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Research article

Screening of aqueous plant extracts for immunomodulatory effects on immune cells and cytokine production: *In vitro* and *in vivo* analyses

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ABSTRACT

This study investigates the immunomodulatory effects of various aqueous plant extracts on immune cells and cytokine production. *In vitro*, several extracts, including holy basil (*Ocimum sanctum*), patawali (*Tinospora crispa*), and Indian borage (*Plectranthus amboinicus* L.), significantly increased $CD3^+$ T-cell populations, while soap pod (*Acacia concinna*), garlic (*Allium sativum* L.), and neem (*Azadirachta indica*) also boosted $CD45RA^+$ B-cells. *In vivo*, the extracts had subtle effects on spleen morphology and Peyer's Patches, with milk bush (*Euphorbia tirucalli* L.) and Indian borage enhancing T-cell responses, while soap pod stimulated B-cells. Additionally, we observed that Neem and milk bush significantly suppressed B-cell populations. Furthermore, cytokine analysis showed that garlic and patawali reduced IL-2, while soap pod, holy basil, and patawali increased TNF-alpha levels. Soap pod also elevated IL-10 and IL-17A, indicating both anti-inflammatory and pro-inflammatory signaling, while patawali induced an increase in IL-4. In conclusion, Thai medicinal plants show strong potential as both immunostimulants and immunosuppressants. They can enhance lymphocyte proliferation, particularly in T-cells, and modulate B-cell populations. Their aqueous extracts play a key role in regulating Th1, Th2, and Th17 cytokine production. Thus, these plants could serve as natural agents and alternative medicines for boosting or modulating immune function.

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1. Introduction

Plant products are widely used as an alternative and complementary medicine to conventional health care worldwide. The various organic compounds produced by plants, also known as secondary metabolites, are often unique to a plant and have specific functions in each species [1]. Nowadays, secondary metabolites have become the main bioactive compounds that are always used for alternative medicines such as alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes, and terpenoids [2,3]. However, these compounds are synthesized in specific parts of the plant to protect the plant from pathogenic or responding environmental stimuli [4].

Plants have been central to traditional medicine for centuries, offering a natural remedy for a variety of health issues. Many plants have played a significant role in traditional medicine. Holy Basil (*O. sanctum*), Patawali (*T. cordifolia*), and Neem (*A. indica*) are well-known for their immune-boosting and anti-inflammatory properties. Holy Basil has been shown to enhance immunity and reduce inflammation, playing a significant role in stress management [5–7]. Additionally, Moringa (*M. oleifera*), Emblic (*P. emblica*), and Indian Borage (*P. amboinicus*) are commonly used for digestive support and overall wellness [8,9,10]. On the other hand, Soap Pod (*H. indicus*), Milk Bush (*E. tirucalli*), and Garlic (*A. sativum*) are valued for their therapeutic effects on skin health and joint support, being traditionally used to treat skin ailments, purify the blood, and support joint health [11,12,13].

Regarding the immunomodulatory properties, the bioactive components of plants have been reported to play a crucial role in different functions of immunostimulants and immuno-suppressants. Many dominant compounds have an immunomodulatory potential including polysaccharides, glycoproteins, fatty acids, phenolics, alkaloids, saponins, terpenoids, and lectin [14,15]. Polysaccharides (including glucans, mannans, pectins, fucoidans, galactans, fructans, and xylans), are major components derived from plants, exhibit significant immunostimulatory effects on both innate immune cells (by promoting cytokine and chemokine secretion in macrophages) and adaptive immune cells (by activating natural killer cells, T lymphocytes, and B lymphocytes) [16,17]. It binds to pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), initiating NF-κB and MAPK signaling cascades that increase cytokine and chemokine production, thereby enhancing immune cell activation [18]. Lectins enhance immune function by promoting antigen recognition and T-cell activation through T-cell receptor cross-linking, playing a key role in immune cell activation and cytokine release [19]. Moreover, saponins can stimulate macrophage activity and enhance antigen presentation, thereby strengthening immune responses and acting as adjuvants in vaccines [20]. Additionally, some secondary metabolites demonstrate immunosuppressive effects by suppressing inflammation and reducing cytokines such as IL-6 and TNF-α [15]. Phenolic compounds, especially flavonoids, reduce oxidative stress and inflammation (such as IL-1β, IL-6, TNF-α, and IL-8) by activating NF-E2-related factor-2 (NRF2) and inhibiting cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) expression, thus protecting immune cells from damage [21]. Tannins also exert anti-inflammatory effects by modulating NF-κB and inhibiting COX-2 and 5-lipoxygenase [22]. Collectively, these metabolites highlight their potential in managing immunomodulation through either immune stimulation or suppression. However, the immunomodulatory properties of these molecules depend on factors such as conformation, molecular weight, functional groups, and branching characteristics [16].

