal of Physics: Conference Series* 2021; **1918**, 032006.

[14] M Suzery, B Cahyono and ND Amalina. Citrus sinensis (L) peels extract inhibits metastasis of breast cancer cells by targeting the downregulation matrix metalloproteinases-9. *Open Access Macedonian Journal of Medical Sciences* 2021; **9**, 464-469.

[15] X Yu, H Lin, Y Wang, W Lv, S Zhang, Y Qian, X Deng, N Feng, H Yu and B Qian. D-limonene exhibits antitumor activity by inducing autophagy and apoptosis in lung cancer. *Onco Targets and Therary* 2018; **11**, 1833-1847.

[16] NE Omari, HN Mrabti, T Benali, R Ullah, A Alotaibi, ADI Abdullah, KW Goh and A Bouyahya. Expediting multiple biological properties of limonene and α-pinene: Main bioactive compounds of pistacia lentiscus L., essential oils. *Frontiers in Bioscience - Landmark* 2023; **28(9)**, 229.

[17] D Mandal, P Patel, SK Verma, BR Sahu and T Parija. Proximal discrepancy in intrinsic atomic interaction arrests G2/M phase by inhibiting Cyclin B1/CDK1 to infer molecular and cellular biocompatibility of d-limonene. *Scientifc Reports* 2022; **12**, 18184.

[18] B Salehi, L Machin, L Monzote, J Sharifi-Rad, SM Ezzat, MA Salem, RM Merghany, NME Mahdy, CS Klllç, O Sytar, M Sharifi-Rad, F Sharopov, N Martins, M Martorell and WC Cho. Therapeutic potential of quercetin: New insights and perspectives for human health. *ACS Omega* 2020; **5(20)**, 11849-11872.

[19] B Baby, P Antony, WA Halabi, ZA Homedi and R Vijayan. Structural insights into the polypharmacological activity of quercetin on serine/threonine kinases. *Drug Design, Development and Therapy* 2016; **10**, 3109-3123.

[20] C Liu, Y Sun, J Sun, J Ma and C Cheng. Protective role of quercetin against lead-induced inflammatory response in rat kidney through the ROS-mediated MAPKs and NF-κB pathway. *Biochimica et Biophysica Acta (BBA) - General Subjects* 2012; **1820(10)**, 1693-1703.

[21] N Andarwulan, D Kurniasih, RA Apriady, H Rahmat, AV Roto and BW Bolling. Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. *Journal of Functional Foods* 2012; **4(1)**, 339-347.

[22] D Lipsa, P Leva, J Barrero-Moreno and M Coelhan. Inflammatory effects induced by selected limonene oxidation products: 4-OPA, IPOH, 4-AMCH in human bronchial (16HBE14o-) and alveolar (A549) epithelial cell lines. *Toxicology Letters* 2016; **262**, 70-79.

[23] NP Kumar, K Moideen, PJ George, C Dolla, P Kumaran and S Babu. Coincident diabetes mellitus modulates Th1-, Th2-, and Th17-cell responses in latent tuberculosis in an IL-10- and TGF-β-dependent manner. *European Journal of Immunology* 2016; **46(2)**, 390-399.

[24] TV Nguyen, CH Piao, YJ Fan, D Shin, SY Kim, H Song, CH Song, HS Shin and OH Chai. Anti-allergic rhinitis activity of α-lipoic acid via balancing Th17/Treg expression and enhancing Nrf2/HO-1 pathway signaling. *Scientific Reports* 2020; **10**, 12528.

[25] R Cheng, Z Wu, M Li, M Shao and T Hu. Interleukin-1β is a potential therapeutic target for periodontitis: A narrative review. *International Journal of Oral Science* 2020; **12**, 2.

[26] S Mursiti, ND Amalina and A Marianti. Inhibition of breast cancer cell development using *Citrus maxima* extract through increasing levels of reactive oxygen species (ROS). *Journal of Physics: Conference Series* 2021; **1918**, 052005.

[27] D Hermansyah, DA Paramita, AM Muhar and ND Amalina. Curcuma longa extract inhibits migration by reducing MMP-9 and Rac-1 expression in highly metastatic breast cancer cells. *Research in Pharmaceutical Sciences* 2024; **19(2)**, 157-166.

[28] M Suzery, B Cahyono and ND Amalina. Antiproliferative and apoptosis effect of hyptolide from *Hyptis pectinata* (L.) Poit on human breast cancer cells. *Journal of Applied Pharmaceutical Science* 2020; **10(2)**, 1-6.

[29] YW Prajoko, A Putra, BT Dirja, AM Muhar and ND Amalina. The ameliorating effects of MSCs in controlling treg-mediated B-Cell depletion by Indoleamine 2, 3-dioxygenase induction in PBMC of SLE patients. *Open Access Macedonian Journal of Medical Sciences* 2022; **10**, 6-11.

[30] K Sharma, N Mahato and YR Lee. Extraction, characterization and biological activity of citrus flavonoids. *Reviews in Chemical Engineering* 2019; **35(2)**, 265-284.

