

production. In this experiment, freeze-dried moringa pod extract at 50-200 $\mu\text{g/ml}$ had polyphenols including genistein (1.99-7.94 $\mu\text{g/ml}$), trans-ferulic acid (0.98-3.9 $\mu\text{g/ml}$), p-Coumaric (0.47-1.88 $\mu\text{g/ml}$), myricetin (0.43-1.71 $\mu\text{g/ml}$), gallic acid (0.36-1.43 $\mu\text{g/ml}$), and syringic acid (0.25-0.98 $\mu\text{g/ml}$) (data from covert calculation). Previous studies showed that these polyphenols had anti-inflammatory activity on LPS-induced Raw264.7 macrophage models. At a concentration of 40 $\mu\text{g/ml}$, genistein has been reported to significantly inhibit the activity of inflammatory mediators such as PGE₂, TNF- α , and IL-1 β [36]. This is similar to a study on ferulic acid, which reported that a concentration of 0.012-0.31 $\mu\text{g/ml}$ of ferulic acid significantly decreased inflammatory activities via NO, IL-1 β , and IL-6 levels without

cytotoxicity [37]. Furthermore, studies have revealed that p-coumaric acid, within the concentration range of 10-100 $\mu\text{g/ml}$, exhibits anti-inflammatory properties by notably suppressing COX-2, iNOS, TNF- α , and IL1 β mRNA expression in a dose-dependent fashion, particularly when compared to LPS-treated Raw264.7 macrophage cells [38]. Furthermore, the study on myricetin, at the concentration 3.98-7.96 $\mu\text{g/ml}$, also significantly inhibited the production of pro-inflammatory cytokines via the decreasing of the mRNA expression level of pro-inflammatory factors (TNF- α , IL-6, IL-1 β , COX-2 and iNOS) [39, 40]. The anti-inflammatory properties of 100 μM gallic acid and 10-20 μM syringic acid have been reported [41, 42]. However, mixed usage of these polyphenols on anti-inflammatory effects showed no evidence.