In previous studies, the immune properties of plant extracts were reported to modulate immune cell responses and cytokine production. Sriwanthana et al. (2007) demonstrated that some Thai herbs, including *Cleistocalyx nervosum* var. *paniala*, *Gynostemma pentaphyllum*, *Gynura procumbens*, *Houttuynia cordata*, *Hyptis suaveolens*, *Portulaca grandiflora*, *Phytolacca americana*, and *Tradescantia spathacea*, can promote human lymphocyte proliferation. They also found that the activity of *C. nervosum* and *H. suaveolen* extract can significantly enhance NK cell activity [23]. Moreover, the stem extract of *Morinda citrifolia* L. has an immunomodulatory effect that promotes the level of CD4/CD3 cells and elevates the level of interleukine-2 (IL-2) and interferon-gamma (IFN-gamma) [24]. Genç and Çelik (2024) found that *Eremurus spectabilis* extracts influenced immune responses in rats with hepatocellular carcinoma (HCC). Specifically, there was a significant reduction in $CD3^+$ and $CD8^+$ T lymphocytes in the CLPLE1 group (50 mg of *E. spectabilis* extract), and a decrease in $CD4^+$ T lymphocytes in the CNPLE2 group (100 mg of *E. spectabilis* extract) [25]. Kulma et al. (2021) report on a randomized, placebo-controlled phase I clinical trial to evaluate the immunomodulatory effects of *Atractylodes lancea* (Thunb) in healthy Thai subjects. They found that *A. lancea* enhanced T lymphocyte and NK cell activity, boosting immune defense. It also modulated cytokine production, increasing both pro-inflammatory (TNF-α and IL-6) and anti-inflammatory (IL-10) cytokines, suggesting a balanced immune response [26].

Thailand is a significant source of herbal agriculture, with around 10,000 plant species recorded [27,28]. Thai herbs contain valuable pharmaceutical substances, and 2187 plant species are used in traditional medicine [29]. Various plant parts, such as leaves,

Table 1
List of Thai local plants used in this study.

Local name	Scientific name	Family	Plant part
1. Holy basil	*Ocimum sanctum*	Lamiaceae	Leaf
2. Moringa	*Moringa oleifera* L.	Moringaceae	Leaf
3. Soap pod	*Acacia concinna*	Fabaceae	Pod
4. Patawali	*Tinospora crispa*	Menispermaceae	Stem
5. Emblic	*Phyllanthus emblica*	Phyllanthaceae	Fruit
6. Neem	*Azadirachta indica*	Meliaceae	Leaf
7. Milk Bush	*Euphorbia tirucalli* L.	Euphorbiaceae	Aerial parts
8. Garlic	*Allium sativum* L.	Amaryllidaceae	Bulb
9. Indian borage	*Plectranthus amboinicus* L.	Lamiaceae	Leaf

stems, and roots, are utilized for medicinal or therapeutic purposes, each containing distinct phytochemical compounds [30]. Despite numerous publications on the biological activities of Thai herbs, there is limited information on their immunomodulatory properties. This study aims to systematically evaluate the immunomodulatory effects of local plants from Thailand, focusing on the proliferation of T-cells and B-cells, which are key components of the immune response. The analysis will also assess cytokine production, specifically IL-2, IL-4, IL-6, IL-10, IL-17A, TNF-α, and IFN-γ, as these cytokines play essential roles in modulating immune cell activity and regulating both pro-inflammatory and anti-inflammatory pathways. The information gathered from these analyses will be integrated to provide a comprehensive understanding of how local plant extracts influence immune responses.

2. Methods

2.1. Materials

Fresh plant materials were purchased from markets in Thailand (Table 1). Ficoll-Paque™ Plus (catalog no. 17-1440-03) was purchased from GE Healthcare Life Sciences (United States). RPMI 1640 medium (catalog no. 11875093), Fetal Bovine Serum (FBS) (catalog no. A5670701), L-glutamine (Gibco) (catalog no. 25030081), and penicillin/streptomycin (Gibco) (catalog no. 15140122) were purchased from Thermo Fisher Scientific (US). Monoclonal antibodies (FITC anti-rat CD3 (catalog no. 201412) and PE/Cyanine7 anti-rat CD45RA (catalog no. 202313) were purchased from BioLegend, Inc. (United States). SUPER-X Plex™ (catalog no. RMX170T) was purchased from Antigenix America Inc. (United States). Materials and equipment were provided by the faculty of pharmaceutical sciences, Khon Kaen University, Thailand.

2.2. Plant extraction

All plants were cleaned and dried at 65 °C in a hot air oven for 48 h. The dried plant was homogenized by a blender and sifted through a mesh sieve (1 mm stainless steel mesh) to make fine powders. The aqueous extraction method was modified from Sawadogo/Ilboudo et al. (2022) [31]. Briefly, powdered plant samples were extracted by dissolving 30 g of plant powder in 300 mL of distilled water. The extraction processes were conducted in an incubator shaker set to 700 rpm at 37 °C for 48 h. The crude extract was then centrifuged at 7500 rpm at 25 °C for 15 min to collect the supernatant. Finally, it was filtered with Whatman No. 1 paper and subjected to freeze-drying.

2.3. In vitro study

2.3.1. Blood collection and peripheral blood mononuclear cell (PMBC) isolation

Fresh blood was collected from the right ventricle of rats under anesthesia and kept in the EDTA tube. For isolating the PBMCs, it was equally diluted with phosphate-buffered saline (PBS) before being isolated with Ficoll-Paque™ following the manufacturer's instructions. The red blood cells were then removed with red blood cell lysis buffer (RBC lysis buffer) by incubating at 37 °C for 8 min, followed by rinsing twice with PBS. The isolated PMBCs were kept in RPMI complete medium (supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin/streptomycin, and 2 mM L-Glutamine) for further use.

