Article

A novel Plasma-Based Ionization Approach to Enhance Herbal Combinations: Application to *Perilla frutescens* and *Taraxacum platycarpum* Dahlst

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Abstract: Herbal medicinal products are gaining attention due to their accessibility and relative safety, yet challenges such as inconsistent efficacy and potential cytotoxicity remain. In this study, to overcome these limitations, we applied a plasma-based ionization method to a combination of *Perilla frutescens* and *Taraxacum platycarpum* Dahlst and evaluated its biological effects. THP-1 and Raw 264.7 cells were treated with either ionized or non-ionized extracts, followed by assessments of cell morphology, confluency, viability, and pro-inflammatory cytokine production. Ionization effectively reduced cytotoxic effects, as evidenced by preserved normal cell morphology and viability, and the absence of elevated proinflammatory cytokines. Notably, non-ionized extracts alone induced significant levels of pro-inflammatory cytokines in both cell types, suggesting a potential risk of undesirable immune activation. Although anti-inflammatory activity was not significantly enhanced, it was maintained in Raw 264.7 cells. In THP-1 cells, the ionized extract showed a trend toward enhanced anti-inflammatory activity, with reduced variability compared to the non-ionized extract. These findings suggest that plasma-based ionization may stabilize and improve the reliability of herbal extract efficacy without introducing adverse effects.

Keywords: traditional medicine; plasma-based ionization; cytotoxicity; anti-inflammatory effect; herbal extracts

1. Introduction

Herbal medicinal products have been used as medicines dating back over 5000 years in ancient India [1]. In the case of East Asia, the origins of botanical medicine are thought to date back to China over 3000 years ago. This medical system was transmitted to Korea in the 6th century, and later this system was named Hanbang [2–4]. Although there are remarkable improvements in biology and pharmacy, the interest in natural product-based therapeutics is increasing. Because these products are inexpensive and easily accessible, they have been used by many people over extended periods and through diverse methods. This long history of widespread use contributes to the general perception that plant-based medicinal products are safe and cause fewer side effects [1,5–7]. However, despite their popularity, there are several issues, such as inconsistent effects, disorders in cellular uptake, and cytotoxicity [6–8].

Various methods have been explored to enhance the medicinal effects of herbs, including the combination of plants [9–11], nano-formulation techniques [12–15], and the isolation of active compounds [16,17]. While each of these approaches offers distinct advantages, they also face notable limitations. Combination therapy, though potentially synergistic, is complicated by the difficulty of selecting compatible medicinal plants, optimizing their ratios, and ensuring efficient cellular uptake [9]. Nano-formulation, despite being an effective delivery system, poses concerns due to its high cost and the risk of toxicity. In particular, non-immunoglobulin E (IgE)-mediated hypersensitivity reactions have been reported, potentially causing cardiopulmonary distress, anaphylaxis, and other adverse effects [15]. Accordingly, nano safety must be thoroughly evaluated through cytotoxicity,

immunotoxicity, and genotoxicity assays, as well as in vivo studies to assess biocompatibility. Active compound isolation, on the other hand, allows for precise targeting but may neglect the synergistic interactions present in the whole medicinal plants' matrix [18,19].

Considering these limitations, ionization methods can be reconsidered not only for analysis but also for their potential to support biological activity. Among various ionization methods, ion-pairing has been widely applied to improve solubility and absorption of highly charged or hydrophilic drugs, such as guanfacine [20], insulin, lumefantrine [21], and alendronate [22]. In contrast, other ionization techniques such as electrospray ionization [23,24], electrospray laser desorption ionization [25], and tissue-spray ionization [26] have traditionally been employed for compound identification, and their application in enhancing therapeutic potential remains largely underexplored. Among these less-studied methods, plasma-based ionization remains particularly rare in the context of herbal research. Nevertheless, it offers a promising route to augment bioactivity in a mild and non-invasive manner [27,28].

As mentioned above, ionization can enhance solubility and absorption and thus may help compensate for the limitations of simple herbal combinations. Furthermore, compared to nano-formulation techniques, ionization methods are generally considered to involve simpler processes. They may offer cost advantages due to the use of readily available materials and less complex equipment. In this study, we applied plasma-based ionization to a newly selected combination of plant extracts, drawing from the approach used in our previous experiments [10], and compared the ionized material with its non-ionized raw forms using two cell lines: human monocyte THP-1 and mouse macrophage Raw 264.7. This allowed us to explore the potential of ionization as a simple and safe strategy to improve the anti-inflammatory activity of botanical mixtures.

