J Herbmed Pharmacol. 2022; 11(2): 278-285.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2022.33

Journal of Herbmed Pharmacology

Antidesma thwaitesianum Müll. Arg. fruit extract rich in 5-hydroxymethylfurfural exhibits anti-inflammatory effects in lipopolysaccharide-stimulated RAW264.7 macrophages

Benchaluk Thongchuai[1], Supaporn Khamchun[1], Wimonrut Insuan[2], Daoyot Daorueang[1], Plubplung Sansai[1], Orapin Insuan[1*]

[1]Department of Medical Technology, School of Allied Health Sciences, University of Phayao, Phayao 56000, Thailand
[2]Department of Veterinary Technology, Faculty of Veterinary Technology, Kasetsart University, Bangkok 10900, Thailand

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 6 January 2022
Accepted: 15 February 2022

Keywords:
Pro-inflammatory cytokines
Inducible nitric oxide synthase
Inflammation
Nitric oxide
Interleukin
Tumour necrosis factor

ABSTRACT

Introduction: *Antidesma thwaitesianum* Müll. Arg is a tropical fruit, which has been commonly used for healthy food and traditional herbal medicine. This study aimed to investigate the anti-inflammatory effects of *A. thwaitesianum* fruit extract (AFE) rich in 5-hydroxymethylfurfural (5-HMF) in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages.

Methods: The chemical composition of AFE was analysed by gas chromatography/mass spectrometry (GC/MS). RAW264.7 cells were used as an *in vitro* inflammatory response model. RAW264.7 cells were pre-treated with various concentrations of AFE or 5-HMF and subsequently treated with LPS. Cell viability was assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The production levels of pro-inflammatory cytokines and mediators, including nitric oxide (NO), interleukin (IL)-1β, and tumour necrosis factor (TNF)-α were determined by the Griess assay and enzyme-linked immunosorbent assay. The protein expression of inducible nitric oxide synthase (iNOS) was examined by western blot analysis.

Results: AFE had a high content of 5-HMF (61.03% ± 0.49%). Pre-treatment with AFE and 5-HMF markedly reduced LPS-induced pro-inflammatory mediators and cytokines, namely NO, IL-1β, and TNF-α, in RAW264.7 cells; this reduction correlated with downregulation of iNOS expression.

Conclusion: This study suggests that *A. thwaitesianum* fruit extract containing 5-HMF could modulate the LPS-induced inflammatory response by inhibiting NO, IL-1β, and TNF-α production and iNOS expression. *A. thwaitesianum* fruit extract rich in 5-HMF could be considered a potential therapeutic agent for the prevention of inflammation.

Implication for health policy/practice/research/medical education:
Antidesma thwaitesianum fruit extract rich in 5-HMF attenuated LPS-stimulated inflammatory response by inhibiting NO, IL-1β and TNF-α production and iNOS expression. Therefore, *A. thwaitesianum* rich in 5-HMF might be considered a potential therapeutic agent for inflammatory disease treatment.

Please cite this paper as: Thongchuai B, Khamchun S, Insuan W, Daorueang D, Sansai P, Insuan O. *Antidesma thwaitesianum* Müll. Arg. fruit extract rich in 5-hydroxymethylfurfural exhibits anti-inflammatory effects in lipopolysaccharide-stimulated RAW264.7 macrophages. J Herbmed Pharmacol. 2022;11(2):278-285. doi: 10.34172/jhp.2022.33.

Introduction

Inflammation is a normal self-protective response to foreign pathogens or tissue injury. However, chronic inflammation leads to the pathogenesis of several diseases, such as arthritis, cardiovascular diseases, diabetes mellitus, metabolic syndromes, and cancers (1). Macrophages are major mononuclear phagocytic cells that play vital roles in the pathogenesis of inflammatory diseases. During the inflammatory process, pro-inflammatory cytokines and inflammatory mediators, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, nitric oxide (NO), and prostaglandin E_2 (PGE_2), are overproduced by macrophages

***Corresponding author**: Orapin Insuan,
Email: orapin.th@up.ac.th

(2-4). A commonly used *in vitro* inflammatory response model is lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages; it allows researchers to assess the anti-inflammatory activities of naturally derived compounds (1). LPS, a component of the gram-negative bacterial cell wall, is one of the most potent activators of macrophages. Activated macrophages transcriptionally express inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, which are the key regulator enzymes associated with the production of NO and PGE_2, respectively (5). iNOS catalyses the oxidative deamination of L-arginine to produce NO. Excessive NO generation leads to the accumulation of reactive oxygen species (ROS) and can cause harmful effects, including inflammatory diseases (6). COX-2 synthesises prostaglandins (PGs), which function as mediators of the inflammatory response to induce pain, fever, and other symptoms (7). The LPS-mediated expression of inflammatory cytokines involved in the expression of specific genes is regulated by nuclear transcription factor kappa B (NF-κB) and the mitogen-activated protein kinase (MAPK) signalling pathways (8,9). NF-κB, a transcription factor composed of the p65 and p50 subunits, regulates the transcription of multiple genes, various inducible enzymes (COX-2 and iNOS), and pro-inflammatory cytokines (10,11). MAPKs, including extracellular signal-regulated kinase (ERK), c-Jun N terminal kinase (JNK), and p38, control the expression of pro-inflammatory mediators and cytokines by sequential phosphorylation (12,13). They are involved in the LPS-induced expression of iNOS and COX-2 in activated macrophages (1,14). Thus, regulating the expression of pro-inflammatory mediators and cytokines is one of the most effective strategies to treat inflammation.