2.3.2. Determination of lymphocyte proliferation

All freeze-dried powder samples were prepared for three concentrations (0.01, 0.1, and 1 mg/mL) by diluting with complete medium RPMI-1640. Each well of 96 well plates contained 100 μL PBMCs of 5×10^5 cells and 100 μL of varying final concentrations (0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL) of each plant extract sample. After 48 h of incubation at 37 °C with 5 % CO_2, PBMCs were washed with PBS and stained with monoclonal antibodies, including anti-rat CD45RA-APC, B-cells, and anti-rat CD3-PE; T-cells, following manufacturers-recommended. It was incubated at 37 °C in total darkness for 30 min and washed twice with PBS to remove unbound antibodies. Finally, it was re-suspended with 250 μL of staining buffer and subjected to measuring with an Attune NxT Flow Cytometer (Thermo Fisher Scientific, USA). The lymphocyte subpopulations were gated and analyzed with the FlowJo software (Version 10.8, TreeStar Inc., OR, USA).

2.4. In vivo study

2.4.1. Animals

The Institutional Animal Care and Use Committee of Khon Kaen University (IACUC KKU) reviewed and approved the animal study protocol (IACUC-KKU-64/63) before initiating the animal research. Male Sparge Dawley rats (*Rattus norvegicus*), 6–8 weeks of age, were purchased from Nomura Siam International Co., Ltd (Thailand) and housed in groups of 3 rats per case at the Northeast Laboratory Animal Center, Khon Kaen University, Thailand. The pelleted food and chlorinated tap water were provided ad libitum to animals. The environmental conditions that were used for maintaining the animal were as follows: a 12:12 h light/dark cycle, room temperature at 23 ± 2 °C, and 30%–60 % relative humidity.

2.4.2. Oral administration of aqueous plant extracts

The animal equivalent dose (AED) equation was used to calculate the dosage of the plant extract samples for oral gavage administration in Sprague Dawley rats, as described by Equation (1) [32].

$$\text{AED (mg/kg)} = \text{Human dose (mg/kg)} \times K_m \text{ ratio} \quad (1)$$

The calculation was based on the recommended daily intake of herbal in humans at 300 mg/60 kg body weight or 5 mg/kg. The correction factor (K_m), which was derived from the ratio of the rat species' body weight (kg) to its surface area (m^2), is 6.2 [32]. Thus, the dosage for oral gavage administration in rats was 31 mg/kg body weight. The plant extract powers were thoroughly dissolved with 1 mL of distilled water and daily fed rats by a ball-tip needle for 14 days.

2.4.3. Sample collection

Whole blood was collected from the left ventricle of the rat during exsanguination while it was under anesthesia. For PBMC isolation, fresh blood was stored in an EDTA tube and kept at 4 °C. Another sample was placed in a non-EDTA tube for serum collection and left at room temperature for 30 min to allow coagulation. Additionally, secondary lymphoid organs, including the spleen and Peyer's patches, were harvested to assess changes in spleen length and weight, as well as the number of Peyer's patches.

2.4.4. The determination of lymphocyte subpopulations

PBMC isolation from whole blood samples was carried out following the Ficoll-Paque manufacturer's instructions. PBMCs were labeled with the specific monoclonal antibodies for lymphocyte subpopulations, anti-rat CD45RA-APC; B-cells and anti-rat CD3-PE; T-cells, for 30 min in the dark. The unbound antibodies were removed by washing twice with PBS before being determined with flow cytometric analysis using Attune NxT Flow Cytometer (Thermo Fisher Scientific, USA). The flow cytometric data analysis was carried out as mentioned above.

2.4.5. Measurement of cytokine production

The serum was collected from clotted whole blood by centrifuging at 1000×*g* for 10 min and stored at −70 °C for further experiment. This study determined the Th1/Th2/Th17 cytokine production, interleukin-2, TNF-alpha, IFN gamma, interleukin-4, interleukin-6, interleukin-10, and interleukin-17a, using SUPER-X Plex™ flow cytometry cytokine assays. Each cytokine was performed in triplicate and carried out according to the manufacturer's recommendation. In brief, 45 μL of capture bead was added in a 96-filter plate, and its residual buffer was removed, followed by adding 30 μL of assay buffer and 15 μL of serum samples. After incubating for 60 min, the plate was washed 3 times with wash buffer and 25 μL of the biotinylated antibody added, followed by incubating and washing step. Finally, 25 μL of streptavidin-PE was added and incubated for 20 min. Each well was washed and resuspended with a reading buffer before being subjected to quantify cytokine levels with the flow cytometer.