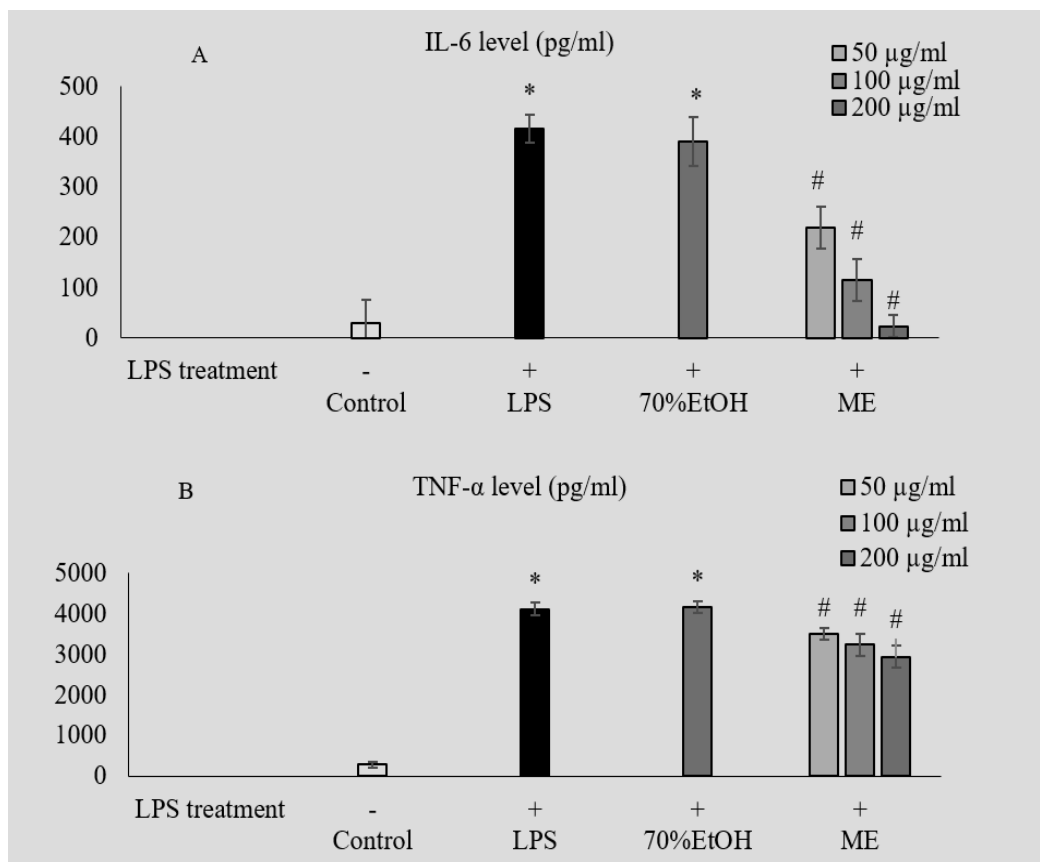


Figure 6. IL-6 (A) and TNF- α (B) cytokine levels were evaluated in RAW 264.7 macrophages following stimulation with lipopolysaccharide for 24 hours. Experimental conditions included Control (Untreated), LPS (treated with LPS at 100 ng/ml), and 70%EtOH (treated with 70% ethanol, serving as the high-value solvent extraction control). Results are presented as mean \pm standard deviation (n=3); * denotes significance at $P < 0.05$ compared to the control, while # indicates significance at $P < 0.05$ compared to the LPS-treated group.

In summary, the results of this study demonstrated that freeze-dried moringa pod extract with bioactive polyphenols could reduce inflammation in LPS-induced Raw264.7 macrophage cells via the suppression of NO, IL-6 and TNF- α levels. However, bioactive absorption, metabolism, and mechanism need further clarification.

CONCLUSION

Freeze-dried moringa pod extract contains various bioactive compounds such as genistein synthetic, trans-ferulic acid, p-Coumaric acid, myricetin, gallic acid, and syringic acid. These compounds have been shown to possess antioxidant properties and exhibit anti-inflammatory effects. The results of this study indicate the functional potential, especially the antioxidant and anti-inflammatory properties, of freeze-dried moringa pods. These raise the local remedy plant to become a new functional ingredient in product development.

List of Abbreviations: DPPH - 2,2 diphenyl-1-picrylhydrazyl; Trolox - 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; DMEM - Dulbecco's Modified Eagle's Medium; FBS - fetal bovine serum; HPLC - High-Performance Liquid Chromatography; MTS - 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; NO – Nitric oxide; IL-6 – Interleukin-6; IL-8 – Interleukin 8.

Authors Contributions: KH conceptualized the study, conducted the experiments, and wrote the original study. SB conceptualized the study, provided supervision and review, and revised the original script.

Completing Interest: The author declares no conflict of interest.

Acknowledgements: The authors thank the CPF Food Research and Development Center for supporting this project.

