Heliyon 10 (2024) e26174

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Anti-inflammatory effect of *Piper longum* L. fruit methanolic extract on lipopolysaccharide-treated RAW 264.7 murine macrophages

Uyen Thi Tu Phan [a], Hai Dang Nguyen [a], Thi Kieu Oanh Nguyen [a], Tuan Hiep Tran [b], Thanh Huong Le [a], Thi Thu Phuong Tran [a,*]

[a] *University of Science and Technology of Hanoi (USTH), Vietnam Academic Science and Technology (VAST), 18 Hoang Quoc Viet, Nghia Do, Cau Giay, Hanoi, Viet Nam*

[b] *Faculty of Pharmacy, PHENIKAA University, Yen Nghia, Ha Dong, Hanoi, 12116, Viet Nam*

A R T I C L E I N F O

Keywords:
Piper longum L.
RAW 264.7 murine macrophage
anti-Inflammation
iNOS
COX-2
MAPKs
HO-1

A B S T R A C T

Context: The *Piper* species was studied several potential properties such as anti-tumor, anti-inflammatory and antioxidant activity. However, the specific anti-inflammatory activity of the extract from the fruits of *P. longum* L. has not been investigated.

Objectives: Our study want to examine the anti-inflammatory effects of *P. longum* L. fruit methanolic extracts (PLE) on lipopolysachharide (LPS)-stimulated RAW 264.7 murine macrophages to understand the mechanism of this effect.

Method: This study examined the chemical profiling of PLE by LC-HRMS analysis and measured the presence of nitric oxide (NO), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) in the supernatant using the Griess reagent assay and enzyme-linked immunosorbent assay (ELISA), respectively. The mRNA expression of IL-6, TNF-α, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) were evaluated by using real-time quantitative polymerase chain reaction (RT-qPCR). Furthermore, the protein expression of COX-2, iNOS and the phosphorylation of MAPK family, c-Jun N-terminal kinase (JNK), p38 in protein level were observed by western blotting.

Result: PLE have detected 66 compounds which belong to different classes such as alkaloids, flavonoids, terpenoids, phenolics, lactones, and organic acids inhibited nitric oxide products with the IC_{50} = 28.5 ± 0.91 μg/mL. Moreover, PLE at 10–100 μg/mL up-regulate HO-1 protein expression from 3 to 10 folds at 3 h. It also downregulated the mRNA and protein expression of iNOS, COX-2, decreased IL-6 and TNF-α secretion by modulating the mitogen-activated protein kinase (MAPK) signaling pathway, specifically by decreasing the phosphorylation of p38 and JNK.

Conclusion: These results shown chemical profiling of PLE and demonstrated that PLE exhibits anti-inflammatory effects by regulating the MAPK family and could be a potential candidate for the treatment of inflammatory diseases.

* Corresponding author.
E-mail address: tran-thi-thu.phuong@usth.edu.vn (T.T.P. Tran).

https://doi.org/10.1016/j.heliyon.2024.e26174
Received 23 October 2023; Received in revised form 7 February 2024; Accepted 8 February 2024
Available online 14 February 2024

1. Introduction

Inflammation is a natural immune response that protects the body against infections caused by pathogens and tissue damage [1]. Inflammation can be triggered by outer factors such as microbial infections (bacterial, fungi, virus), physical factors (trauma, radiation, temperature), chemical substances (irritant and corrosive chemicals) or inner factors such as tissue necrosis, and hypersensitive reactions [2]. This immune response is normally initiated by antigen presenting cells like macrophages. Macrophages possess the ability to identify and transmit antigens, triggering immune responses that involve the secretion of different inflammatory cytokines and chemokines [3].

Macrophages are exposed to stimuli like LPS, they activate intracellular signaling pathways, including nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinases (MAPKs), resulting in the release of pro-inflammatory mediators such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), nitric oxide (NO), cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) [4,5]. MAPK pathways, which coordinately regulate gene expression, mitosis, metabolism, motility, survival, apoptosis, and differentiation. Conventional MAPKs comprise the extracellular signal-regulated kinases ½ (ERK1/2), c-Jun amino (N)-terminal kinases 1/2/3 (JNK1/2/3), p38 isoforms, and ERK5. Atypical MAPKs have nonconforming particularities and comprise ERK3/4, ERK7, and Nemo-like kinase (NLK). However, the most extensively studied groups of mammalian MAPKs are the ERK1/2, JNKs, and p38 isoforms [6–11]. The three primary MAPK families are p38, extracellular signal-regulated protein kinase (ERK), and c-Jun amino-terminal kinase (JNK), and they regulate various physiological processes, including inflammation.