Antidesma thwaitesianum Müll. Arg (family: Euphorbiaceae), commonly known as mamao luang, is a tropical fruit that is typically used for soft drinks and healthy food. Moreover, it has been used as a traditional herbal medicine because it contains high amounts of phenolic acids, flavonoids, and anthocyanins. Vanillic acid, gallic acid, caffeic acid, *p*-coumaric acid, ferulic acid, sinapinic acid, and cinnamic acid are the phenolic acids in mamao (15). The most abundant flavonoids are (-)-epicatechin and (+)-catechin (flavan-3-ols). Cyanidin-3-*O*-glucoside, cyanidin 3-rutinoside, and malvidin 3,5-diglucoside are the main anthocyanin glycosides presented in mamao fruits (15). Fresh and dried fruits of *A. thwaitesianum* have many biological properties, including antioxidant, anticancer, antimicrobial, and anti-inflammatory activities (16-20). Several studies have reported that *A. thwaitesianum* extract remarkably reduced the expression of pro-inflammatory genes such as TNF-α, IL-6, vascular cell adhesion molecule-1 (VCAM-1), monocyte chemoattractant protein-1 (MCP-1), and endothelial nitric oxide synthase (eNOS) (19). Besides, it also induced apoptosis in human breast epithelial (MCF10A) cells by inhibiting poly(ADP-ribose) polymerase (PARP)/caspase-3 cleavage, inducing anti-apoptotic Bcl-2 expression, and downregulating pro-apoptotic Bax (21). According to recent reports, the biological activities of *A. thwaitesianum* are associated with phenolic composition. Recently, it has been reported that various food products such as dried fruits and fruit juices are also rich in 5-hydroxymethylfurfural (HMF), which acts as an anticarcinogenic, antioxidant, antiproliferative, and anti-apoptotic agent (22-24). In addition, 5-HMF has been reported to inhibit the release of NO, PGE_2, and pro-inflammatory cytokines in LPS-stimulated macrophages (25). Hence, we explored the anti-inflammatory effects of *A. thwaitesianum* fruit extract rich in 5-HMF in LPS-stimulated RAW264.7 macrophages. To correlate 5-HMF with bioactivity, we used gas chromatography/mass spectrometry (GC/MS) to analyse the active component of *A. thwaitesianum* fruit extract.

Materials and Methods

Plant material

Fruits of *A. thwaitesianum* were harvested from Phayao Province, Thailand (19°05′29″N, 99°48′33″E; 515 m altitude) in December 2020. A voucher specimen was deposited at Queen Sirikit Botanic Garden Herbarium, Chiang Mai, Thailand (QBG No. 128865).

Chemicals and reagents

5-HMF, LPS from *Escherichia coli* serotype O111:B4, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum, streptomycin, and penicillin were obtained from Invitrogen Gibco (Grand Island, NY, USA). The Griess reagent was purchased from Invitrogen, Thermo Fisher Scientific, Inc. (Eugene, OR, USA). TNF-α and IL-1β enzyme-linked immunosorbent assay (ELISA) kits were purchased from R&D Systems, Inc. (Minneapolis, MN, USA). Antibodies against iNOS and β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). All other chemicals were analytical grade.

Preparation of *A. thwaitesianum* fruit extract and GC/MS analysis

The ripe dried fruit of *A. thwaitesianum* was ground to powder and mixed with ethanol (50% v/v) at the ratio of 1:3. The extraction was performed with a magnetic stirrer at 50°C for 45 minutes. After filtering through folded paper, the filtrate was evaporated and lyophilised. This ethanol extract was subjected to GC/MS analysis and anti-inflammatory studies. The GC/MS analysis of *A. thwaitesianum* fruit extract (AFE) was performed by using a GCMS-QP2010 apparatus (Shimadzu, Kyoto, Japan) and an SH-Rxi-5Si-MS column (30 m × 0.25 mm, 0.25 μm film thickness; Zebron, CA, USA) and a method modified from Sarpate et al (26). GC/MS was carried out

by using split injection (split ratio 5:1) for a 1 µL sample, with the following temperature settings: injector set at 250°C, column set at 60°C, heating ramp of 10°C/min, final temperature of 280°C, and detector set at 200°C. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. Fragmentation was performed by electron impact (70 eV), and the mass range was between 40 and 500 amu. Identification of compounds was based on comparisons of the mass spectra with those of the NIST (NIST14) mass spectral library data standard of the GC/MS system.

