Indian Journal of Experimental Biology
Vol. 64, January 2026, pp. 62-67
DOI: 10.56042/ijeb.v64i01.20840

Inhibitory effect of ethanolic extracts of *Sargassum confusum* C. Agardh on atopic dermatitis in DNCB-stimulated mice

Ga Eun Woo[1], Min Ji Kim[1], Mi Jeong Jo[2,3], Sook Young Lee[4] & Dong Hyun Ahn[1]*

[1]Department of Food Science and Technology, Pukyong National University, Busan 48513, Republic of Korea

[2]Industry-University Cooperation Foundation, Pukyong National University, Busan 48513, Republic of Korea

[3]Department of Microbiology, Pukyong National University, Busan 48513, Republic of Korea

[4]Marine Bio Research Center, Chosun University, Wando 59146, Republic of Korea

Received 27 June 2025; revised 06 November 2025

The present study aimed to evaluate the inhibitory effects of an ethanolic extract of *Sargassum confusum* (SCEE) on acute atopic dermatitis (AD) using a murine model. AD-like symptoms were induced in BALB/c mice by repeated application of 2,4-dinitrochlorobenzene (DNCB), followed by topical administration of SCEE. Clinical evaluation revealed that SCEE treatment significantly reduced dermatitis severity scores and white blood cell counts. Moreover, the levels of pro-inflammatory markers such as TNF-α, total IgE, and Th2 cytokines (IL-4 and IL-5) were markedly suppressed, while the anti-inflammatory cytokines IL-10 and interferon-γ were significantly elevated in both serum and splenocytes. These results suggest that SCEE may have therapeutic potential as a natural functional material for the prevention or treatment of atopic dermatitis through modulation of immune responses.

Keywords: *Sargassum*, Atopic dermatitis, T lymphocytes, 2,4- dinitrochlorobenzene (DNCB), Mouse model

It is known that immune imbalance from exogenous factors such as diet, chemicals and environmental pollution, and destruction of the acquired skin barrier are main causes of atopic dermatitis (AD)[1]. AD, a multifactorial disease, is a common skin disease that affects about 30% of children and adults worldwide, and the prevalence rate is continuously increasing[2]. Clinical symptoms of AD include pruritus, psoriasis, eczema, and histopathologic symptoms like excessive infiltration of inflammatory cells such as lymphocytes, macrophages, activated mast cells, causing chronic inflammation[3,4]. Acute AD is characterised by Th2 cell predominance and an environment rich in Th2 cytokines such as interleukin (IL)-4 and IL-13, excessive immunoglobulin E (IgE) production, and peripheral eosinophilia. Th2 cytokines are overproduced in skin lesions due to antigenic invasion, and increased IgE stimulates and activates B cells. This also causes mast cell degranulation and release of inflammatory mediators such as the chemicals histamine and heparin. They further contribute to the Th2 response by producing cytokines such as IL-4 and IL-13[5-7]. This process causes an imbalance of Th1/Th2 cells in AD, and skin lesions have a Th2 cell-dominant environment. On the other hand, IFN-γ, a Th1 cytokine, acts as a potent inhibitor of IgE synthesis, Th2 cell proliferation, and IL-4 receptor expression in Th2 cells in acute AD lesions. In addition, IL-10 cytokine produced by regulatory T (Treg) cells is known to play an important role in regulating the immune response associated with atopic disease.

Recently, synthetic drugs such as immunosuppressants and anti-inflammatory drugs are used as therapeutic agents for AD. However, synthetic drugs are difficult to use for a long period of time since many expensive drugs, and external steroids have a symptomatic effect, but have clinical limitations due to various side effects such as skin atrophy and growth retardation when used for a long time[8-10]. Therefore, it is necessary to search for safe and effective natural materials to minimize side effects and treat AD. Sargassum, a type of brown algae (Phaeophyceae) belonging to the Sargassaceae family, are known to have various pharmacological properties and ar e a rich source of antioxidant, antiviral, antibacterial, and anticancer effects. It also includes health maintenance substances such as