On the other hand, heme oxygenase-1 (HO-1) is an enzyme that provides cellular protection by responding to oxidative and/or inflammatory stimuli. Heme is catalytically destroyed by HO-1 to biliverdin and iron, with the production of carbon monoxide (CO) as a byproduct [12]. Compelling experimental evidence strongly supports the cytoprotective attributes of CO, encompassing the reduction of inflammation, modulation of cell apoptosis and proliferation, and impact on various cellular processes [13]. In addition, activation of HO-1 has been observed as a natural defense mechanism used by cells to mitigate inflammation and minimize tissue damage in certain injury models [14]. Therefore, HO-1 has garnered significant attention as a mechanism to combat oxidative stress and apoptosis.

Inflammation response is needed, however, when the balance between host defenses and multiple stimuli is disrupted, uncontrolled inflammation can lead to various acute and chronic conditions such as arthritis, diabetes, and autoimmune diseases [15–18]. Seeking the anti-inflammatory effect of natural products is still promising due to the diversity of plant resources and the safety of those compounds. In this study, we investigate the potential of *Piper longum* L. extract in managing the inflammation by examining the anti-inflammatory effects of the methanolic extract of the fruits of *Piper longum* (PLE) on LPS-stimulated murine macrophage RAW 264.7 cells, to elucidate the signaling pathways involved and gain a better understanding of the mechanism of action of this extract. *Piper longum* L. is a plant belonging of the *Piperaceae* family growing in India, Malaysia, Nepal, Sri Lanka, and Vietnam and used as traditional medicine. This plant has been utilized for treating various conditions including bronchitis, abdominal pain, spleen disorders, tumors, ascites, and biliousness relief. *P. longum* fruits were used as analgesic and anti-inflammatory [19]. However, a systemic investigation comprising *in vitro* and *in vivo* tests which explains the mechanisms in molecular level is absent. In the context of this work, MAPK pathways and HO-1 are main focus then based on the understanding of PLE on those, we will expand the investigation to provide a comprehensive knowledge that could contribute scientifically to clinical use of this plant.

2. Material and methods

2.1. Sample collection and preparation

Piper longum L. fruits were collected at the Me Linh Station for Biodiversity, Me Linh, Vinh Phuc in September 2022, and were identified by Dr. Nguyen The Cuong, the Institute of Ecology and Biological Resources. A voucher for specimens was deposited at the Department of Life Sciences, University of Science and Technology of Hanoi, VAST. Fruits of *Piper longum* L. were dried and extracted by methanol three times (with 30 min sonicate each time). The solvent was removed by rotary evaporation method. The extract (PLE) was dissolved in 99.9% DMSO into the concentration 20 mg/mL as a stock and stored at 4 °C until used.

2.2. LC-HRMS analysis

For LC-HRMS analysis, 10 mg of the dried extract was diluted with 1 mL of MeOH and filtered through 0.22 μm membrane before injecting to the system. The analysis was performed by ACQUITY UPLC I-Class Plus system coupled to a high-resolution Xevo G3 ESI/QTOF mass spectrometer (Waters Corporation). Chromatographic separation was achieved on an ACQUITY UPLC BEH C18, 130 Å, 1.7 μm, 2.1 mm × 100 mm, with the mobile phase as composition of solvent A (H_2O + 0.1%FA) and solvent B (ACN). The gradient elution program starts with 1 % B at 0.5 min, and linearly increased to 35 % B within 15.5 min, ramped to 100 % B in 2 min, then the column was re-equilibrated at 1 % B for 5 min before the next injection. The mobile phase was pumped at a flow rate of 0.6 mL/min. The sample was injected with a volume of 10 μL.

The QTOF parameters were set up under MSe mode which scan from *m/z* 100 to 1500. The scan was taken each 0.100s. In a low energy mode, the cone voltage of 6 V was applied while in a high energy, a range of voltage ramped from 15 to 40 V. Besides, the capillary voltage 2.00 kV, the sample cone 100 V, source temperature 120 °C, desolvation temperature 550 °C, cone gas 30 L/h, desolvation gas 1000 L/h were set for ESI positive mode of ionization.

The acquisition was done under the direction of Masslynx 4.2 software, and the obtained raw files were processed, visualized, and

reported by the UNIFI software (Waters Corporation). To dereplicate known compounds in the PLE, the QTOF based chromatogram was processed by UNIFY software using Waters Traditional Medicine Library (consist of 6308 compounds) (Waters corporation, US).

2.3. Cell culture

RAW 264.7 cells were purchased from ATCC, USA. Cells were cultured in DMEM (Gibco, Invitrogen, USA), supplemented with 10% FBS, 1% penicillin/streptomycin solution (penicillin 10000 units/mL and streptomycin 10000 μg/mL) (Gibco, Invitrogen, USA), at 37 °C in a humidified 5% CO_2 incubator.