The selected plant extracts, *Perilla frutescens* and *Taraxacum platycarpum* Dahlst, were chosen based on their well-recognized anti-inflammatory activity, both in Korean medicine and supported by international scientific studies [29–36]. In addition to their known pharmacological potential, they are readily available and easy to cultivate, making them practical choices for this investigation. Given their accessibility, effective utilization of these herbs may contribute to improving public healthcare when integrated with our plasma-ionization method. The comparison focused on evaluating cytotoxicity and the maintenance or enhancement of anti-inflammatory activity in a modern cell-based system.

2. Materials and Methods

2.1. Plant Extracts and Ionization

Perilla frutescens and *Taraxacum platycarpum* Dahlst (Figure S1) were prepared from Bluechem (Anyang, Republic of Korea). The 1:1 (*w*/*w*) mixture was composed of whole or partial components derived from the aforementioned plants. After equally dividing the mixture into two portions, one remained crude, and the other was subjected to our plasma-based ionization method developed in-house. The ionization process utilized nano-oxygen and an electrolyte solution containing water-soluble calcium salts as the conductive buffer. This ionization was promoted through multiple cycles of plasma discharge to enhance electron-ion separation. As expected, a pH shift from 8.4 to 8.1 was observed after ionization; however, this change alone does not demonstrate ionization. However, this slight acidification may suggest proton displacement resulting from ionic interactions, such as calcium ion (Ca^{2+}) binding, indicating potential physicochemical alterations relevant to the extract's improved biological activity. Such ionic modifications, particularly those involving calcium-mediated proton displacement, may contribute to improved extract functionality by affecting the solubility, structural stability, or membrane permeability of certain bioactive constituents. Both mixtures were prepared as solutions and designated as non-ionized extract and ionized extract. Due to the complex properties of the plant extracts, the exact concentration of active compounds could not be calculated. Therefore, relative dilutions (1/50 and 1/100) were used for comparison.

2.2. Cytotoxicity and Inflammatory Response Assay

Human monocyte THP-1 (ATCC, Manassas, VA, USA) and mouse macrophage Raw 264.7 (ATCC) were selected due to their established roles in innate immunity and their high sensitivity to inflammatory stimuli, such as lipopolysaccharide (LPS). The use of both human and mouse immune cell lines enables assessment of cross-species consistency. Cells were treated with the ionized and non-ionized extracts at various dilutions. THP-1 cells were seeded at a density of 5000 cells/well, and Raw 264.7 cells at 10,000 cells/well in 96-well plates. Three days after treatment with the two extracts, each well was examined under a microscope to assess cell viability, confluency (as an indicator of cell growth), detect contamination, and observe morphological changes. Images were captured, and Raw 264.7 cells were stained with crystal violet solution.

As cytotoxic stress may induce an inflammatory response, the degree of inflammation was evaluated to assess the cellular effects of the extracts. Accordingly, THP-1 (25,000 cells/well) and Raw 264.7 (50,000 cells/well) cells were seeded in 96-well plates. Eighteen hours after extract treatment, the concentration of human interleukin-8 (hIL-8) in the THP-1 supernatant and mouse tumor necrosis factor alpha (mTNFα) in the Raw 264.7 supernatant were measured using ELISA kits (R&D Systems, Minneapolis, MN, USA). Absorbance was measured using a microplate reader, and cytokine concentrations were calculated based on standard curves generated from known concentrations of recombinant proteins. Cells were cultured in RPMI 1640 supplemented with 10% Fetal Bovine Serum (FBS), 100 units/mL penicillin, and 100 µg/mL streptomycin at 37 °C in a humidified incubator with 5% CO_2. All cell culture reagents, including RPMI 1640, FBS, and penicillin-streptomycin mixture, were purchased from Welgene (Gyeongsan, Republic of Korea).

2.3. Anti-Inflammatory Assay

THP-1 cells (25,000/well) and Raw 264.7 cells (50,000/well) were seeded in 96-well plates. The two types of extracts were first applied, and six hours later, LPS O127: B8 (3 ng/mL) was added. This LPS O127: B8 was selected for its well-established ability to stimulate toll-like receptor 4 (TLR4), which is a key pathway in innate immune activation. Among various LPS serotypes tested in our preliminary screening, O127: B8 consistently induced a strong cytokine response with minimal cytotoxicity in both THP-1 and Raw 264.7 cell lines, making it optimal for this assay. The concentration (3 ng/mL) was determined through in-house optimization to elicit a robust inflammatory response with minimal cytotoxic effects. Eighteen hours after LPS treatment, the culture supernatants were collected to measure inflammatory cytokine levels. hIL-8 in THP-1 cell supernatants and mTNFα in Raw 264.7 cell supernatants were quantified using ELISA kits. Absorbance was measured using a microplate reader, and cytokine concentrations were calculated based on standard curves generated from known concentrations of recombinant proteins.