*Correspondence:
Phone:+82 51 629 5831
E-mail: dhahn@pknu.ac.kr

polysaccharides and polyphenols. *Sargassum confusum* C. Agardh (*S. confusum*), belonging to the brown algae family, grows widely in China, Sakhalin, and the east and south coasts of Korea, and has been consumed by Korean coastal residents as an appetite stimulant since ancient times[11-13]. Studies conducted to date using *S. confusum* include the effect of Fucoidan in suppressing UVB-induced changes in the skin barrier of human keratinocytes, the anticancer effect of polysaccharide, and the anti-obesity and body fat reduction effects, but there is still minimal research using the extract of *S. confusum*. Therefore, this study aimed to confirm the anti-atopic activity of *S.confusum* ethanol extracts, explore data for the development of treatments for atopic diseases, and verify the applicability of these extracts as functional material.

Materials and Methods

Reagent

Fetal Bovine Serum (FBS), penicillin/streptomycin were purchased from Hyclone (UT, USA), RPMI 1640 was purchased from Gibco BRL (Eggenstein, Germany). The olive oil, 2,4- Dinitro chlorobenzene (DNCB), dexamethasone (DEX), and MTT were purchased from Sigma (MO, USA). Mouse ELISA kits were used by BD Bioscience (CA, USA).

Preparation of ethanolic extracts of *S. confusum* (SCEE)

S. confusum collected from Yeonhwa-ri, Gijang, Busan was washed with fresh water, freeze-dried and grinded. The *S. confusum* powder was suspended in 95% ethanol and extracted for 24 hours. The supernatant was taken by centrifugation, and the residue was additionally extracted twice. The supernatant was concentrated with a vacuum concentrator and dried at room temperature.

Animals

A 5-week-old male, BALB/c mouse was purchased from Orient Bio (Orient Co., Gyeong-gi, Korea). The mice were used in the experiment after undergoing a preliminary breeding period for one week in an animal breeding room where a temperature of 20±2℃, humidity of 50±10%, and a 12-hour light/dark cycle was maintained.

Atopic dermatitis induction and SCEE treatment

The mice were divided into 4 groups (n=5): Non-treat, DNCB-only, DNCB with DEX, and DNCB with SCEE groups. The back of a 6-week-old male BALB/c mouse was cleanly removed and left for 24 hours to heal fine wounds and soothe the skin. 200 μL of 1% (w/v) DNCB dissolved in an acetone:olive oil (3:1) was administered a week at two-day intervals. After 1 week, 200 μL of 0.3% (w/v) DNCB solution was applied once a day, and at 12-hour intervals, DEX (0.01%) and SCEE (10, 50 mg/mL) were applied once a day. 200 μL at a time was evenly applied to the same area for 2 weeks. The mice were sacrificed 21 days after DNCB application (Fig. 1).

Visual evaluation was performed starting 0 day after application of the test substance using a method generally used for clinical visual evaluation in AD. The evaluation items were divided into lichemification, erosion, edema & excoriation, erythema, pruritus & dry skin, and the severity of AD was expressed as the total score of each of the five items. Each item was scored as none (0), mild (1), moderate (2), and severe (3) and then added to give a score ranging from a minimum of 0 to a maximum of 15 points per day. Then, the scores for each week were added to show the change in the total evaluation score over the three weeks.

Blood analysis and plasma isolation

The mice were anesthetized with diethyl ether, and approximately 1.0 mL of blood was collected from the aorta using a disposable syringe. The collected blood was shaken in an EDTA tube and then analyzed using a HEMAVET HV950 Multispecies