2.4. Cell viability

Cell viability was determined by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. Cells were grown in 96-well plates (5×10^4 cells/well) with/without the various concentrations of extract treatment. After incubation for 24 h, cells were treated with the MTT solution for 4 h, and blue-violet formazan crystals formed in intact cells were dissolved with isopropanol. Absorbance was measured at 570 nm using a microplate reader (SpectraMax iD5, Molecular Devices, USA).

2.5. Estimation of nitric oxide level

Cells were grown in 96-well plates at a concentration of 5×10^4 cells/well and incubated in a humidified incubator at 37 °C, 5% CO_2 for 24 h. Cells were pre-activated for 30 min with different concentrations of PLE, then added 1 μg/mL LPS (*Escherichia coli* 0111: B4; Sigma Aldrich, USA) and incubated for the next 24 h. 100 μL of the supernatant of each well was transferred to another 96-well plate and 100 μL of Griess reagent was added. The absorbance of the assay was read at 540 nm using an iMark microplate reader (SpectraMax iD5, Molecular Devices, USA).

2.6. ELISA

Concentration of IL-6, TNF-α secretion in the supernatant was evaluated using an ELISA kit (Thermo Fisher, USA) according to the experimental procedure of the manufacturer. The absorbance of solution was detected at 450 nm by iMark microplate reader (SpectraMax iD5, Molecular Devices, USA). The level of cytokines were calculated based on the standard curve.

2.7. Real-time quantitative PCR (RT-qPCR)

Total mRNA was isolated using easy-BLUE Total RNA Extraction Kit (iNtRON, Korea). 1000 ng of total RNA was used to synthesis first stranded cDNA using Maxime RT PreMix (Oligo (dT)15 Primer) ((iNtRON, Korea). Real-time quantitative PCR (qPCR) was performed to measure mRNA expression levels of iNOS, COX-2, IL-6, HO-1, IL-6 and TNF-α. The primers for iNOS, COX-2, IL-6, TNF-α, HO-1 and GAPDH were as follows: iNOS, 5′AGTGAAGCAAAGCCCAACAA3′(sense) and 5′- TGG AAC GGG TCG ATG TCA- 3' (anti-sense), HO-1, 5'- CGC AAC AAG CAG AAC CCA-3' (sense) and 5'- GCG TGC AAG GGA TGA TTT CC-3' (antisense); TNF-α 5′- AGC CGA TGG GTT GTA CCT TG-3' (sense), and 5′ATAGCAAATCGGCTGACGGT3' (antisense); COX-2 5′-TGA GTA CCG CAA ACG CTT CT-3' (sense) and 5' -CTC CCC AAA GAT AGC ATC TGG-3' (antisense); GAPDH 5′- CAT CAC TGC CAC CCA GAA GAC TG-3′(sense) and 5′-ATG CCA GTG AGC TTC CCG TTC AG-3' (anti-sense). Relative gene expression levels of each respective gene were calculated using the threshold cycle ($2^{-\Delta\Delta CT}$) method and normalized to GAPDH mRNA levels.

2.8. Western blot

RAW 264.7 cells were lysed by RIPA buffer containing protease inhibitor and were sonicated for complete cell disruption. The total protein lysis concentration was determined through the Bradford experiment (SERVA Electrophoresis GmbH, Germany). Protein samples were separated by SDS-PAGE, then transferred to polyvinylidene fluoride membranes (PVDF, Millipore, Bedford, MA, USA), and blocking in TBS (50 mmol/L Tris-HCL pH 7.6), 150 mmol/L NaCl containing 0.1% Tween-20 (TBS-T) with 3% BSA in TBS for 1 h. Membranes were incubated with different antibodies (Thermo Fisher, USA) overnight at 4 °C. Membranes were washed in TBS-T and labeled with an HRP-conjugated second antibody, 15 min each (Thermo Fisher, USA), washed 3 times and measured using an ECL luminescence enhancer (GE Healthcare, UK). Tubulin (Invitrogen, USA) was used as a loading control to ensure that the amount of protein was evenly distributed among the samples. Expression images of proteins were captured by ImageQuant LAS 500.

2.9. Statistical analysis

The results are presented as mean ± SD of three independent experiments. Statistically significant differences between multiple groups were assessed by ANOVA. Then the results are used in the Dunnett method. P value < 0.05 was statistically significant.

Table 1
LC-QTOFMS annotation of compounds in the PLE.