2.5. Statistical analysis

All experiments were conducted at least in triplicate, and data were expressed as mean ± standard deviation. A significant

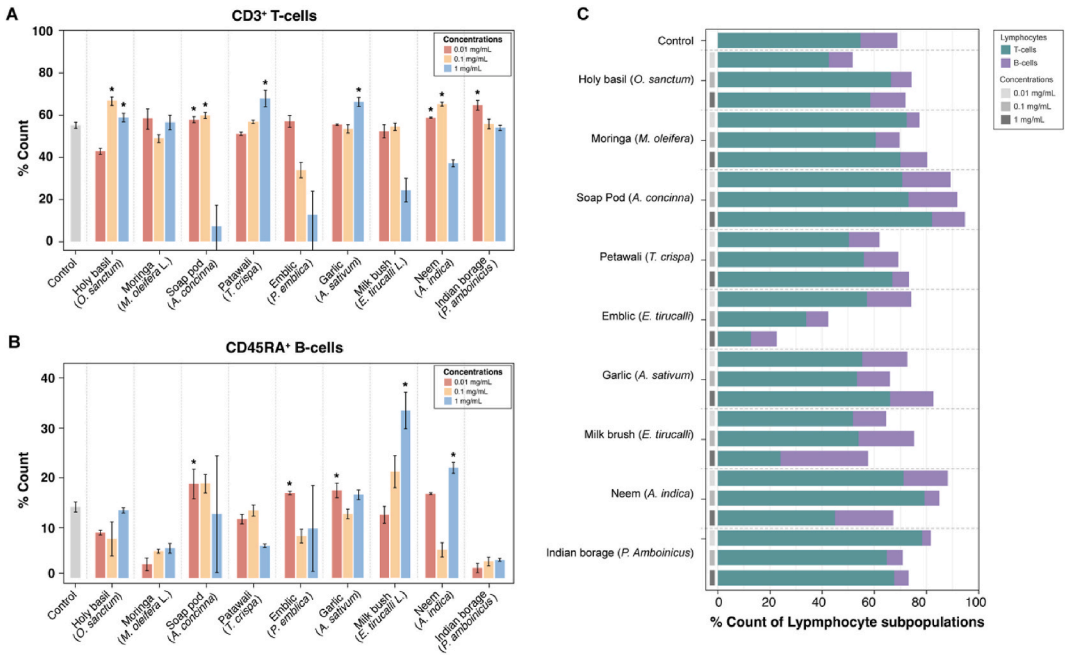

Fig. 1. The bar graphs represent the percentage of lymphocyte subpopulations: (**A**) $CD3^+$ T-cells, (**B**) $CD45RA^+$ B-cells. Significant differences compared to the control group are indicated by *; $p < 0.05$. (**C**) A stacked bar chart displays the distribution of lymphocyte subpopulations in plant extract treatments at various concentrations.

difference between the control and treatment groups was assessed using a parametric test: a one-way ANOVA, followed by Dunnett's test for multiple comparisons. The statistically significant difference between the control group and the treatment group was considered when the p-value was less than 0.05. The statistical analysis was performed using RStudio (version 2022.07.1).