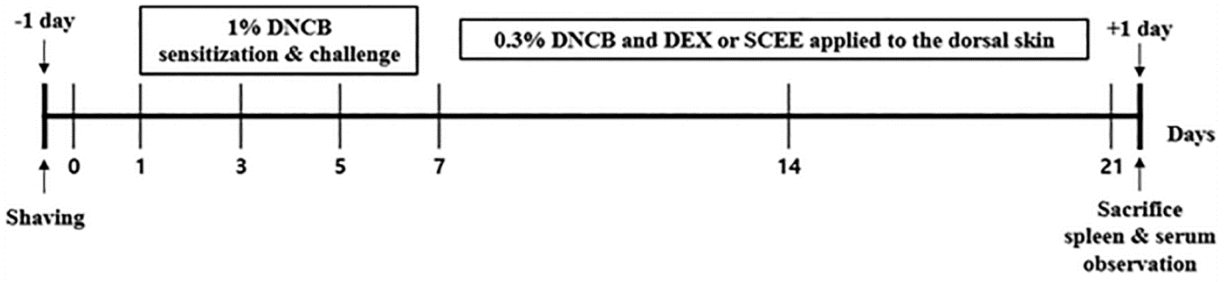

Fig. 1 — Schematic diagram of plan.

Hematologic Analyzer (Drew Scientific, CT, USA). Plasma was separated by centrifugation and used for ELISA.

Splenocyte isolation and culture

Male BALB/c mice were anesthetized and sacrificed with diethyl ether, and then the spleen was aseptically removed. The spleen was washed with 10% FBS-RPMI medium and disrupted using a tissue grinder and then passed through a cell strainer to separate only the cell suspension. RBC lysis buffer (Tris-buffered ammonium chloride; 0.87% (w/v) NH_4Cl, pH 7.2) was added on cell suspension to remove erythrocytes. After removing the RBC lysis buffer by centrifugation, each splenocytes suspension was seeded in a 48-well plate (2×10^6cells/mL) and cultured in a 5% CO_2 incubator at 37°C for 72 hours.

Measurement of cytokine secretion

The contents of cytokines (IL-4, -5, -10, IgE, TNF- α, or IFN-γ) of splenocytes and plasma were measured using an ELISA kit following the manufacturer′s instruction. Absorbance was measured at a wavelength of 490 nm using a microplate reader.

Statistical analysis

Statistical processing for all results was performed using SAS software (SAS Institute Inc., NC, USA), followed by analysis of variance using one-way ANOVA, and significance testing between survey items using Duncan's multiple range test. All data are expressed as mean ± S.D. and means between letters above bars are significantly different ($P< 0.05$).

Results

Visual evaluation of atopic dermatitis

In the first week of treatment with DNCB alone, lichenification, erythema, hematoma, and hemorrhage appeared at the application site in all experimental groups except the control group (Fig. 2A). In both groups, the DNCB with DEX or SCEE, when the test substance was continuously applied for 1 week, the dermatitis of the affected area recovered to some extent compared to the DNCB-only group. Moreover, clinical symptoms were almost completely recovered in the 3rd week when the test substance was applied continuously for 2 weeks. In addition, to confirm the severity of AD in DNCB-treated mice, the total clinical severity score was measured according to the AD clinical visual evaluation method (Fig. 2B). The increased score after treatment of DNCB was significantly reduced in the 2nd and 3rd weeks by applying DNCB and SCEE compared to the DNCB-only group.

Whole blood analysis

To determine whether the application of SCEE is involved in the production of inflammatory cells related to AD, blood collected from the aorta was analysed. As a result (Table 1) it was confirmed that total white blood cells (WBCs), including neutrophils, eosinophils, monocytes, and lymphocytes in mice blood, increased in the DNCB-only group. On the other hand, in the experimental group which SCEE was applied, it was shown that the total WBCs was decreased, and the levels of lymphocytes and eosinophils were significantly reduced as well.