Cell culture

The RAW264.7 mouse macrophage cell line was obtained from American Type Culture Collection (ATCC® number TIB-71) and cultured in DMEM supplemented with 10% fetal bovine serum and 100 units/mL penicillin–streptomycin under a humidified (95%) atmosphere of 5% CO_2 at 37°C.

Cell viability test

RAW264.7 cells were plated into a 96-well plate at a density of 2×10^4 cells/well (cell viability >95%). After 24 hours, the cells were pre-treated with various concentrations of AFE or 5-HMF for 2 hours and subsequently treated with 1 µg/mL LPS for 22 hours. Next, the cells were incubated with MTT solution at the final concentration of 0.5 mg/mL for 4 hours at 37°C. Then, the supernatant was removed, 100 µL of dimethyl sulfoxide was added to dissolve the formazan crystals, and absorbance at 540 nm was determined by using a microplate reader. Untreated cells with a cell viability of 100% were used as the control. The cell viability of the treated wells was calculated as a percentage relative to that of the control.

Measurement of NO and cytokine production

RAW264.7 cells (1×10^6 cells/well) were plated into a 6-well plate and treated with various concentrations of AFE or 5-HMF. After 2 hours of treatment, the cells were incubated with LPS (1 µg/mL) for 22 hours. The NO production in the cell culture supernatant was measured by using a Griess reagent kit (Invitrogen, Thermo Fisher Scientific, Inc., Eugene, OR, USA), whereas the levels of IL-1β and TNF-α were detected by using ELISA kits (R&D Systems, Minneapolis, MN, USA) following the manufacturer's instructions.

Western blot analysis

RAW264.7 cells were plated into 6-well plate at a concentration of 1×10^6 cells/mL. After 24 hours, the cells were treated with different concentrations of AFE or 5-HMF for 2 hours and subsequently treated with LPS (1 µg/mL) for 22 hours. The cells were harvested and washed with cold phosphate buffer saline. Total proteins were extracted from the cells using Laemmli lysis buffer (Bio-Rad Laboratories, Inc., Hercules, CA, USA), and the protein concentration was quantified by using a Bradford assay kit (Thermo Fisher Scientific, Inc., IL, USA). Western blot analysis was performed according to a standard protocol. Briefly, 30 µg of proteins was separated by 10% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and transferred to nitrocellulose membranes. The membranes were incubated in 5% non-fat dry milk in Tris-buffered saline (TBS) to block nonspecific protein binding. The membranes were subsequently incubated with primary rabbit monoclonal antibodies against iNOS and β-actin, the latter served as a loading control, at 4°C overnight. The membranes were then washed three times with 0.05% Tween 20-TBS and incubated with horseradish peroxidase–conjugated secondary antibodies at room temperature for 1 hour. Protein bands were detected by using an enhanced chemiluminescence reagent (Bio-Rad Laboratories) and then exposed to X-ray film. Band intensity data were obtained by using ImageJ software (https://imagej.nih.gov/ij).

Statistical analysis

All results are expressed as mean ± standard deviation (SD). Differences between the treatments were determined by one-way analysis of variance (ANOVA). Statistical significance was accepted for P values < 0.05.

Results

Chemical characteristics of *A. thwaitesianum* fruit extract

The chemical composition of *A. thwaitesianum* fruit extract (AFE) is shown in Table 1. The AFE contained 22 compounds; the main compound was 5-HMF (61.03% ± 0.49%). Other abundant compounds were D-allose (aldohexose) (9.16% ± 0.33%), 3-methyl-2,5-furandione (6.25% ± 0.09%), 1,6-anhydro-beta-D-glucofuranose (5.22% ± 0.25%), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (4.72% ± 0.15%).

Cytotoxic effects of *A. thwaitesianum* fruit extract and 5-HMF on RAW264.7 cells

The MTT assay was used to determine the cytotoxicity of AFE and 5-HMF in RAW264.7 cells. Treatment with both AFE (12.5-400 µg/mL) and 5-HMF (12.5-400 µg/mL) with or without LPS (1 µg/mL) had no effect on the viability of RAW264.7 cells. The percentage of cell survival under all conditions relative to the control exceeded 80% (Figure 1). Therefore, the 100-400 µg/mL AFE and 50-200 µg/mL 5-HMF were used in the subsequent experiments.

Effects of *A. thwaitesianum* fruit extract and 5-HMF on LPS-induced NO production and iNOS expression in RAW264.7 cells

To investigate the anti-inflammatory properties of AFE and 5-HMF, we initially measured the production of NO in LPS-stimulated RAW264.7 cells. As shown in Figures 2A and 2B, the NO level in the LPS-treated group showed a significant increase relative to that in the control group

Table 1. Chemical constituents and their percentages in *Antidesma thwaitesianum* fruit extract identified by gas chromatography/mass spectrometry (GC/MS)