REFERENCES

1. Furman D. Campisi J., V.E., Carrera-Bastos P., Targ S., Franceschi C., Ferrucci L., et al., Chronic inflammation in the etiology of disease across the life span. *Nature medicine*, 2019. 25(12): 1822-1832
DOI: <https://doi.org/10.1038/s41591-019-0675-0>.
2. Chiş, A.; Noubissi, P.A.; Pop, O.-L.; Mureşan, C.I.; Fokam Tagne, M.A.; Kamgang, et al. Bioactive Compounds in Moringa oleifera: Mechanisms of Action, Focus on Their Anti-Inflammatory Properties. *Plants* 2024, 13, 20.
DOI: <https://doi.org/10.3390/plants13010020>.
3. Xu, Y., et al., Most Recent Research Progress in Moringa oleifera: Bioactive Phytochemicals and Their Correlated Health Promoting Effects. *Food Reviews International*, 2023: 1-31. DOI: <https://doi.org/10.1080/87559129.2023.2195189>.
4. El-Massry, F.H., M.E. MOSSA, and S.M. YOUSSEF, Moringa oleifera plant. *Egyptian Journal of Agricultural Research*, 2013. 91(4): 1597-1909
DOI: <https://doi.org/10.21608/EJAR.2013.166383>.
5. Bokhad, M.N. and U.B. Jagtap, Bioactive Phytochemicals from Moringa (M. oleifera) Seed Oil Processing By-Products, in *Bioactive Phytochemicals from Vegetable Oil and Oilseed Processing By-products*. 2023, Springer. 1-17
DOI: https://doi.org/10.1007/978-3-030-91381-6_32
6. Su, X., et al., Moringa oleifera Lam.: a comprehensive review on active components, health benefits and application. *RSC advances*, 2023. 13(35):24353-24384
DOI: <https://doi.org/10.1039/D3RA03584K>
7. Martirosyan, D., H. Kanya, and C. Nadalet, Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. *Functional Foods in Health and Disease*, 2021. 11(5): 213-221 DOI: <https://www.doi.org/10.31989/ffhd.v11i5.788>.
8. Martirosyan, D., T. Lampert, and M. Ekblad, Classification and regulation of functional food proposed by the Functional Food Center. *Functional Food Science-Online* ISSN: 2767-3146, 2022. 2(2):25-46
DOI: <https://doi.org/10.31989/ffs.v2i2.890>.
9. Martirosyan, D. and S. Stratton, Advancing functional food regulation. *Bioactive Compounds in Health and Disease-Online* ISSN: 2574-0334; Print ISSN: 2769-2426, 2023. 6(7): 166-171 DOI: <https://doi.org/10.31989/bchd.v6i7.1178>.
10. Nowak, D. and E. Jakubczyk, The freeze-drying of foods—The characteristic of the process course and the effect of its