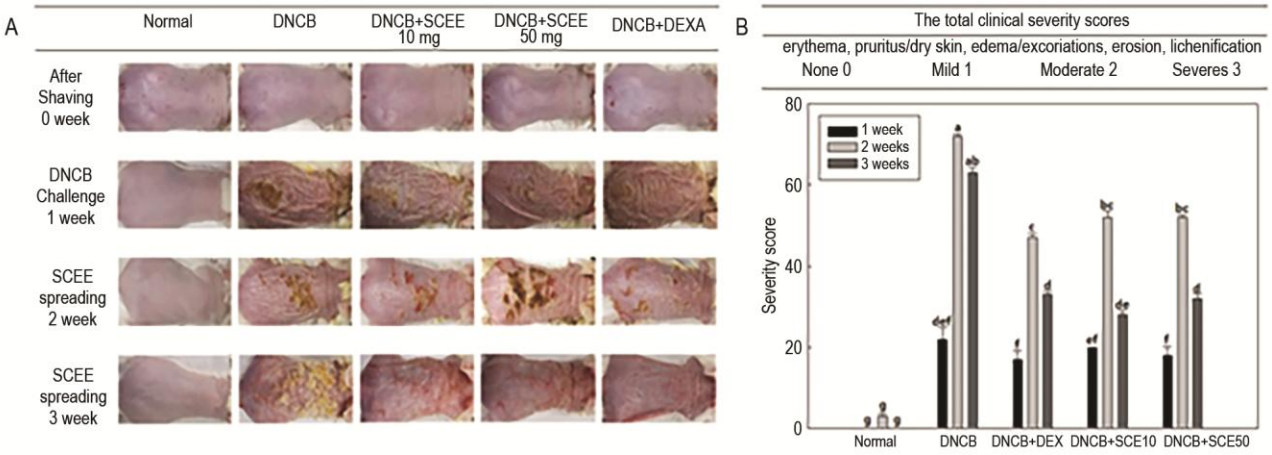

Fig. 2 — Clinical effects of SCEE on clinical skin features (A) and total clinical score (B) of DNCB-applied atopic dermatitis-like skin lesion of BALB/c mice (n=5). Normal, Negative control; DNCB, DNCB and vehicle; DNCB+DEX, DNCB and dexamethasone; DNCB+SCE10, DNCB and SCEE 10 mg/mL; DNCB+SCE50, DNCB and SCEE 50 mg/ mL.

Table 1 —*In vivo* analysis of white blood cells levels in mice whole blood

Cells	Normal range	Normal	DNCB	DNCB+DEX	DNCB+SCEE 10 mg/kg	DNCB+SCEE 50 mg/kg
WBCs	1.8-10.7	2.48 ± 0.17^{d}	7.66 ± 1.52^{a}	5.65 ± 0.94^{b}	4.47 ± 0.82^{c}	4.41 ± 0.44^{c}
Neutrophils	0.1-2.4	0.67 ± 0.24^{b}	2.5 ± 0.24^{a}	2.28 ± 0.53^{a}	1.93 ± 0.48^{a}	1.98 ± 0.67^{a}
Lymphocytes	0.9-9.3	1.73 ± 0.31^{d}	5.57 ± 0.19^{a}	2.62 ± 0.55^{c}	3.45 ± 1.07^{b}	3.23 ± 0.55^{bc}
Eosinophils	0.0-0.2	0.15 ± 0.10^{b}	0.94 ± 0.32^{a}	0.1 ± 0.00^{b}	0.07 ± 0.05^{b}	0.08 ± 0.05^{b}
Monocytes	0.0-0.2	0.32 ± 0.08^{c}	0.9 ± 0.09^{a}	0.65 ± 0.02^{b}	0.43 ± 0.15^{c}	0.83 ± 0.33^{ab}

Determination of the content of total IgE and cytokines in plasma

Compared to DNCB-only group, IgE was significantly decreased in the DNCB with SCEE groups (Fig. 3A). The level of TNF-α was significantly increased in the DNCB-only group but decreased in the DNCB with SCEE groups (Fig. 3B). In the case of IL-10, the amount of secretion was low in the DNCB-only group but the DNCB with SCEE groups were elevated (Fig. 3C).

Secretion of cytokines in splenocytes

The secretion of IL-4 was significantly increased in the DNCB-only group, but it was reduced in the DNCB with SCEE groups (Fig. 4A). The IL-5 was significantly increased in the DNCB-only group, but respectively decreased in the DCNB with SCEE groups (Fig. 4B). Moreover, a lower secretion amount of IFN-γ was confirmed in the DNCB-only group, but it was significantly higher in the DNCB with SCEE groups (Fig. 4C).