Retention time (min)	Peak area (%)	Chemical formula	Chemical composition
3.52	6.25 ± 0.09	$C_5H_4O_3$	3-Methyl-2,5-furandione
3.78	0.89 ± 0.03	$C_6H_6O_2$	5-Methyl-2-furancarboxaldehyde
4.00	0.89 ± 0.11	$C_6H_8O_4$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4.36	0.21 ± 0.02	$C_8H_{16}BNO_3$	2-Butyl-, methyl ester, L-1,3,2-oxazaborolane-4-carboxylic acid
4.61	1.34 ± 0.10	$C_5H_4O_3$	Dihydro-3-methylene-2,5-furandione
4.82	0.91 ± 0.01	$C_5H_8O_3$	4-Oxo-pentanoic acid
5.21	0.27 ± 0.02	$C_3H_6N_6$	1,3,5-Triazine-2,4,6-triamine
5.24	0.44 ± 0.01	$C_6H_4O_3$	2,5-Furandicarboxaldehyde
5.33	1.50 ± 0.08	$C_6H_6O_3$	Furyl hydroxymethyl ketone
5.70	0.21 ± 0.01	$C_6H_{12}O_2$	Butyl acetate
5.72	0.29 ± 0.02	$C_6H_6O_3$	Levoglucosenone
6.19	4.72 ± 0.15	$C_6H_8O_4$	2,3-Dihydro-3,5-dihydroxy-6-methyl -4H-pyran-4-one
6.61	0.43 ± 0.05	$C_7H_{12}O_3$	Ethyl propionylacetate
6.95	0.43 ± 0.03	$C_8H_{16}O_2$	2-Pentanol, propanoate
7.05	0.26 ± 0.01	$C_6H_{10}O4$	1,2:5,6-dianhydrogalactitol
7.35	61.03 ± 0.49	$C_6H_6O_3$	5-Hydroxymethylfurfural
7.84	1.38 ± 0.08	$C_5H_6O_4$	Methylene-butanedioic acid
9.40	2.84 ± 0.11	$C_{14}H_{26}O_4$	Succinic acid, 3-methylbutyl pentyl ester
10.26	0.79 ± 0.01	$C_4H_9NO_5$	2-(hydroxymethyl)-2-nitro-1,3-propanediol
10.91	9.16 ± 0.33	$C_6H_{12}O_6$	D-allose
11.68	0.57 ± 0.09	$C_4H_4N_2O_2$	1-(Beta-d-ribofuranosyl)-5-fluoro-4-O-difluoromethyl uracil
12.12	5.22 ± 0.25	$C_6H_{10}O_5$	1,6-Anhydro-beta-D-glucofuranose

Each value is expressed as mean ± SD of three measurements.

($P < 0.005$). NO release was inhibited dramatically by both AFE and 5-HMF in a dose-dependent manner ($P < 0.005$). NO production is related directly to upregulation of the expression of iNOS. Therefore, the effect of AFE and 5-HMF on LPS-induced iNOS protein expression was examined by western blot analysis. The iNOS protein level was markedly increased by LPS stimulation and suppressed by AFE (400 μg/mL) and 5-HMF (100 and 200 μg/mL) treatment (Figure 2C-F).

Effects of *A. thwaitesianum* fruit extract and 5-HMF on LPS-induced IL-1β and TNF-α in RAW264.7 cells

As the treatment of AFE and 5-HMF inhibited the expression of the pro-inflammatory mediators NO

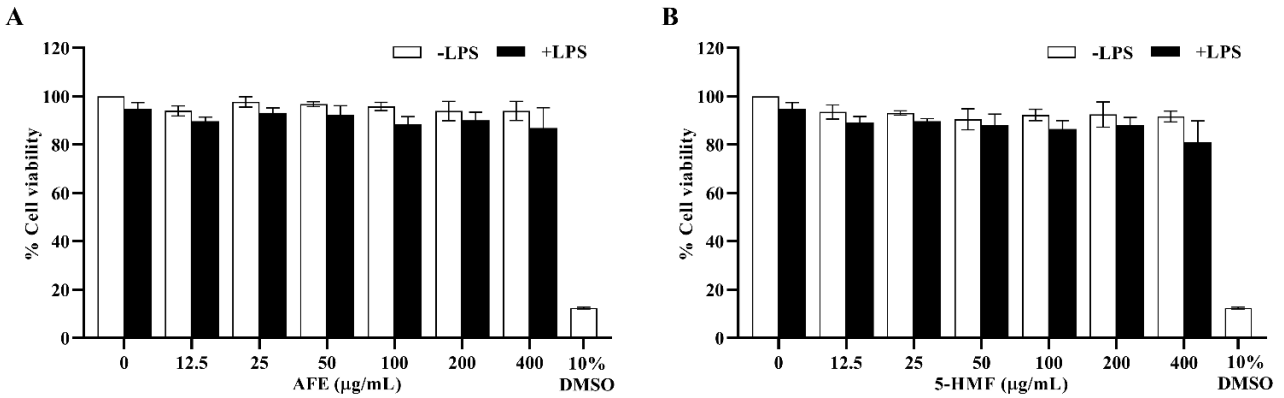

Figure 1. The effect of *Antidesma thwaitesianum* fruit extract (AFE) and 5-hydroxymethylfurfural (5-HMF) on the viability of RAW264.7 macrophages. The cells were treated with indicated concentrations of A: AFE and B: 5-HMF in the absence or presence of lipopolysaccharide (LPS). The results are expressed as the mean ± SD (n = 3).