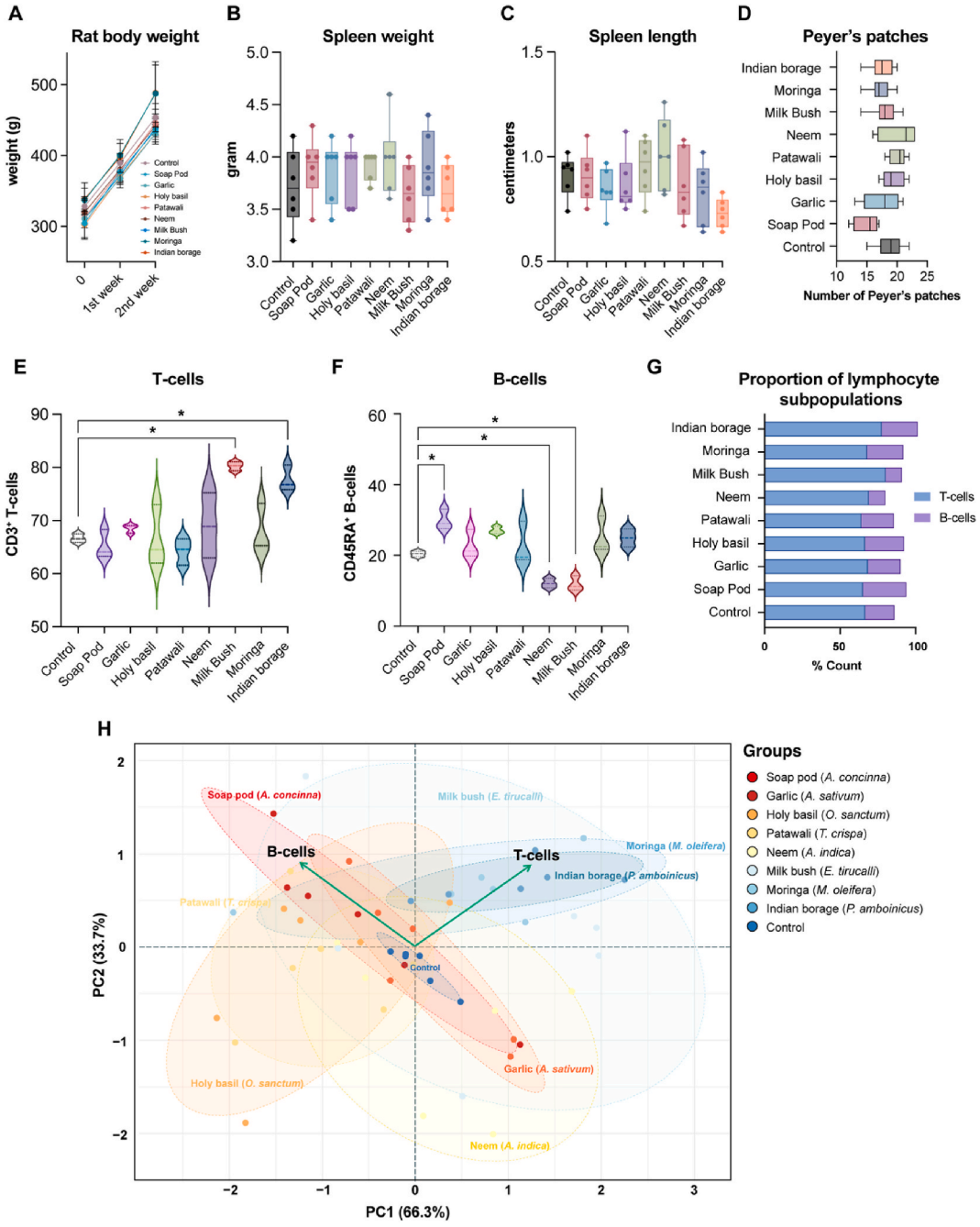

Fig. 2. Effects of various plant extracts on immunomodulatory effects in animal models. (**A**) Rat body weight was monitored over two weeks. (**B**, **C**) After two weeks, spleen weight and length were measured across treatment groups, and (**D**) the number of Peyer's patches was recorded for each group. (**E**) Violin plots show $CD3^+$ T-cell counts and (**F**) $CD45RA^+$ B-cell counts. Significant differences between groups are indicated as *; $p < 0.05$. (**G**) The proportion of T-cell and B-cell populations is represented for each group. (**H**) Principal component analysis (PCA) illustrates the clustering of groups based on their B-cell and T-cell profiles.

3. Results

3.1. In vitro lymphocyte response to aqueous plant extracts

The PBMCs were treated with aqueous plant extracts at varying concentrations for 48 h to determine the frequency of lymphocyte subpopulations. The results showed the effects of various plant extracts on the percentages of $CD3^+$ T-cells and $CD45RA^+$ B-cells at three different concentrations: 0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL. Holy basil, soap pod, patawali, garlic, neem, and Indian borage significantly increased the percentage of $CD3^+$ T-cells at different concentrations. In contrast, extracts like moringa, emblic, and milk