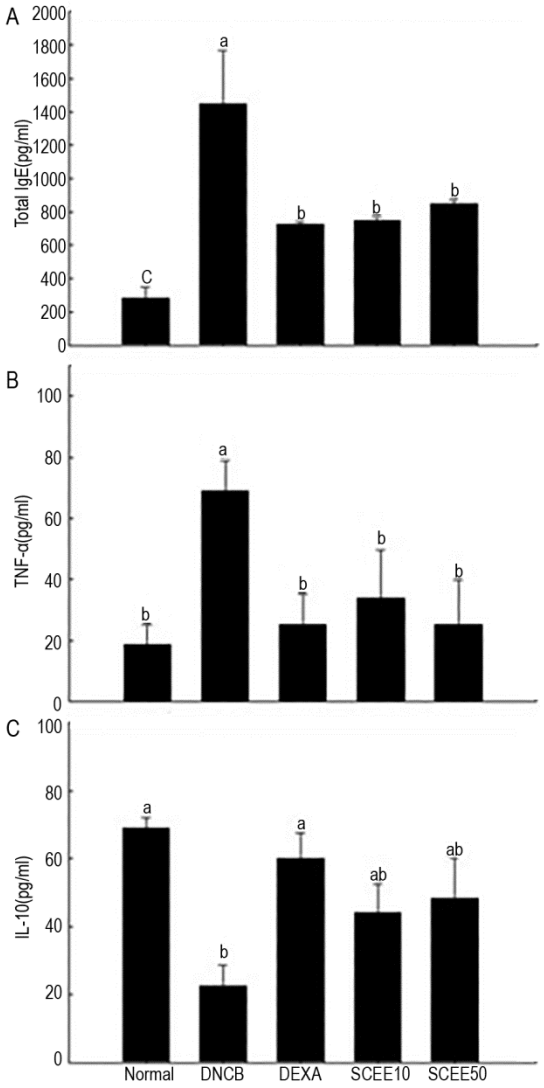

Fig. 3 —*In vivo* regulatory effect of SCEE on the production of total IgE (A), TNF-α (B), and IL-10 (C) in SCEE and DNCB-applied BALB/c mice plasma (n=5).

Discussion

Activation of $CD4^{+}$ T cells results in the generation of two distinct subsets: Th1 and Th2 cells[14]. Th1 cells are responsible for the production of IL-2 and IFN-γ, while Th2 cells produce IL-4, 5, 6, and 13[15]. In atopic dermatitis (AD), memory Th2 cells targeting specific tissues induce the migration and infiltration of T lymphocytes expressing skin-homing receptors, thereby promoting skin inflammation and lesion development[16]. IL-4, a representative cytokine of Th2 cells, plays a central role in B cell proliferation and differentiation, ultimately increasing IgE production[17,18]. Elevated levels of IgE subsequently activate mast cells, contributing to the exacerbation of AD symptoms. Moreover, IL-5 is closely associated with eosinophilia, and increased eosinophils levels are linked to further IgE production and symptom aggravation[19].

Clinically, AD is characterised by severe itching, epidermal desquamation, erythema, and psoriasis lesions, which often follow a pattern of chronic relapse show chronic relapse[20]. Clinical visual scoring is a commonly used and objective method to evaluate the severity of AD. In this study, we observed that the clinical score in mice were reduced following the application of SCEE, a result consistent with previous findings where natural products such as tuna heart ethanol extract, chamomile, lavender, and sandalwood oil improved AD symptoms in murine models.