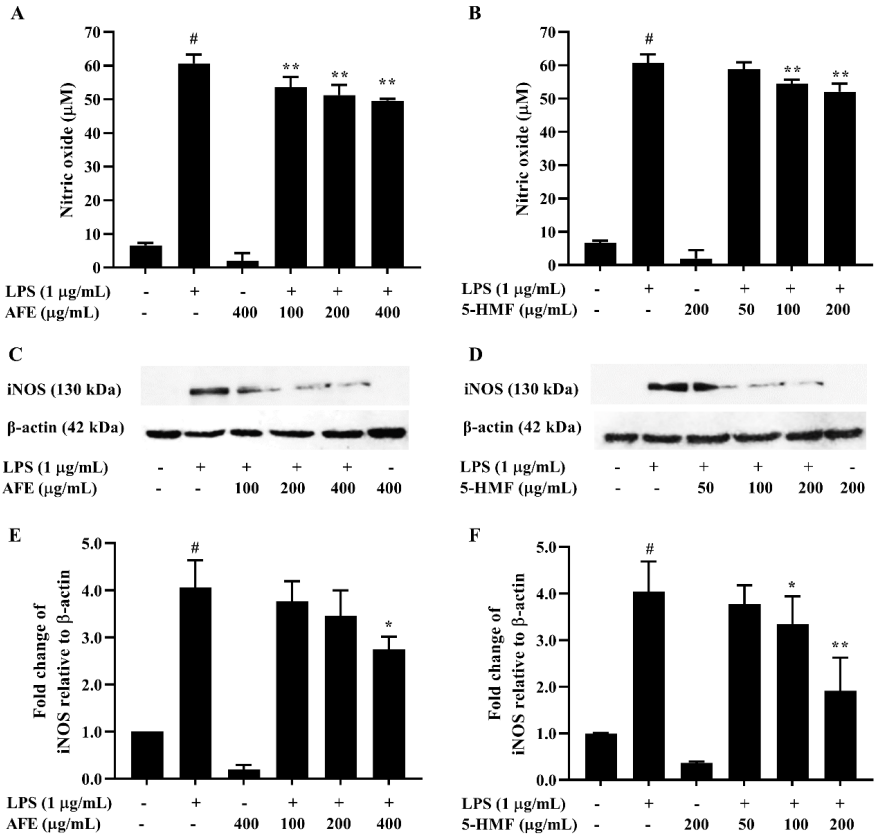

Figure 2. The effect of *Antidesma thwaitesianum* fruit extract (AFE) and 5-hydroxymethylfurfural (5-HMF) on the production of NO and the expression of iNOS proteins in LPS-induced RAW264.7 macrophages. (A,B) The level of NO in cell culture supernatant was determined by the Griess assay. (C-F) The expression of iNOS protein was determined by western blot analysis. The results are expressed as mean ± SD (n = 3). $^{\#}P < 0.005$ indicates a significant difference from the LPS-untreated cells; $^{*}P < 0.05$ and $^{**}P < 0.005$ indicate significant differences from the lipopolysaccharide (LPS) treatment alone.

and iNOS, the effects of AFE and 5-HMF on the pro-inflammatory cytokines, including IL-1β and TNF-α, were examined by ELISA. The levels of IL-1β and TNF-α were significantly higher in the LPS-treated group than in the control group (Figure 3). Pre-treatment with various concentrations of 5-HMF (50-200 μg/mL) significantly decreased LPS-stimulated IL-1β and TNF-α production in a dose-dependent manner (Figure 3B and 3D). The produced level of TNF-α was dramatically and dose-dependently reduced by AFE relative to that in the LPS-treated group (Figure 3C). However, the production level of IL-1β was not changed (Figure 3A).

Discussion

Dietary components have notable biological effects, including the ability to ameliorate inflammation. While steroids and anti-inflammatory non-steroidal compounds are commonly used to treat chronic inflammatory diseases, these drugs may have undesirable side effects (27,28). Research on the anti-inflammatory effects of natural compounds aims to provide alternative therapies. There have been a number of investigations on *A. thwaitesianum* fruit and it anti-inflammatory actions. In this study, we focussed on the anti-inflammatory effects of AFE rich in 5-HMF in LPS-stimulated macrophages. GC/MS was used to analyse the chemical constituents of AFE. The major constituent detected in AFE was 5-HMF (61.03% ± 0.49%). 5-HMF can be found naturally in honey and processed foods, including fruit juices, UHT milk, and baked foods (29). Besides, it can be produced by acid-catalysed thermal dehydration of fructose (30). Thus, our result demonstrated the potential of *A. thwaitesianum* fruit extract as a natural source of 5-HMF. Several beneficial effects of 5-HMF have been reported, including anti-allergic, antioxidative, anticancer, and anti-inflammatory properties (25,31). Maillard reaction products containing 5-HMF exert antioxidant and anti-inflammatory effects in interferon-γ and phorbol ester–induced Caco-2 cells (32). Researchers have also explored the anti-inflammatory activities of 5-HMF in LPS-induced RAW264.7 cells via suppression of MAPK, NF-κB, and Akt/mTOR signalling pathways.