[31] A Kottaiswamy, A Kizhakeyil, AM Padmanaban, FB Mirza, VR Vijay, PS Lee, NK Verma, P Kalaiselvan and S Samuel. The Citrus flavanone hesperetin induces apoptosis in CTCL Cells via STAT3/Notch1/NFκB-Mediated signaling axis.

Anti-Cancer Agents in Medicinal Chemistry 2020; **20(12)**, 1459-1468.

[32] J Lim, B Kim, D Ryu, TW Kim, G Park and C Min. The therapeutic efficacy of mesenchymal stromal cells on experimental colitis was improved by the IFN-γ and poly (I:C) priming through promoting the expression of indoleamine 2,3-dioxygenase. *Stem Cell Research & Therapy* 2021; **12**, 37.

[33] FK Hartati, SB Widjanarko, TD Widyaningsih and M Rifa'i. Anti-Inflammatory evaluation of black rice extract inhibits TNF-α, IFN-γ and IL-6 cytokines produced by immunocompetent cells. *Food and Agricultural Immunology* 2017; **28(6)**, 1116-1125.

[34] CK Rahardja, SP Mulyani and A Putra. Secretome of hypoxic mesenchymal stem cells improves fluconazole-induced alopecia in rats via immunoregulatory modulation of IL-15 and IFN-γ. *Journal of Biology & Biology Education* 2024; **16(1)**, 23-36.

[35] SR Bailey, MH Nelson, RA Himes, Z Li, S Mehrotra and CM Paulos. Th17 cells in cancer: The ultimate identity crisis. *Frontiers in Immunology* 2014; **5**, 1-13.

[36] J Tabarkiewicz, K Pogoda, A Karczmarczyk, P Pozarowski and K Giannopoulos. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. *Archivum Immunologiae et Therapiae Experimentalis* 2015; **63**, 435-449.

[37] AN Kamali, SM Noorbakhsh, H Hamedifar, F Jadidi-Niaragh, R Yazdani, JM Bautista and G Azizi. A role for Th1-like Th17 cells in the pathogenesis of inflammatory and autoimmune disorders. *Molecular Immunology* 2019; **105**, 107-115.

[38] R Ginwala, R Bhavsar, DGI Chigbu, P Jain and ZK Khan. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. *Antioxidants* 2019; **8(2)**, 35.

[39] CS Ma and TG Phan. Here, there and everywhere: T follicular helper cells on the move. *Immunology* 2017; **152(3)**, 382-387.

[40] AG Zaretsky, C Konradt, F Depis, JB Wing, DG Atria, JS Silver, S Cho, AI Wolf, J Quinn, JB Engiles, DC Brown, D Beiting, J Erikson, MP Cancro, S Sakaguchi, L Lu, CO Benoist and CA Hunter. T regulatory cells support plasma cell populations in the bone marrow. *Cell Reports* 2017; **18(8)**, 1906-1916.

[41] S Veenbergen, P Li, HC Raatgeep, DJ Lindenbergh-Kortleve, Y Simons-Oosterhuis, A Farrel, LMM Costes, ME Joosse, LAV Berkel, LFD Ruiter, MA Leeuwen, D Winter, SM Holland, AF Freeman, Y Wakabayashi, J Zhu, LD Ridder, GJ Driessen, JC Escher, WJ Leonard and JN Samsom. IL-10 signaling in dendritic cells controls IL-1β-mediated IFNγ secretion by human CD4+ T cells: Relevance to inflammatory bowel disease. *Mucosal Immunology* 2019; **12(5)**, 1201-1211.

[42] D Schmidt-Arras and S Rose-John. Endosomes as signaling platforms for IL-6 family cytokine receptors. *Frontiers in Cell and Developmental Biology* 2021; **9**, 688314.

[43] L Xie, W Gong, J Chen, H Xie, M Wang, X Yin and W Wu. The flavonoid kurarinone inhibits clinical progression of EAE through inhibiting Th1 and Th17 cell differentiation and proliferation. *International Immunopharmacology* 2018; **62**, 227-236.

[44] JM Al-Khayri, GR Sahana, P Nagella, BV Joseph, FM Alessa and MQ Al-Mssallem. Flavonoids as potential Anti-inflammatory molecules: A review. *Molecules* 2022; **279(90)**, 2901.

[45] K Hirahara and T Nakayama. CD4+ T-cell subsets in inflammatory diseases: Beyond the Th1/Th2 paradigm. *International Immunology* 2016; **28**, 163-171.

[46] DM Darlan, D Munir, A Putra and NK Jusuf. MSCs-released TGFβ1 generate CD4+CD25+Foxp3+ in T-reg cells of human SLE PBMC. *Journal of the Formosan Medical Association* 2021; **120(1)**, 602-608.

[47] L Han, Q Fu, C Deng, L Luo, T Xiang and H Zhao. Immunomodulatory potential of flavonoids for the treatment of autoimmune diseases and tumour. *Scandinavian Journal of Immunology* 2022; **95(1)**, e13106.