2.4. Statistical Analysis

All quantitative data are presented as mean ± standard error of the mean (SEM), based on at least three independent experiments. Statistical analyses were performed using two-way ANOVA, followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$ and are indicated in the figures with an asterisk (*).

3. Results

3.1. Comparison of Cytotoxic and Inflammatory Responses between Ionized and Non-Ionized Extracts

The cytotoxicity effects of ionized and non-ionized extracts were evaluated in THP-1 cells and Raw 264.7 cells (Figures 1 and 2). Microscopic examination of THP-1 cells treated with non-ionized extract at 1/50 dilution, but not at 1/100 dilution, revealed a substantial number of darkened and shrunken cells, indicative of cell death (Figure 1A). No dead cells were observed in the wells treated with the ionized extract, even after 72 h of incubation, under identical seeding and medium conditions. In Raw 264.7 cells, morphological changes indicating the onset of macrophage-like differentiation, such as cellular elongation and increased adherence, were more pronounced in wells treated with the non-ionized extract compared to both the untreated control and ionized extract-treated groups (Figure 2A). This was further supported by increased crystal violet staining intensity in non-ionized extract-treated wells, particularly at 1/100 and 1/1000 dilutions, suggesting a higher degree of adherence and possible differentiation. The staining pattern was visibly denser and more clustered compared to ionized extract-treated groups (Figure 2B).

Two ELISA results showed that pro-inflammatory cytokines were induced by non-ionized extract in a dilution-dependent manner (Figures 1B and 2C). Although mTNFα induction increased in Raw 264.7 cells treated with ionized extract at 1/50 dilution compared to the control, the level was still more than three times lower than that observed with the non-ionized extract. These findings suggest that ionization reduces the unintended pro-inflammatory activity of the extract, thereby minimizing cellular stress or cytotoxic effects under the tested conditions (Figure 2C).

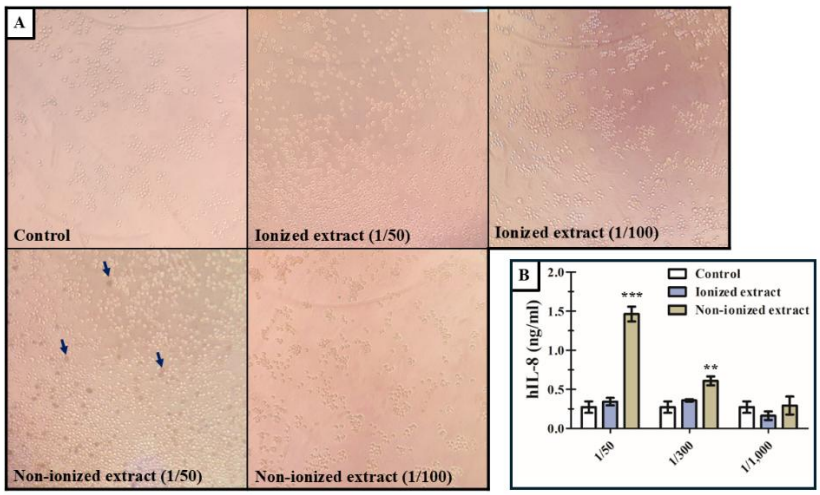

Figure 1. Comparison of cytotoxicity between ionized extract and non-ionized extracts using THP-1 cells. (**A**) shows representative images of THP-1 cells (100× magnification). Untreated cells served as the negative control. THP-1 cells were treated with ionized extracts at 1/50 and 1/100 dilutions and with non-ionized extract at 1/50 and 1/100 dilutions. Among multiple dead cells observed, representative ones are marked with dark blue arrows. In suspension cultures, dead cells appear darker and less refractile than viable cells. The hIL-8 levels in the culture supernatant were measured 18 h after treatment (**B**). Data were expressed as mean ± SEM (n = 4). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. ** $p < 0.01$ and *** $p < 0.001$ compared to the untreated control.