Macrophages are innate immune cells that play a role as sensors and responders to inflammation (33,34). The model of LPS-induced inflammatory responses in RAW264.7 cells has been widely used for anti-inflammatory drug

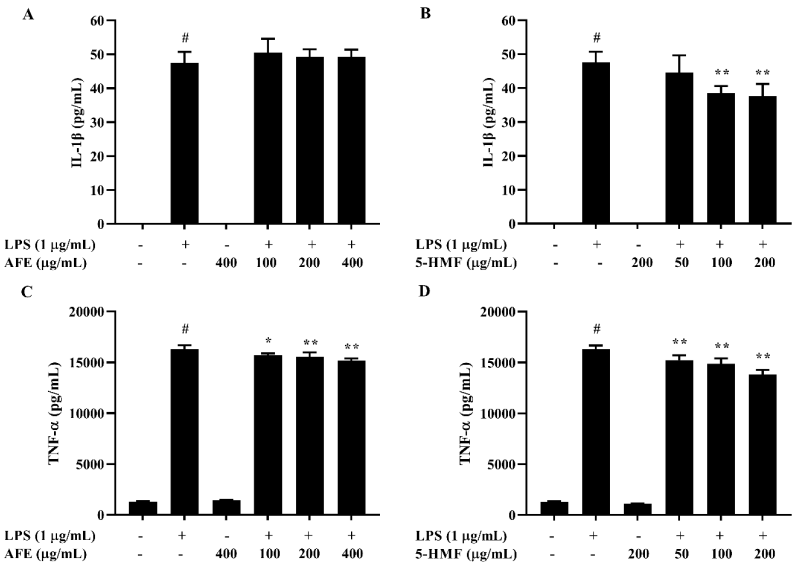

Figure 3. The effect of *Antidesma thwaitesianum* fruit extract (AFE) (A,C) and 5-hydroxymethylfurfural (5-HMF) (B,D) on the production of pro-inflammatory cytokines in LPS-induced RAW264.7 macrophages. The results are expressed as mean ± SD (n = 3). $^{\#}p < 0.005$ indicates a significant difference from the LPS-untreated cells; $^{*}P < 0.05$ and $^{**}P < 0.005$ indicate significant differences from the lipopolysaccharide (LPS) treatment alone.

screening. In the present study, we confirmed that AFE rich in 5-HMF had an anti-inflammatory effect on the LPS-stimulated inflammatory response of RAW 264.7 cells. During inflammation, macrophage activation results in increased production of NO, a reactive free radical, by iNOS. High NO levels are cytotoxic in inflammation (35). Therefore, the inhibition of NO and iNOS overexpression is important for anti-inflammation. We found that AFE rich in 5-HMF and 5-HMF alone effectively inhibited NO production by downregulating iNOS protein expression in LPS-stimulated RAW 264.7 cells. Hence, AFE rich in 5-HMF and 5-HMF can inhibit the production of inflammatory mediators. LPS-induced macrophage activation can lead to the production of pro-inflammatory cytokines and mediators, including IL-1β, IL-6, and TNF-α (1). TNF-α is central in the inflammatory process and is normally found in chronic inflammatory diseases (36), while IL-1β is important for the initiation and enhancement of the inflammatory response (37). Therefore, blocking pro-inflammatory cytokine production is one of the most effective strategies for the treatment of inflammation. Our results support the anti-inflammatory effects of AFE rich in 5-HMF and 5-HMF in LPS-stimulated RAW264.7 cells. AFE and 5-HMF significantly inhibited the release of TNF-α, whereas only 5-HMF inhibited IL-1β production. The potential of AFE rich in 5-HMF to reduce LPS-induced inflammation was confirmed by its ability to reduce NO, TNF-α, and IL-1β, which are central in inflammation.

Conclusion

The fruit extract of *A. thwaitesianum* containing 5-HMF and 5-HMF alone exerted anti-inflammatory properties by suppressing the production of NO, TNF-α, and IL-1β, reductions that correlated with downregulation of iNOS expression. *A. thwaitesianum* rich in 5-HMF might be considered a potential therapeutic agent for inflammatory disease treatment. Despite the fact that we have established the anti-inflammatory effect of *A. thwaitesianum* rich in 5-HMF, the molecular mechanisms underlying the inhibitory effect of the extract need to be elucidated.

Authors' contributions

BT and WI performed the experiments, analysed the data, and wrote the manuscript. SK, DD, and PS performed the experiments and analysed the data. OI designed and performed the experiments, analysed the data, and wrote and edited the manuscript. All co-authors revised and approved the final version of the manuscript.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

Ethical issues, including text plagiarism, misconduct, data fabrication, falsification, double submission or publication, redundancy have been carefully observed by authors.

Funding/Support

This work was supported by the Thailand Science Research and Innovation Fund and the University of Phayao (Grant No. FF64-RIM040).