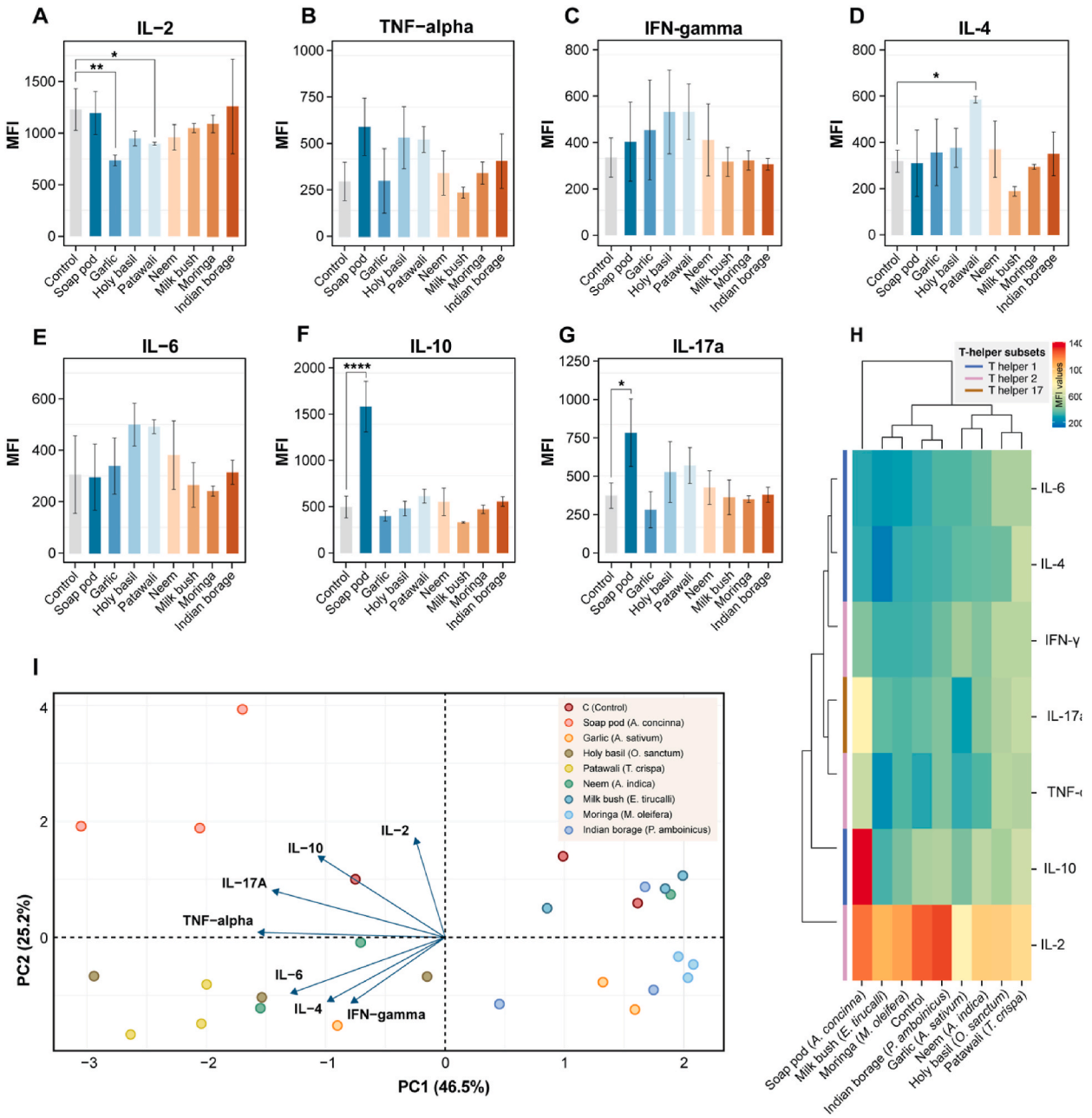

Fig. 3. Cytokine production and clustering analysis of different plant extract treatments. Bar graphs show the median fluorescence intensity (MFI) of cytokines (**A**) IL-2, (**B**) TNF-α, (**C**) IFN-γ, (**D**) IL-4, (**E**) IL-6, (**F**) IL-10, and (**G**) IL-17a across different treatment groups. Significant differences between groups are indicated (*; $p < 0.05$, **; $p < 0.01$, ****; $p < 0.0001$). (**H**) A heatmap showing hierarchical clustering of treatment groups based on cytokine expression levels, highlighting distinct cytokine profiles among treatments. (**I**) Principal component analysis (PCA) of cytokine data reveals clustering and separation of plant extract treatments based on their cytokine signatures.

bush, showed no significant differences in T-cell percentages compared to the control, the high concentration (1 mg/mL) of these treatment groups appeared to suppress the T-cell population, particularly in the soap pod and neem groups (Fig. 1A).

For $CD45RA^+$ B cells, soap pod, emblic, garlic, milk bush, and neem significantly increased B-cell percentages at concentrations of 0.01 or 1 mg/mL, but not at 0.1 mg/mL. Milk bush showed a dose-dependent effect in enhancing B-cell activity, while other extracts, including holy basil, moringa, petawali, and Indian borage, did not produce any statistically significant changes in B-cell percentages (Fig. 1B). In addition, the graph highlighted the differential influence of each plant extract on lymphocyte subpopulations, with soap pod and garlic demonstrating a more balanced modulation of both T-cells and B-cells compared to the other treatments (Fig. 1C).

3.2. In vivo immunomodulatory activity

3.2.1. The effect of aqueous plant extracts on secondary lymphoid organs

The results provided detailed insights into the physiological and immunological effects of various plant extracts on different parameters. The body weight of rats steadily increased across all groups (Fig. 2A), with no significant differences observed between the control and treatment groups at the start, first, and second weeks. Spleen weights varied slightly across the different groups, with some groups like neem and moringa showing a slight increase compared to the control (Fig. 2B). Most spleen lengths were consistent across the treatment groups; however, some plant extracts, such as patawali and neem milk bush, had a slight impact on increasing this parameter. (Fig. 2C).

The number of Peyer's patches (PPs) across different treatment groups reflected the varying effects on PPs. While there was no significant increase in the number of PPs, a slight increase was observed in the neem, patawali, and holy basil groups, whereas a notable decrease was seen in the soap pod group (Fig. 2D).

3.2.2. Effect of plant extracts on $CD3^+$ T-cell and $CD45RA^+$ B-cell populations

Milk bush and Indian borage extracts significantly increased T-cell populations compared to the control (Fig. 2E). Holy basil, neem, and moringa also elevated T-cell percentages, though these increases were not statistically significant. For B-cells, the percentage of $CD45RA^+$ cells increased notably in response to soap pod, garlic, holy basil, patawali, moringa, and Indian borage, with soap pod showing the most significant effect. In contrast, neem and milk bush caused a substantial decrease compared to the control (Fig. 2F). Other extracts, such as holy basil, patawali, milk bush, and moringa, showed only slight, non-significant increases.

The overall balance between T-cells and B-cells was modulated by all plant extracts to varying extents. Milk bush and Indian borage resulted in a higher T-cell to B-cell ratio compared to the control group, whereas garlic and patawali maintained a more balanced distribution (Fig. 2G).

3.2.3. Principal component analysis (PCA)

The principal component analysis (PCA) biplot illustrated the distribution of immune responses, specifically T-cells and B-cells, across the various treatment groups. The two principal components (PC1 and PC2) accounted for 66.3 % and 33.7 % of the total variance, respectively (Fig. 2H). The loading vectors for T-cells and B-cells revealed distinct immune modulations induced by the different plant extracts. Treatments such as soap pod and garlic were closely associated with B-cell responses, clustering along the B-cell vector. In contrast, moringa and Indian borage aligned with the T-cell vector, indicating a stronger influence on T-cell proliferation. Other treatments, including control, neem, garlic, and holy basil, were positioned closer to the origin, suggesting no significant immunostimulatory effect.