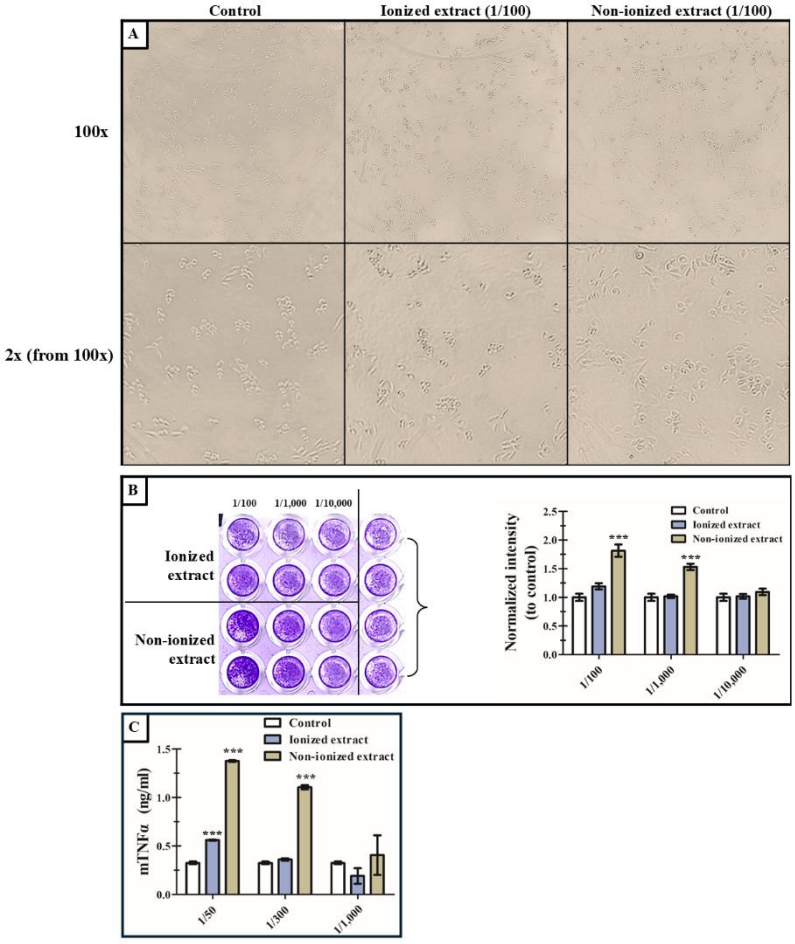

Figure 2. Comparison of cytotoxicity between ionized extract and non-ionized extracts using Raw 264.7 cells. (**A**) shows representative images of Raw 264.7 cells (100× magnification) and 2× magnified regions from the same fields. Untreated cells served as the negative control. Raw 264.7 cells were treated with an ionized extract at a

1/100 dilution and a non-ionized extract at a 1/100 dilution. The brightness and contrast were uniformly adjusted for all images. Background subtraction was applied using ImageJ. No structural alterations were made. To visualize the degree of differentiation, the cells were stained with crystal violet solution (**B**). The staining intensity of each well was quantified using ImageJ (n = 4). The mTNFα levels in the culture supernatant were measured 18 h after treatment (**C**). Data were expressed as mean ± SEM (n = 3). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. *** $p < 0.001$ compared to the untreated control.

3.2. Comparison of Anti-Inflammatory Activity between Ionized and Non-Ionized Extracts

To assess the anti-inflammatory activity potentially derived from the original plant materials, THP-1 and Raw 264.7 cells were pre-treated with either ionized or non-ionized extracts. Six hours later, LPS O127: B8 3 ng/mL was added. The levels of pro-inflammatory cytokines hIL-8 and mTNFα were subsequently measured in the culture supernatants of THP-1 and Raw 264.7 cells, respectively, using ELISA kits. In THP-1 cells, a trend toward enhanced anti-inflammatory activity was observed with the ionized extract compared to the non-ionized form. Although the mean hIL-8 levels appeared lower in the ionized group across all tested dilutions, the difference did not reach statistical significance ($p > 0.05$) based on two-way ANOVA and Tukey's post hoc test. This lack of significance may be due to the relatively high variability observed in the non-ionized extract group, which potentially masked consistent but modest effects induced by ionization (Figure 3A). In Raw 264.7 cells, although ionization did not lead to a marked improvement in anti-inflammatory activity compared to the non-ionized extract, the original suppressive effect on mTNFα induction was still maintained. All tested dilutions of the ionized extract significantly reduced cytokine levels compared to the control group, indicating that the ionization process did not impair the extract's functional integrity (Figure 3B).