References

1. Dong L, Yin L, Zhang Y, Fu X, Lu J. Anti-inflammatory effects

of ononin on -stimulated RAW 264.7 cells. Mol Immunol. 2017;83:46-51.doi:10.1016/j.molimm.2017.01.007.
2. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol. 2008;8(12):958-69. doi: 10.1038/nri2448.
3. Wirtz PH, von Känel R. Psychological stress, inflammation, and coronary heart disease. Curr Cardiol Rep. 2017;19(11):111. doi: 10.1007/s11886-017-0919-x.
4. McInnes IB, Schett G. Pathogenetic insights from the treatment of rheumatoid arthritis. Lancet. 2017; 389(10086):2328-37. doi: 10.1016/s0140-6736(17)31472-1.
5. Aktan F. iNOS-mediated nitric oxide production and its regulation. Life Sci. 2004;75(6):639-53. doi: 10.1016/j.lfs.2003.10.042.
6. Jung YS, Kim DH, Hwang JY, Yun NY, Lee YH, Han SB, et al. Anti-inflammatory effect of tricin 4'-O-(threo-β-guaiacylglyceryl) ether, a novel flavonolignan compound isolated from Njavara on in RAW264.7 cells and in ear mice edema. Toxicol Appl Pharmacol. 2014;277(1):67-76. doi: 10.1016/j.taap.2014.03.001.
7. Jin M, Suh SJ, Yang JH, Lu Y, Kim SJ, Kwon S, et al. Anti-inflammatory activity of bark of *Dioscorea batatas* DECNE through the inhibition of iNOS and COX-2 expressions in RAW264.7 cells via NF-κB and ERK1/2 inactivation. Food Chem Toxicol. 2010;48(11):3073-9. doi: 10.1016/j.fct.2010.07.048.
8. Guha M, Mackman N. LPS induction of gene expression in human monocytes. Cell Signal. 2001;13(2):85-94. doi: 10.1016/s0898-6568(00)00149-2.
9. Zhang JY, Jin H, Wang GF, Yu PJ, Wu SY, Zhu ZG, et al. Methyl-1-hydroxy-2-naphthoate, a novel naphthol derivative, inhibits lipopolysaccharide-induced inflammatory response in macrophages via suppression of NF-κB, JNK and p38 MAPK pathways. Inflamm Res. 2011;60(9):851-9. doi: 10.1007/s00011-011-0345-2.
10. Vermeulen L, De Wilde G, Notebaert S, Vanden Berghe W, Haegeman G. Regulation of the transcriptional activity of the nuclear factor-kappaB p65 subunit. Biochem Pharmacol. 2002;64(5-6):963-70. doi: 10.1016/s0006-2952(02)01161-9.
11. Shih RH, Wang CY, Yang CM. NF-kappaB signaling pathways in neurological inflammation: a mini review. Front Mol Neurosci. 2015;8:77. doi: 10.3389/fnmol.2015.00077.
12. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiol Mol Biol Rev. 2004;68(2):320-44. doi: 10.1128/mmbr.68.2.320-344.2004.
13. Thalhamer T, McGrath MA, Harnett MM. MAPKs and their relevance to arthritis and inflammation. Rheumatology (Oxford). 2008;47(4):409-14. doi: 10.1093/rheumatology/kem297.
14. Lee SH, Kwak CH, Lee SK, Ha SH, Park J, Chung TW, et al. Anti-inflammatory effect of ascochlorin in LPS-stimulated RAW 264.7 macrophage cells is accompanied with the down-regulation of iNOS, COX-2 and proinflammatory cytokines through NF-κB, ERK1/2, and p38 signaling pathway. J Cell Biochem. 2016;117(4):978-87. doi: 10.1002/jcb.25383.
15. Jorjong S, Butkhup L, Samappito S. Phytochemicals and antioxidant capacities of Mao-Luang (*Antidesma bunius* L.) cultivars from Northeastern Thailand. Food Chem. 2015;181:248-55. doi: 10.1016/j.foodchem.2015.02.093.
16. Puangpronpitag D, Areejitranusorn P, Boonsiri P, Suttajit M, Yongvanit P. Antioxidant activities of polyphenolic compounds isolated from *Antidesma thwaitesianum* Müll. Arg. seeds and marcs. J Food Sci. 2008;73(9):C648-53. doi: 10.1111/j.1750-3841.2008.00951.x.
17. Pheungsomphane S, Tinchan P, Tayuan C. Total phenolic content and biological activity of *Antidesma thwaitesianum* Müll. Arg. fruit extracts. Khon Kean Agric J. 2019;47(Suppl 1):495-500.
18. Hansakul P, Dechayont B, Phuaklee P, Prajuabjinda O, Juckmeta T, Itharat A. Cytotoxic and antioxidant activities of *Antidesma thwaitesianum* Müll Arg (Euphorbiaceae) fruit and fruit waste extracts. Trop J Pharm Res. 2015;14(4):627-34. doi: 10.4314/tjpr.v14i4.10.
19. Udomkasemsab A, Ngamlerst C, Kwanbunjun K, Krasae T, Amnuaysookkasem K, Chunthanom P, et al. Maoberry (*Antidesma bunius*) improves glucose metabolism, triglyceride levels, and splenic lesions in high-fat diet-induced hypercholesterolemic rats. J Med Food. 2019;22(1):29-37. doi: 10.1089/jmf.2018.4203.
20. Anantachoke N, Lomarat P, Praserttirachai W, Khammanit R, Mangmool S. Thai fruits exhibit antioxidant activity and induction of antioxidant enzymes in HEK-293 Cells. Evid Based Complement Alternat Med. 2016;2016:6083136. doi: 10.1155/2016/6083136.
21. Puangpronpitag D, Yongvanit P, Boonsiri P, Suttajit M, Areejitranusorn P, Na H-K, et al. Molecular mechanism underlying anti-apoptotic and anti-inflammatory effects of Mamao (*Antidesma thwaitesianum* Müll. Arg.) polyphenolics in human breast epithelial cells. Food Chem. 2011;127(4):1450-8. doi: 10.1016/j.foodchem.2011.01.099.
22. Capuano E, Fogliano V. Acrylamide and 5-hydroxymethylfurfural (HMF): a review on metabolism, toxicity, occurrence in food and mitigation strategies. LWT Food Sci Technol. 2011;44(4):793-810. doi: 10.1016/j.lwt.2010.11.002.
23. Hardt-Stremayr M, Mattioli S, Greilberger J, Stiegler P, Matzi V, Schmid MG, et al. Determination of metabolites of 5-hydroxymethylfurfural in human urine after oral application. J Sep Sci. 2013;36(4):670-6. doi: 10.1002/jssc.201200768.
24. Abraham K, Gürtler R, Berg K, Heinemeyer G, Lampen A, Appel KE. Toxicology and risk assessment of 5-hydroxymethylfurfural in food. Mol Nutr Food Res. 2011;55(5):667-78. doi: 10.1002/mnfr.201000564.
25. Kong F, Lee BH, Wei K. 5-hydroxymethylfurfural mitigates lipopolysaccharide-stimulated inflammation via suppression of MAPK, NF-κB and mTOR activation in RAW 264.7 cells. Molecules. 2019;24(2):275. doi: 10.3390/molecules24020275.
26. Sarpate RV, Deore TK, Patil MV, Tupkari SV. Characterization of anthocyanins by GCMS. Int J Chem Sci. 2010;8(1):415-23.
27. Checker R, Sharma D, Sandur SK, Khanam S, Poduval TB. Anti-inflammatory effects of plumbagin are mediated by inhibition of NF-kappaB activation in lymphocytes. Int Immunopharmacol. 2009;9(7-8):949-58. doi: 10.1016/j.intimp.2009.03.022.
28. Pinho BR, Sousa C, Valentão P, Andrade PB. Is nitric oxide