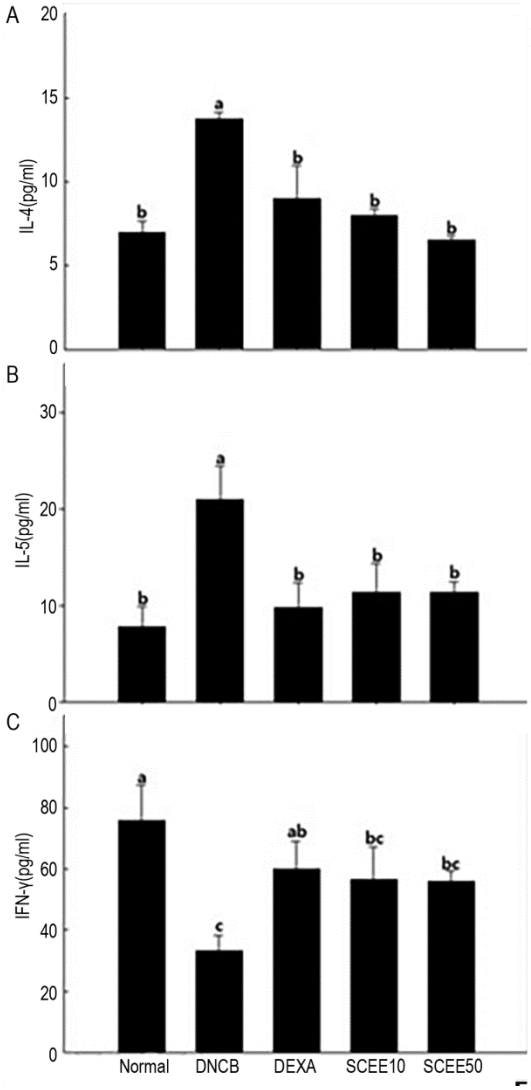

Fig. 4 —*In vivo* regulatory effect of SCEE on the production of total IL-4 (A), IL-5 (B), IFN-γ (C) in SCEE and DNCB-applied BALB/c mice splenocytes (n=5).

In atopic skin lesions, Th2 cells activation leads to the recruitment and infiltration of inflammatory cells. Monocytes, which circulate in the bloodstream, migrate to sites of inflammation and differentiate into macrophages capable of phagocytosis[21]. Neutrophils, the most abundant white blood cells, play a pivotal role in mediating tissue destruction in inflammatory conditions. Eosinophils, which are elevated in type 1 allergic reactions including AD, are correlated with disease severity and IgE levels. In our study, administration of SCEE significantly reduced total white blood cell counts, particularly neutrophils and eosinophils, suggesting an anti-inflammatory effect that may underlie the observed clinical improvement [22].

Following sensitisation to specific antigens, patients with AD exhibit heightened activation of various immune cells, including macrophages, B cells, and keratinocytes. B cells are particularly elevated in AD and are responsible for the increased levels of both specific and total IgE detected in patients′ blood[20]. IgE binds to mast cells, triggering histamine release via an IgE-dependent mechanism. Histamine contributes to intense itching, and persistent scratching can cause skin damage and secondary infection, exacerbating inflammation[18]. Keratinocytes further amplify inflammation by producing pro-inflammatory cytokines such as IL-4, IL-6, and TNF-α. Among these, TNF-α promotes the expression of Th2 cytokines, cell proliferation, and necrosis during early AD, and facilitates the recruitment of neutrophils to lesion sites. Conversely, IL-10, a potent anti-inflammatory cytokine secreted by Treg cells and lymphocytes, suppress the production of IgE, Th2 cytokine, and TNF-α [23]. Our findings suggest that SCEE ameliorates AD symptoms by reducing the levels of pro-inflammatory cytokines and IgE while enhancing IL-10 expression in serum.

The cells are differentiated into Th1 and Th2 subsets, which produce IFN-γ/IL-2 and IL-4/IL-5, respectively. IFN-γ, predominantly secreted by Th1 cells, regulates delayed-type hypersensitivity reactions, whereas IL-4 promotes class switching to the IgE isotype by stimulating B cells[20]. In acute AD, an imbalance is observed where Il-4, IL-5, and IL-13 are upregulated, and IFN-γ is markedly decreased[24]. This Th2-biased cytokine profile is implicated in enhanced IgE production. Previous studies on hydrolyzed celery extract and Gracillin have demonstrated that increased Th2 cytokine production is positively correlated with elevated serum IgE levels[25]. IL-5 also contributes to AD severity by inducing eosinophilia and stimulating histamine secretion by basophils. Conversely, IFN-γ plays an immunomodulatory role by interfering with IL-4-derived class switching and suppressing Th2 cell proliferation. Elevated IFN-γ levels are therefore associated with symptom alleviation in AD, reinforcing the importance of restoring Th1/Th2 balance.