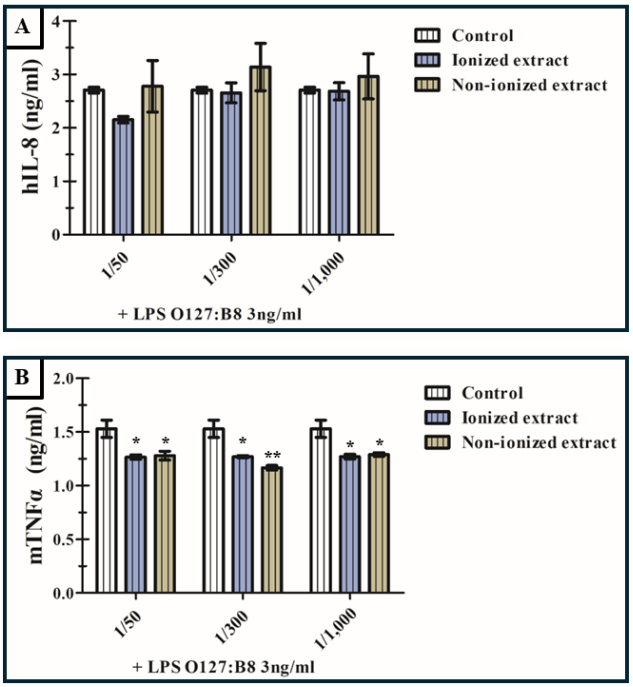

Figure 3. Comparison of anti-inflammatory activity between ionized and non-ionized extracts using THP-1 cells and Raw 264.7. Ionized and non-ionized extracts were pretreated at various dilutions (1/50, 1/300, and 1/1000) in THP-1 and Raw 264.7 cells 6 h prior to stimulation with LPS O127:B8 (3 ng/mL). The hIL-8 levels in the THP-1 culture supernatant (**A**) and mTNFα levels in Raw 264.7 culture supernatant (**B**) were measured 18 h after LPS treatment. Data are shown as mean ± SEM (n = 6 for THP-1, n = 3 for Raw 264.7). Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$ and ** $p < 0.01$ compared to the LPS O127:B8 only treatment control.

4. Discussion

In this study, we investigated the impact of our novel plasma-based ionization method for herbal extracts on cytotoxicity and biological activity. Compared to the non-ionized extract, the ionized extract reduced the cellular stress response while maintaining or improving anti-inflammatory activity in both THP-1 and Raw 264.7 cells. During the 7-day treatment duration, no visible indicators of contamination, such as microbial growth, crystal

formation, or particulate matter, were observed under light microscopy. In our previous study [10], we showed that the appropriate combination of medicinal plants could increase the biological activity, and this time, we used this combination strategy in addition to the new ionization method. The selected plants, *Perilla frutescens* and *Taraxacum platycarpum* Dahlst, were chosen due to their well-documented anti-inflammatory properties [29–36], providing a meaningful contrast for evaluating pro-inflammatory responses as indirect indicators of toxic effects on cells.

Although cell viability was not quantitatively assessed using trypan blue or other viability assays in THP-1 cells, consistent signs of cell death, such as cell shrinkage and darkening, were observed in wells treated with a high concentration of non-ionized extract (Figure 1A). In Raw 264.7 cells, treatment with the non-ionized extract led to noticeable morphological alterations, including increased size, enhanced adhesion, and an elongated appearance, suggesting a pro-inflammatory activation state in Figure 2A,B [37]. Such dendritic-like morphological changes are often associated with inflammatory activation in macrophages and may reflect a stress-induced cytopathic effect. This alteration was further supported by crystal violet solution staining (Figure 2B). In addition, the significantly higher induction of pro-inflammatory cytokines in non-ionized extract-treated cells compared to ionized extract-treated cells suggests that ionization may reduce the cytotoxic effects of herbal extracts.

Additionally, as shown in Figure 3A, in THP-1 cells, the ionized extract exhibited a modest downward trend in hIL-8 production compared to the non-ionized extract, although this reduction was not statistically significant. This subtle difference may reflect greater consistency or potential robustness in the anti-inflammatory response of the ionized form, possibly due to the high variability observed in the activity of the non-ionized extract. In Figure 3B, in Raw 264.7 cells, both demonstrated comparable anti-inflammatory effects. These findings suggested that while ionization did not markedly improve anti-inflammatory activity under LPS-inflammatory conditions, it may help preserve the original efficacy of botanical agents without introducing adverse effects.

The herbal combination strategy has been explored to achieve synergistic effects and reduce cell toxicity [9–11]. However, this strategy faces challenges in selecting compatible medicinal plants, optimizing their ratios, and ensuring effective cellular uptake. To overcome these limitations, we adopted ionization methods commonly used in analytical chemistry that are simpler, more cost-effective, and potentially safer than nano-formulation. While ionization has been widely applied to improve the solubility and absorption of various pharmaceutical compounds, its application to plant-derived treatments remains limited [20–22].