3.2.4. The effect of aqueous plant extracts on cytokine production

On day 14, Th1/Th2/Th17 cytokine production was measured in rat blood serum samples following treatment with various plant extracts. The cytokine levels were assessed using bead-based flow cytometric analysis and represented as mean fluorescence intensity (MFI) values. The results indicated that most plant extracts did not significantly affect IL-2 levels, except for garlic and patawali extracts, which led to a significant reduction ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 3A). TNF-alpha production was influenced by soap pod, holy basil, and patawali extracts, though the changes were not statistically significant ($p > 0.05$) (Fig. 3B). IFN-gamma levels slightly increased in rats treated with soap pod, garlic, holy basil, patawali, and neem extracts, but these changes were also not significant ($p > 0.05$) (Fig. 3C). Notably, patawali extract significantly enhanced IL-4 levels (Fig. 3D) and showed a trend toward increasing IL-6 and IL-17a levels ($p > 0.05$) (Fig. 3E and G). Soap pod extract demonstrated a significant ability to elevate IL-10 ($p < 0.001$) (Fig. 3F) and IL-17a levels ($p < 0.05$). However, milk bush treatment did not affect any cytokine levels (Fig. 3H).

The principal component analysis (PCA) plot (Fig. 3I) illustrates the effects of various plant extracts on cytokine production in rat serum, with PC1 and PC2 accounting for 46.5 % and 25.2 % of the variance, respectively. Soap pod, positioned on the positive side of PC2, had a strong impact, particularly in enhancing IL-10 and IL-17A levels. Garlic and patawali, aligned with IL-2 on the negative side of PC1, significantly reduced IL-2 levels. Patawali was also associated with increases in IL-4 and IL-6. Other extracts, such as holy basil, neem, and Indian borage, clustered near the origin, indicating more moderate effects. Neem, located near the center, had minimal impact on cytokine levels. Overall, some extracts showed subtle or no significant effects on cytokine production.

4. Discussion

Although numerous studies have documented the immunomodulatory effects of Thai herbs, pinpointing their exact mechanisms remains challenging. Our study aimed to further reveal the immunomodulatory properties of aqueous plant extracts through both *in*

vitro and *in vivo* assessments. For the *in vitro* evaluation of immunostimulatory responses, it is crucial to challenge peripheral blood mononuclear cells (PBMCs) with varying concentrations of plant extracts. This is because the optimal dosage for stimulating or inhibiting immune cell responses differs among extracts. T-cell mitogenic activities were observed in extracts from holy basil (*O. sanctum*), soap pod (*A. concinna*), patawali (*T. crispa*), garlic (*A. sativum*), neem (*A. indica*), and Indian borage (*P. amboinicus*). However, emblica extract was found to increase cell toxicity, particularly affecting T-cell counts as its concentration increased. Additionally, at the highest concentrations, soap pod and neem extract reduced lymphocyte subpopulations, though this effect was not seen at middle or low doses. Interestingly, these plant extracts were more effective in enhancing T-cell proliferation compared to other lymphocyte subsets. Soa pod, garlic, and neem extract not only increased T-cell numbers but also promoted B-cell proliferation. Conversely, moringa, neem, and Indian borage extracts showed immunosuppressive effects on B-cell counts. Therefore, these plant extracts demonstrated both immunostimulatory and immunosuppressive properties.

An intriguing aspect of our study was the differentiation between *in vitro* and *in vivo* immunological responses to plant-based immunomodulation. We found that the efficacy of certain local medicinal plants in cell culture did not correspond to the results observed in animal model studies. One possible reason could be the environmental differences between *in vitro* and *in vivo* conditions. The animal digestive tract is highly complex, and bioactive compounds from plant extracts must be extracted and absorbed. Factors like pH, enzymes, and chemical composition in the gastrointestinal tract could impact the degradation of bioactive compounds. Qin, Ketnawa, & Ogawa (2022) reported a decrease of about 65 %–70 % in the total polyphenol content (TPC) of green tea when using a simulated *in vitro* gastrointestinal digestion model [33]. The largest reduction, around 60 %, occurred during the intestinal phase (pH 6.8), while the gastric phase (pH 1.2) caused a 25 % reduction in TPC. As a result, plant polyphenols often have low bioavailability due to their sensitivity to gastrointestinal conditions [34]. This may explain why *in vitro* and *in vivo* results regarding immunostimulation properties did not correlate, particularly in terms of lymphocyte profiles. After 14 days of administering plant extracts to rat models, both milk bush and Indian borage extracts significantly increased the $CD3^+$ T-cell population. This finding is consistent with a study by Avelar et al. (2011), which reported that crude latex from milk bush (*E. tirucalli* L.) stimulates $CD4^+$ T-lymphocyte proliferation and induces Type I cytokine production (IFN-γ, TNF-α, and IL-10) [35]. Similarly, Ibrahim et al. (2018) found that *E. tirucalli* L. promotes the production of cytokines and chemokines such as IL-1β, IL-2, IL-6, GM-CSF, and RANTES [36]. However, our study did not detect an increase in these cytokines in blood serum samples, though we did observe a suppression of IL-4 and IL-10 levels. The immunomodulatory potential of Indian borage remains limited in the literature, particularly regarding lymphocyte responses. Akinbo et al. (2018) noted the anti-inflammatory properties of Indian borage, which decreased pro-inflammatory cytokine expression (TNF-α and IL-8) in the blood circulation of LPS-induced Wistar rats [37]. However, in our study, no changes in cytokine levels were observed following treatment with Indian borage extract. Remarkably, B-cell proliferation was noted in rats treated with holy basil extract, aligning with earlier reports by Goel et al. (2010), which found that an aqueous holy basil extract induces both T-cell and B-cell proliferation. It also stimulates inflammatory cytokines, including TNF-α, IFN-γ, and IL-6 [38]. Moringa extract similarly enhances B-cell numbers without affecting IL-6 levels, which play a role in B-cell differentiation and antibody secretion [39]. Previous research has shown that *M. oleifera* extract downregulates inflammatory cytokines (IL-2, IL-6, and TNF-α) and elevates serum levels of IgA and IgM [40,41]. As a result, moringa extract may contribute to both humoral responses and anti-inflammatory activity.