- parameters on the physical properties of food materials. *Foods*, 2020. 9(10):1488
DOI: <https://doi.org/10.3390/foods9101488>.
11. WHO, W.H.O. Healthy Diet. 2020 [retrieved on 2020 April, 29]; Available from: <https://www.who.int/news-room/fact-sheets/detail/healthy-diet>.
 12. Adainoo, B., A.L. Thomas, and K. Krishnaswamy, Correlations between color, textural properties and ripening of the North American pawpaw (*Asimina triloba*) fruit. *Sustainable Food Technology*, 2023. 1(2): 263-274
DOI: <https://doi.org/10.5586/aa/168236>.
 13. Paisey, E.K. and E. Santosa, Self-pruning in lime (*Citrus aurantifolia* Swingle) after treatments with ichiphon, abscisic acid and nitrogen, phosphorus, potassium fertilizers. *Acta Agrobotanica*, 2023. 76: 168236
DOI: <https://doi.org/10.5586/aa/168236>.
 14. Seal, T., Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. *Journal of Applied Pharmaceutical Science*, 2016. 6(2): 157-166 DOI: <https://doi.org/10.7324/JAPS.2016.60225>.
 15. Tranquilino-Rodríguez, E. and H.E. Martínez-Flores, Ultrasound-assisted extraction of phenolic compounds from *Moringa oleifera* leaves by response surface methodology. *Bioactive Compounds in Health and Disease-Online* ISSN: 2574-0334; Print ISSN: 2769-2426, 2023. 6(11): 325-337 DOI: <https://doi.org/10.31989/bchd.v6i11.1229>.
 16. Kok, T., Anti-inflammatory activity of *Lactobacillus* spp. and *Rhodopseudomonas palustris* probiotics. *Bioactive Compounds in Health and Disease (BCHD)*, 2023. 6(4): 63-72
DOI: <https://doi.org/10.31989/bchd.v6i4.1067>.
 17. Bhawamai, S., Lin SH., Hou YY. and Chen YH., Thermal cooking changes the profile of phenolic compounds, but does not attenuate the anti-inflammatory activities of black rice. *Food & nutrition research*, 2016. 60(1): 32941 DOI: <https://doi.org/10.3402/fnr.v60.32941>.
 18. Ebrahimi, P., et al., Chlorophylls as natural bioactive compounds existing in food by-products: A critical review. *Plants*, 2023. 12(7): 1533
DOI: <https://doi.org/10.3390/plants12071533>.
 19. Erkmen, O. and T.F. Bozoglu, Food preservation by reducing water activity. *Food microbiology: Principles into practice*, 2016. 2: 44-58
DOI: <https://doi.org/10.1002/9781119237860.ch30>.
 20. Owon, M., Osman M., Ibrahim A., Salama M.A. and Matthäus B., Characterisation of different parts from *Moringa oleifera* regarding protein, lipid composition and extractable phenolic compounds. *OCL*, 2021. 28: 45
DOI: <https://doi.org/10.1051/ocl/2021035>.
 21. Llorent-Martínez, E.J., et al., Preliminary phytochemical screening and antioxidant activity of commercial *Moringa oleifera* food supplements. *Antioxidants*, 2023. 12(1):110
DOI: <https://doi.org/10.3390/antiox12010110>.
 22. Prabakaran, M., et al., Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of *Moringa oleifera*. *Food bioscience*, 2018. 26: 23-29
DOI: <https://doi.org/10.1016/j.fbio.2018.09.003>.
 23. Goh, Y.X., Jalil J., Lam K.W., Husain K. and Premakumar C.M., Genistein: a review on its anti-inflammatory properties. *Frontiers in Pharmacology*, 2022. 13: 820969
DOI: <https://doi.org/10.3389/fphar.2022.820969>.
 24. Srinivasan, M., A.R. Sudheer, and V.P. Menon, Ferulic acid: therapeutic potential through its antioxidant property. *Journal of clinical biochemistry and nutrition*, 2007. 40(2): 92-100 DOI: <https://doi.org/10.3164/jcfn.40.92>.
 25. Mussatto, S.I., G. Dragone, and I.C. Roberto, Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain. *Industrial Crops and Products*, 2007. 25(2): 231-237 DOI: <https://doi.org/10.1016/j.indcrop.2006.11.001>.
 26. Roychoudhury, S., Sinha B., Choudhury B.P., Jha N.K., Palit P., Kundu S., Mandal S.C., et al., Scavenging properties of plant-derived natural biomolecule para-coumaric acid in the prevention of oxidative stress-induced diseases. *Antioxidants*, 2021. 10(8): 1205
DOI: <https://doi.org/10.3390/antiox10081205>
 27. Kahkeshani, N., Farzaei F., Fotouhi M., Alavi S.S., Bahramsoltani R., Naseri R., Momtaz S., et al., Pharmacological effects of gallic acid in health and diseases: A mechanistic review. *Iranian journal of basic medical sciences*, 2019. 22(3): 225
DOI: <https://doi.org/10.22038/ijbms.2019.32806.7897>.
 28. Imran, M.S.F., Hussain G., Imran A., Mehmood Z., Gondal T.A., El-Ghorab A., et al., Myricetin: A comprehensive review on its biological potentials. *Food science and nutrition*, 2021. 9(10): 5854-5868
DOI: <https://doi.org/10.1002/fsn3.2513>.
 29. Srinivasulu, C., et al., Syringic acid (SA)—a review of its occurrence, biosynthesis, pharmacological and industrial