With concerns about the side effects of synthetic drugs, interest in herbal and natural product-based therapies is increasing due to their perceived safety. Nonetheless, botanical medicines still suffer from low cellular uptake, inconsistent efficacy, and difficulties in achieving reproducible experimental results due to their complex composition [1,5–8]. The use of ionization in this study offers a promising strategy to address some of these challenges. In particular, a plasma-based ionization method emerging applications in plant science improved the bioactivity and reduced cytotoxicity of the herbal mixture [27,28].

In summary, this study highlights the potential of plasma-based ionization as a relatively simple and cost-effective strategy to reduce cytotoxicity and preserve or enhance the predicted biological activity. Since the findings are based on in vitro experiments, further in vivo studies are required to confirm the efficacy and safety of this approach. Nevertheless, our results suggest that ionization could offer a practical and scientifically grounded tool for modernizing and advancing various traditional herbal techniques, such as Hanbang medicines, thereby contributing to the advancement of overall healthcare quality.

Given the promising results, future research should explore the broader applications of plasma-based ionization to enhance the bioactivity and safety of herbal treatments. It would be valuable to investigate the generalizability of plasma-based ionization methods across different herbal mixtures. Establishing such standardized conditions would promote the reproducibility and consistency of plasma-based ionization processes, facilitating their effective integration into diverse herbal therapies. Additionally, long-term safety studies and clinical trials are essential for confirming the broader applicability and safety of plasma-based ionization in both traditional and modern medicine.

Supplementary Materials: The following supporting information can be downloaded at: https://media.sciltp.com/articles/others/250618113327698 1/JIIM-1083-Supplementary-Materials.pdf, Figure S1: Photos of *Perilla frutescens* and *Taraxacum platycarpum* Dahlst.

Author Contributions: M.C.: conceptualization, methodology, formal analysis, investigation; H.L.: investigation; Y.K., B.K. and T.J.: data curation, formal analysis; G.C. and G.K.: conceptualization; K.H. and H.N.: conceptualization, resources; S.S.: conceptualization, supervision, writing, reviewing, and editing. M.C. and S.S. share corresponding authorship. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

ELISA, enzyme-linked immune specific assay; hIL-8, human interleukin-8; IgE, immunoglobulin E; LPS, lipopolysaccharide; mTNFα, mouse tumor necrosis factor alpha