A decrease in IL-2 levels was observed in rats treated with garlic and patawali extracts. IL-2 is primarily produced by activated $CD4^+$ helper T-cells and plays a role in T-cell proliferation, survival, and differentiation [42]. Garlic extract's effect on cytokine production has been previously reported, with findings showing reduced production of Th1 and inflammatory cytokines (IL-1α, IL-6, IL-8, IFN-γ, IL-2, and TNF-α) [43]. The key bioactive compounds in garlic, diallyl disulfide, diallyl thiosulfonate (allicin), diallyl sulfide (DAS), and S-allyl-cysteine sulfoxide (alliin), are involved in the NF-κB signaling pathway, which inhibits pro-inflammatory responses [44,45]. Ahmad et al. (2015) also reported that the ethanolic extract of *Tinospora crispa* promotes T-cells and B-cell proliferation and elevates both Th1 (TNF-α, IL-2, IFN-γ) and Th2 (IL-4) cytokine levels [46]. Our study similarly found that patawali extract increased IL-4 levels, which are crucial for the differentiation of naive $CD4^+$ T-cells into Th2 cells. The decrease in IL-2 levels suggests that patawali extract may specifically enhance Th2 cytokine production. Therefore, both garlic and patawali extracts exhibit anti-inflammatory properties by reducing IL-2 cytokine levels. Notably, soap pod extract demonstrated strong anti-inflammatory activity by elevating IL-10 levels, a cytokine primarily produced by Th2 cells and essential for immune homeostasis [47]. Additionally, soap pod significantly increased IL-17a levels, which may contribute to the differentiation of naive $CD4^+$ T-cells into Th17 cells, activation of innate immunity, and enhancement of B-cell function [48,49].

From our perspective, the crude extracts of each plant likely contain various bioactive compounds that target multiple immune cells. The choice of plant parts for medicinal use is influenced by their therapeutic properties, with different parts, such as roots, leaves, or flowers, containing specific bioactive compounds that are useful for treating various ailments [50]. Polysaccharides, in particular, are major components that can broadly trigger immune cells, including monocytes, macrophages, dendritic cells, neutrophils, and lymphocytes [17]. They function through both direct and indirect mechanisms. According to our findings, the predominant effect of plant extracts was observed in the T-cell population. In adaptive immunity, naive T-cells differentiate into effector T-cells, which include CD4 and CD8 effector T-cells that perform various functions depending on the stimuli. Effector T-cells are crucial in modulating B-cell activation and enhancing CD8 T-cell development [51,52]. Interestingly, fully active effector T-lymphocytes can be distinguished by their cytokine production profiles [53]. Our study revealed that plant extracts modulated Th1, Th2, and Th17 cytokine production, both stimulating and suppressing cytokine levels. Th1 cytokines, such as IFN-γ, TNF, and IL-2, are involved in cell-mediated immunity, while Th2 cytokines (IL-4, IL-5, IL-10, and IL-13) are key players in humoral immune responses [54,55]. Th17 cytokines are important for host defense mechanisms against various infections [56]. Therefore, our study not only found the effects of aqueous plant extracts on lymphocyte responses but also demonstrated their ability to modulate Th1, Th2, and Th17 cytokine production.

5. Conclusions

The utilization of Thai local plants as alternative medicine or supplementary food is becoming increasingly popular. Our findings suggest that these plants have high potential as immunostimulants to enhance lymphocyte proliferation, particularly T-cells. Some local plants also act as immunosuppressants, modulating the population of B-cells. Additionally, the aqueous extracts of these plants play a crucial role in the modulation of Th1, Th2, and Th17 cytokine production. Therefore, Thai medicinal plants offer effective, plant-derived medicines that could be used to improve the adaptive immune response, either by enhancing or suppressing it.

CRediT authorship contribution statement

Theerawat Dobutr: Writing – original draft, Visualization, Validation, Software, Funding acquisition, Conceptualization. **Nisachon Jangpromma:** Methodology, Conceptualization. **Rina Patramanon:** Funding acquisition. **Jureerut Daduang:** Validation, Methodology, Funding acquisition. **Sirinan Kulchat:** Funding acquisition. **Jringjai Areemit:** Funding acquisition, Conceptualization. **Komsorn Lomthaisong:** Funding acquisition. **Sakda Daduang:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition.

Ethics statement

This study was conducted in accordance with ethical guidelines for the care and use of animals in research, ensuring adherence to all relevant standards and regulations that promote the welfare and humane treatment of the animals involved. The protocol for the animal study was thoroughly reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Khon Kaen University, under approval number IACUC-KKU-64/63.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Theerawat Dobutr reports financial support was provided by Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B), Thailand. Sakda Daduang reports financial support was provided by The Fundamental Fund of KKU received funding support from the National Science, Research and Innovation Fund (NSRF), Thailand. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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