- importance. *Biomedicine & Pharmacotherapy*, 2018. 108: p. 547-557.
DOI: <https://doi.org/10.1016/j.biopha.2018.09.069>.
30. Conner, E.M. and M.B. Grisham, Inflammation, free radicals, and antioxidants. *Nutrition*, 1996. 12(4): 274-277
DOI: [https://doi.org/10.1016/S0899-9007\(96\)00000-8](https://doi.org/10.1016/S0899-9007(96)00000-8).
31. Wangcharoen, W. and S. Gomolmanee, Antioxidant capacity and total phenolic content of *Moringa oleifera* grown in Chiang Mai, Thailand. *Thai Journal of Agricultural Science*, 2011. 44(5).
32. Facchin, B.M., Dos Reis G.O., Vieira, G.N., Mohr E.T.B., da Rosa J.S., Kretzer I.F., Demarchi I.G. and E.M. Dalmarco, Inflammatory biomarkers on an LPS-induced RAW 264.7 cell model: A systematic review and meta-analysis. *Inflammation Research*, 2022. 71(7-8): 741-758
DOI: <https://doi.org/10.1007/s00011-022-01584-0>.
33. Sharma, J., A. Al-Omran, and S. Parvathy, Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, 2007. 15: 252-259 DOI: <https://doi.org/10.1007/s10787-007-0013-x>.
34. Alderton, W.K., C.E. Cooper, and R.G. Knowles, Nitric oxide synthases: structure, function and inhibition. *Biochemical journal*, 2001. 357(3):593-615
DOI: <https://doi.org/10.1042/0264-6021:3570593>.
35. Muangnoi, C., Chingsuwanrote P., Praengamthanachoti P., Svasti S. and Tuntipopipat S., *Moringa oleifera* pod inhibits inflammatory mediator production by lipopolysaccharide-stimulated RAW 264.7 murine macrophage cell lines. *Inflammation*, 2012. 35:445-455
DOI: <https://doi.org/10.1007/s10753-011-9334-4>.
36. Widowati, W.P.S., Ekayanti NLW., Munshy UZ., Kusuma HSW., Wibowo SHB., Amalia A., W. Widodo, and R. Rizal. Anti-inflammation assay of black soybean extract and its compounds on lipopolysaccharide-induced RAW 264.7 cell. in *Journal of Physics: Conference Series*. 2019. IOP Publishing. p. 012052
DOI: <https://doi.org/10.1088/1742-6596/1374/1/012052>.
37. Shi, Y., Chen X., Qiang S., Su J. and Li J., Anti-oxidation and anti-inflammatory potency evaluation of ferulic acid derivatives obtained through virtual screening. *International Journal of Molecular Sciences*, 2021. 22(21): 11305 DOI: <https://doi.org/10.3390/ijms222111305>.
38. Zhao, Y., Liu J., Liu C., Zeng X., Li X. and Zhao J., Anti-inflammatory effects of p-coumaric acid in LPS-stimulated RAW264. 7 cells: Involvement of NF- κ B and MAPKs pathways. *Med. Chem*, 2016. 6:327-330
DOI: <https://doi.org/10.4172/2161-0444.1000365>.
39. Hou, W., Hu S., Su Z., Wang Q., Meng G., Guo T., Zhang J. and P. Gao, Myricetin attenuates LPS-induced inflammation in RAW 264.7 macrophages and mouse models. *Future medicinal chemistry*, 2018. 10(19):2253-2264
DOI: <https://doi.org/10.4155/fmc-2018-0172>.
40. Mao, T. and J. Fan, Myricetin Protects Against Rat Intervertebral Disc Degeneration Partly Through the Nrf2/HO-1/NF- κ B Signaling Pathway. *Biochemical Genetics*, 2023:1-18
DOI: <https://doi.org/10.1007/s10528-023-10456-z>.
41. Li, Y., Zhu Y., Li W., Liu L. Shen G., Zhu Y. and Tu Q., Syringic acid (SA) inhibits the IL-1 β -induced inflammation in mice chondrocytes and ameliorates the progression of osteoarthritis via the PTEN/AKT/NF- κ B pathway. *Journal of Functional Foods*, 2023. 107: 105683
DOI: <https://doi.org/10.1016/j.jff.2023.105683>.
42. Li, K., Gong Q., Lu B., Huang K., Tong Y., Mutsvene T.E., Lin M., et al., Anti-inflammatory and antioxidative effects of gallic acid on experimental dry eye: in vitro and in vivo studies. *Eye and Vision*, 2023. 10(1):17
DOI: <https://doi.org/10.1186/s40662-023-00334-5>.