References

1. Petrovska, B.B. Historical review of medicinal plants' usage. *Pharmacogn. Rev.* **2012**, *6*, 1–5. https://doi.org/10.4103/0973-7847.95849.
2. Park, H.; Kim, H.S. Korean traditional natural herbs and plants as immune enhancing, antidiabetic, chemopreventive, and antioxidative agents: A narrative review and perspective. *J. Med. Food* **2014**, *17*, 21–27. https://doi.org/10.1089/jmf.2013.3059.
3. Yuan, H.; Ma, Q.; Ye, L.; Piao, G. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules* **2016**, *21*, 559. https://doi.org/10.3390/molecules21050559.
4. Kumar, H.; Song, S.Y.; More, S.V.; Kang, S.M.; Kim, B.W.; Kim, I.S.; Choi, D.K. Traditional Korean East Asian medicines and herbal formulations for cognitive impairment. *Molecules* **2013**, *18*, 14670–14693. https://doi.org/10.3390/molecules181214670.
5. Wanjari, A.S.; Wanjari, D.S. An Overview on Herbal Medicine. *Res. J. Pharmacogn. Phytochem.* **2019**, *11*, 14–17. https://doi.org/10.5958/0975-4385.2019.00003.7.
6. Sanjoy Kumar Pal, Y.S. Herbal Medicine: Current Status and the Future. *Asian Pac. J. Cancer Prev.* **2003**, *4*, 281–288.
7. Ekor, M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol.* **2014**, *4*, 177. https://doi.org/10.3389/fphar.2013.00177.
8. Ben-Shabat, S.; Yarmolinsky, L.; Porat, D.; Dahan, A. Antiviral effect of phytochemicals from medicinal plants: Applications and drug delivery strategies. *Drug Deliv. Transl. Res.* **2020**, *10*, 354–367. https://doi.org/10.1007/s13346-019-00691-6.
9. Che, C.T.; Wang, Z.J.; Chow, M.S.; Lam, C.W. Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. *Molecules* **2013**, *18*, 5125–5141. https://doi.org/10.3390/molecules18055125.
10. Choi, M.; Hwang, J.; Han, K.; Noh, H.; Kang, B.; Jeong, T.; Choi, G.; Lee, H.; Kim, Y.; Ryu, H.; et al. Natural Plant Extracts as Novel Antiviral Candidates: Citrus extracts. *J. Inflamm. Infect. Med.* **2025**, *1*, 3. https://doi.org/10.53941/jiim.2025.100003.
11. Wang, Y.; Yang, H.; Chen, L.; Jafari, M.; Tang, J. Network-based modeling of herb combinations in traditional Chinese medicine. *Brief. Bioinform.* **2021**, *22*, bbab106. https://doi.org/10.1093/bib/bbab106.
12. Ai, S.; Li, Y.; Zheng, H.; Zhang, M.; Tao, J.; Liu, W.; Peng, L.; Wang, Z.; Wang, Y. Collision of herbal medicine and nanotechnology: A bibliometric analysis of herbal nanoparticles from 2004 to 2023. *J. Nanobiotechnol.* **2024**, *22*, 140. https://doi.org/10.1186/s12951-024-02426-3.
13. Bonifacio, B.V.; Silva, P.B.; Ramos, M.A.; Negri, K.M.; Bauab, T.M.; Chorilli, M. Nanotechnology-based drug delivery systems and herbal medicines: A review. *Int. J. Nanomed.* **2014**, *9*, 1–15. https://doi.org/10.2147/IJN.S52634.
14. Moradi, S.Z.; Momtaz, S.; Bayrami, Z.; Farzaei, M.H.; Abdollahi, M. Nanoformulations of Herbal Extracts in Treatment of Neurodegenerative Disorders. *Front. Bioeng. Biotechnol.* **2020**, *8*, 238. https://doi.org/10.3389/fbioe.2020.00238.
15. Teja, P.K.; Mithiya, J.; Kate, A.S.; Bairwa, K.; Chauthe, S.K. Herbal nanomedicines: Recent advancements, challenges, opportunities and regulatory overview. *Phytomedicine* **2022**, *96*, 153890. https://doi.org/10.1016/j.phymed.2021.153890.
16. Sasidharan, S.; Chen, Y.; Saravanan, D.; Sundram, K.M.; Yoga Latha, L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *Afr. J.Tradit. Complement. Altern. Med.* **2011**, *8*, 1–10.
17. Atanasov, A.G.; Waltenberger, B.; Pferschy-Wenzig, E.M.; Linder, T.; Wawrosch, C.; Uhrin, P.; Temml, V.; Wang, L.; Schwaiger, S.; Heiss, E.H.; et al. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnol. Adv.* **2015**, *33*, 1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001.
18. Zhao, Q.; Luan, X.; Zheng, M.; Tian, X.H.; Zhao, J.; Zhang, W.D.; Ma, B.L. Synergistic Mechanisms of Constituents in Herbal Extracts during Intestinal Absorption: Focus on Natural Occurring Nanoparticles. *Pharmaceutics* **2020**, *12*, 128. https://doi.org/10.3390/pharmaceutics12020128.
19. Wagner, H.; Ulrich-Merzenich, G. Synergy research: Approaching a new generation of phytopharmaceuticals. *Phytomedicine* **2009**, *16*, 97–110. https://doi.org/10.1016/j.phymed.2008.12.018.
20. Hou, Z.; Shi, D.; Lin, J.; Zhao, X.; Zhang, H.; Ding, J. Effect of ion pair strategy on transdermal delivery of guanfacine:

Which factor dominates drug permeation? *Int. J. Pharm.* **2024**, *652*, 123835. https://doi.org/10.1016/j.ijpharm.2024.123835.