Taken together, our results demonstrate that *S. confusum* ethanolic extract (SCEE) effectively alleviates atopic dermatitis symptoms in a murine model by modulating key immunological parameters. SCEE reduced the infiltration of inflammatory cells

such as eosinophils and neutrophils, suppressed pro-inflammatory cytokines including IL-4, IL-5, and TNF-α, and elevated anti-inflammatory cytokines such as IL-10 and IFN-γ. These results suggest that SCEE ameliorates AD through restoration of Th1/Th2 balance and downregulation of IgE-mediated immune response. Given its efficacy and natural origin, SCEE has strong potential as a functional material for the development of alternative therapeutics for atopic dermatitis.

Ethical statement

Animal experimentation was conducted with the approval of the Animal Experimentation Ethics Committee and complied with the Pukyong National University Animal Experimentation Ethics Guidelines (PKNUIACUC-2022-14).

Acknowledgment

This research was supported by Korea Institute of Marine Science & Technology Promotion (KIMST) funded by the Ministry of Oceans and Fisheries (202106567).

Conflict of interest

The authors have no financial conflicts of interest to declare.

References

1 Ständer S, Luger T, Kim B, Lerner E, Metz M, Adiri R, Canosa JM, Cha A & Yosipovitch G. Cutaneous components leading to pruritus, pain, and neurosensitivity in atopic dermatitis: a narrative review. *Dermatol Ther*, 14 (2024) 45.

2 Das P, Mounika P, Yellurkar ML, Prasanna VS, Sarkar S, Velayutham R & Arumugam S. Keratinocytes: An enigmatic factor in atopic dermatitis. *Cells*, 11 (2022) 1683.

3 Kim AR, Kim MJ, Seo J, Moon KM & Lee B. The beneficial roles of seaweed in atopic dermatitis. *Mar Drugs*, 22 (2024) 566.

4 Mihindukulasooriya SP, Kim HJ, Cho J, Herath KH, Yang J, Dinh DT, Ko MO, Jeon YJ, Ahn G & Jee Y. Polyphenol-rich Sargassum horneri alleviates atopic dermatitis-like skin lesions in NC/Nga mice by suppressing Th2-mediated cytokine IL-13. *Algae*, 37 (2022) 331.

5 Zheng R, Ren Y, Liu X, He C, Liu H, Wang Y, Li J, Xia S, Liu Z, Ma Y & Wang D. Exogenous drug-induced mouse models of atopic dermatitis. *Cytokine Growth Factor Rev*, 77 (2024) 104.

6 Jayasinghe AMK, Kirindage KGIS, Fernando IPS, Han EJ, Oh GW, Jung WK & Ahn G. Fucoidan Isolated from Sargassum confusum suppresses inflammatory responses and oxidative stress in TNF-α/IFN-γ-stimulated HaCaT keratinocytes by activating Nrf2/HO-1 signaling pathway. *Mar Drugs*, 20 (2022) 117.

7 Tang A, Wei ML, Haemel A, La C, Sirota M & Lee EY. Artificial intelligence-enabled precision medicine for inflammatory skin diseases. *ArXiv*, (2025).

8 Han EJ, Fernando IP, Kim HS, Jeon YJ, Madusanka DM, Dias MK, Jee Y & Ahn G. Oral administration of Sargassum horneri improves HDM/DNCB-induced atopic dermatitis in NC/Nga mice. *Nutrients*, 12 (2020) 2482.