decrease observed with naphthoquinones in LPS stimulated RAW 264.7 macrophages a beneficial property? PLoS One. 2011;6(8):e24098. doi: 10.1371/journal.pone.0024098.
29. Shapla UM, Solayman M, Alam N, Khalil MI, Gan SH. 5-hydroxymethylfurfural (HMF) levels in honey and other food products: effects on bees and human health. Chem Cent J. 2018;12(1):35. doi: 10.1186/s13065-018-0408-3.
30. Zirbes L, Nguyen BK, de Graaf DC, De Meulenaer B, Reybroeck W, Haubruge E, et al. Hydroxymethylfurfural: a possible emergent cause of honey bee mortality? J Agric Food Chem. 2013;61(49):11865-70. doi: 10.1021/jf403280n.
31. Zhao L, Chen J, Su J, Li L, Hu S, Li B, et al. In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. J Agric Food Chem. 2013;61(44):10604-11. doi: 10.1021/jf403098y.
32. Kitts DD, Chen XM, Jing H. Demonstration of antioxidant and anti-inflammatory bioactivities from sugar-amino acid Maillard reaction products. J Agric Food Chem. 2012;60(27):6718-27. doi: 10.1021/jf2044636.
33. Stewart AG, Beart PM. Inflammation: maladies, models, mechanisms and molecules. Br J Pharmacol. 2016;173(4):631-4. doi: 10.1111/bph.13389.
34. Brüne B, Dehne N, Grossmann N, Jung M, Namgaladze D, Schmid T, et al. Redox control of inflammation in macrophages. Antioxid Redox Signal. 2013;19(6):595-637. doi: 10.1089/ars.2012.4785.
35. Nussler AK, Billiar TR. Inflammation, immunoregulation, and inducible nitric oxide synthase. J Leukoc Biol. 1993;54(2):171-8.
36. Andreakos ET, Foxwell BM, Brennan FM, Maini RN, Feldmann M. Cytokines and anti-cytokine biologicals in autoimmunity: present and future. Cytokine Growth Factor Rev. 2002;13(4-5):299-313. doi: 10.1016/s1359-6101(02)00018-7.
37. El-Omar EM, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature. 2000;404(6776):398-402. doi: 10.1038/35006081.