21. Ristroph, K.D.; Prud'homme, R.K. Hydrophobic ion pairing: Encapsulating small molecules, peptides, and proteins into nanocarriers. *Nanoscale Adv.* **2019**, *1*, 4207–4237. https://doi.org/10.1039/c9na00308h.
22. Samiei, N.; Foroutan, S.M.; Razipour, F.; Zarghi, A.; Shafaati, A. An investigation into the ability of alendronate ion pairs to increase oral absorption. *Int. J. Pharm.* **2017**, *527*, 184–190. https://doi.org/10.1016/j.ijpharm.2017.05.042.
23. Schütz, K.; Kammerer, D.R.; Carle, R.; Schieber, A. Characterization of phenolic acids and flavonoids in dandelion (Taraxacum officinale WEB. ex WIGG.) root and herb by high-performance liquid chromatography/electrospray ionization mass spectrometry. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 179–186. https://doi.org/10.1002/rcm.1767.
24. Qiu, Z.D.; Chen, J.L.; Zeng, W.; Ma, Y.; Chen, T.; Tang, J.F.; Lai, C.J.; Huang, L.Q. Real-time toxicity prediction of Aconitum stewing system using extractive electrospray ionization mass spectrometry. *Acta Pharm. Sin. B* **2020**, *10*, 903–912. https://doi.org/10.1016/j.apsb.2019.08.012.
25. Su, H.; Liu, K.T.; Chen, B.H.; Lin, Y.P.; Jiang, Y.M.; Tsai, Y.H.; Chang, F.R.; Shiea, J.; Lee, C.W. Rapid identification of herbal toxins using electrospray laser desorption ionization mass spectrometry for emergency care. *J. Food Drug Anal.* **2019**, *27*, 415–427. https://doi.org/10.1016/j.jfda.2018.11.001.
26. Chan, S.L.; Wong, M.Y.; Tang, H.W.; Che, C.M.; Ng, K.M. Tissue-spray ionization mass spectrometry for raw herb analysis. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 2837–2843. https://doi.org/10.1002/rcm.5177.
27. Bora, J.; Khan, T.; Mahnot, N. Cold Plasma Treatment Concerning Quality and Safety of Food: A Review. *Curr. Res. Nutr. Food Sci. J.* **2022**, *10*, 427–446. https://doi.org/10.12944/CRNFSJ.10.2.3.
28. Pogorzelska-Nowicka, E.; Hanula, M.M.; Brodowska-Trębacz, M.; Górska-Horczyczak, E.; Jankiewicz, U.; Mazur, T.; Marcinkowska-Lesiak, M.; Półtorak, A.; Wierzbicka, A. The Effect of Cold Plasma Pretreatment on Water-Suspended Herbs Measured in the Content of Bioactive Compounds, Antioxidant Activity, Volatile Compounds and Microbial Count of Final Extracts. *Antioxidants* **2021**, *10*, 1740.
29. Jin Su, Y.; Sang Yong, K.; Sang Hyun, K.; Tae Yong, S. Antiallergic and Anti-inflammatory Effects of Perilla frutescens var. acuta. *Korean J. Pharmacogn.* **2012**, *43*, 163–166.
30. Son, H.-U.; Heo, J.-C.; Seo, M.-S.; Lee, S.-H. Effects of Perilla frutescens L. on anti-oxidant and anti-inflammation activity. *Korean J. Food Preserv.* **2010**, *17*, 757–761.
31. Youn, I.; Han, S.; Jung, H.J.; Noh, S.G.; Chung, H.Y.; Koo, Y.K.; Shin, S.; Seo, E.K. Anti-Inflammatory Activity of the Constituents from the Leaves of Perilla frutescens var. acuta. *Pharmaceuticals* **2023**, *16*, 1655.
32. Huang, B.P.; Lin, C.H.; Chen, Y.C.; Kao, S.H. Anti-inflammatory effects of Perilla frutescens leaf extract on lipopolysaccharide-stimulated RAW264.7 cells. *Mol. Med. Rep.* **2014**, *10*, 1077–1083. https://doi.org/10.3892/mmr.2014.2298.
33. Kangwan, N.; Pintha, K.; Khanaree, C.; Kongkarnka, S.; Chewonarin, T.; Suttajit, M. Anti-inflammatory effect of Perilla frutescens seed oil rich in omega-3 fatty acid on dextran sodium sulfate-induced colitis in mice. *Res. Pharm. Sci.* **2021**, *16*, 464–473. https://doi.org/10.4103/1735-5362.323913.
34. Yon-Suk, K.; Mi-Yeun, J.; Beom-Seok, R.; Pyo-Jam, P.; Jae-Hyun, J. Anti-Inflammatory Activities of Extracts from Fermented Taraxacum platycarpum D. Leaves Using Hericium erinaceum Mycelia. *J. Korean Soc. Food Sci. Nutr.* **2016**, *45*, 20–26.
35. Talapphet, N.; Palanisamy, S.; Li, C.; Ma, N.; Prabhu, N.M.; You, S. Polysaccharide extracted from Taraxacum platycarpum root exerts immunomodulatory activity via MAPK and NF-κB pathways in RAW264.7 cells. *J. Ethnopharmacol.* **2021**, *281*, 114519. https://doi.org/10.1016/j.jep.2021.114519.
36. Cheong, H.; Choi, E.J.; Yoo, G.S.; Kim, K.M.; Ryu, S.Y. Desacetylmatricarin, an anti-allergic component from Taraxacum platycarpum. *Planta Med.* **1998**, *64*, 577–578. https://doi.org/10.1055/s-2006-957520.
37. Saxena, R.K.; Vallyathan, V.; Lewis, D.M. Evidence for lipopolysaccharideinduced differentiation of RAW264.7 murine macrophage cell line into dendritic like cells. *J. Biosci.* **2003**, *28*, 129–134. https://doi.org/10.1007/BF02970143.