9 Mitroi GG, Pleșea EL, Mitroi GF, Mitroi MR, Neagoe CD & Ianoși SL. IL-4 and IL-13 plasma levels as biomarkers in atopic dermatitis. *Life*, 14 (2024) 352.

10 Li H, Zhang Z, Zhang H, Guo Y & Yao Z. Update on the pathogenesis and therapy of atopic dermatitis. *Clin Rev Allergy Immunol*, 61 (2021) 324.

11 Oh SJ, Joung EJ, Kwon MS, Lee B, Utsuki T, Oh CW &Kim HR. Anti-Inflammatory Effect of Ethanolic Extract of Sargassum serratifolium in Lipopolysaccharide-Stimulated BV2 Microglial Cells. *J Med Food*, 19 (2016) 1023.

12 Suleman M, Sayaf AM, Moltrasio C, Tricarico PM, Giambuzzi F, Rimondi E, Melloni E, Secchiero P, Marcuzzi A, Marzano AV & Crovella S. Phytocompounds in Precision Dermatology: COX-2 Inhibitors as a Therapeutic Target in Atopic-Prone Skin. *Biomolecules*, 15 (2025) 998.

13 Kim AR, Kim MJ, Seo J, Moon KM & Lee B. The beneficial roles of seaweed in atopic dermatitis. *Mar Drugs*, 22 (2024) 566.

14 Bieber T. Atopic dermatitis: An expanding therapeutic pipeline for a complex disease. *Nat Rev Drug Discov*, 21 (2022) 21.

15 Kim J, Kim BE & Leung DYM. Pathophysiology of atopic dermatitis: Clinical implications. *Allergy Asthma Proc*, 40 (2019) 84.

16 Samat AA, van der Geest J, Vastert SJ, van Loosdregt J & van Wijk F. Tissue–Resident Memory T Cells in Chronic Inflammation—Local Cells with Systemic Effects? *Cells*, 10 (2021) 409.

17 Lin YJ, Goretzki A & Schülke S. Immune metabolism of IL-4-activated B cells and Th2 cells in the context of allergic diseases. *Front Immunol*, 12 (2021) 790658.

18 Ogulur I, Mitamura Y, Yazici D, Pat Y, Ardicli S, Li M, D'Avino P, Beha C, Babayev H, Zhao B, Zeyneloglu C, Viscardi OG, Ardicli O, Kiykim A, Garcia-Sanchez A, Lopez JF, Shi LL, Yang M, Schneider SR, Dhir R, Radzikowska U, Nadeau KC & Akdis CA. Type 2 immunity in allergic diseases. *Cell Mol Immunol*, 22 (2025) 211.

19 Mitroi GG, Pleșea EL, Mitroi GF, Mitroi MR, Neagoe CD & Ianoși SL. Exploring the potential of IL-4 and IL-13 plasma levels as biomarkers in atopic dermatitis. *Life*, 14 (2024) 352.

20 Ruimin B, Yan Z & Xiaofeng D. Atopic dermatitis: diagnosis, pathogenesis, and therapeutic insights. *Mol Biomed*, 6 (2025) 71.

21 Christopoulos PF, Gjølberg TT, Krüger S, Haraldsen G, Andersen JT & Sundlisæter E. Targeting the notch signaling pathway in chronic inflammatory diseases. *Front Immunol*, 12 (2021) 668207.

22 Li W & Yosipovitch G. The role of the microbiome and microbiome-derived metabolites in atopic dermatitis and non-histaminergic itch. *Am J Clin Dermatol*, 21 (2022) 44.

23 Kim AR, Kim MJ, Seo J, Moon KM & Lee B. The beneficial roles of seaweed in atopic dermatitis. *Mar Drugs*, 22 (2024) 566.

24 Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J Allergy Clin Immunol*, 113 (2004) 395.

25 Jegal J, Park NJ, Jo BG, Bong SK, Jegal H, Yang MH & Kim SN. Anti-Atopic properties of gracillin isolated from Dioscorea quinqueloba on 2, 4-dinitrochlorobenzene-induced skin lesions in mice. *Nutrients*, 10 (2018) 1205.