No	Class	Component name	Observed RT (min)	Adducts	Expected mass (Da)	Observed mass (Da)	Mass error (mDa)	Formula
1	Alkaloid	Anhalinine	9.3	+H	2.231.208	2.231.199	−0.93901	C12H17NO
2		Anhalonine	14.8	+H	2.211.052	2.211.041	−108.144	C13H17NO3
3		Arecoline	2.2	+Na	1.550.946	1.550.955	0.877834	C8H13NO2
4		beta-Colubrine	14.6	+Na	3.641.787	3.641.806	1.904.241	C22H24N2O3
5		beta-Hydrastine	10.6	+H	3.831.369	3.831.364	−0.46624	C21H21NO6
6		Canadine	6.9	+H	3.391.471	339.146	−102.984	C20H21NO4
7		Chloroquine	6.3	+Na	3.191.815	3.191.801	−145.733	C18H26ClN3
8		Colchicine	8.9	+H	3.991.682	3.991.677	−0.45141	C22H25NO6
9		Cytisine	13.8	+Na	1.901.106	1.901.115	0.930349	C11H14N2O
10		Emetine	11.6	+H	4.802.988	4.802.969	−190.114	C29H40N2O4
11		Piperine	5.7	+H	2.851.365	2.851.354	−109.745	C17H19NO3
12		Piperlongumine	3.1	+H	3.171.263	3.171.256	−0.69863	C17H19NO5
13		Quinidine	9.1	+H	3.241.838	3.241.832	−0.55721	C20H24N2O2
14		Reserpine	13.7	+Na	6.082.734	6.082.765	3.084.983	C33H40N2O9
15		Sempervirine	14.5	+Na	2.731.392	2.731.381	−103.233	C19H16N2
16		Tropacocain	8.7	+H	2.451.416	2.451.408	−0.76798	C15H19NO2
17	Amino acid	Hippuryl-L-arginine	1.1	+Na	3.351.594	3.351.596	0.236947	C15H21N5O4
18	Anthranoid	Aloe emodin	5.7	+H	2.700.528	2.700.521	−0.73858	C15H10O5
19		Rhein	9.5	+H	2.840.321	2.840.328	0.693515	C15H8O6
20	Benzenoid	4,5-Dihydropiperlonguminine	14.9	+Na	2.751.521	2.751.536	1.481.794	C16H21NO3
21		Dillapiole	5.4	+Na	2.220.892	2.220.901	0.895086	C12H14O4
22		*para*-Hydroxyphenylacetic acid	6	+Na	1.520.473	1.520.482	0.865767	C8H8O3
23		Salidroside	6.1	+Na	3.001.209	300.121	0.119715	C14H20O7
24		Syringic acid methyl ester	6.3	+H	2.120.685	2.120.693	0.848925	C10H12O5
25		Veratric acid	7.2	+H	1.820.579	182.057	−0.89244	C9H10O4
26	Caffeic acid derivatives	Cichoric acid	5.4	+Na	4.740.798	4.740.811	126.245	C22H18O12
27	Curcuminoid	Curcumin	9	+Na	368.126	3.681.255	−0.47792	C21H20O6
28	Flavonoid	(−)-Pinocembrin	1.8	+H	2.560.736	2.560.723	−123.525	C15H12O4
29		Apigenin-4,5,7-trimethylether	3.5	+Na	3.120.998	3.120.992	−0.60589	C18H16O5
30		Baicalein	6	+H	2.700.528	270.052	−0.77877	C15H10O5
31		Bergenin	14.9	+Na	3.280.794	3.280.793	−0.14718	C14H16O9
32		Eriodictyol	8.4	+H	2.880.634	2.880.643	0.951714	C15H12O6
33		Flavokawain A	14.8	+H	3.141.154	3.141.146	−0.86642	C18H18O
34		Kaempferitrin	8.5	+H	5.781.636	5.781.642	0.619434	C27H30O14
35		Narcissoside	7.1	+H	624.169	6.241.701	1.033.333	C28H32O16
36		Nicotiflorin	8	+H	5.941.585	5.941.588	0.362795	C27H30O15
37		Rutin	6.1	+H	6.101.534	6.101.539	0.50437	C27H30O16
38	Hydroxycinnamic acid	Ferulic acid	12.3	+Na	1.940.579	1.940.568	−107.011	C10H10O4
39		*para*-Coumaroylputrescine	4.9	+Na	2.341.368	234.138	1.195.125	C13H18N2O2
40		Rosavin	10.6	+H	4.281.682	4.281.661	−212.469	C20H28O10
41	Kavalactone	Desmethoxyyangonin	4.3	+Na	2.280.786	2.280.778	−0.80743	C14H12O3
42		Methysticin	7.5	+H	2.740.841	2.740.852	1.079.083	C15H14O5
43	Lactone	N-Hexanoyl-L-homoserine lactone	1.3	+Na	1.991.208	1.991.219	1.049.931	C10H17NO3
44	Organic acid	(−)-Abietic acid	11.6	+H	3.022.246	3.022.253	0.677725	C20H30O2
45		(−)-Shikimic acid	1.9	+Na	1.740.528	1.740.534	0.580615	C7H10O5
46		(+)-Dethiobiotin	1.2	+Na	2.141.317	2.141.328	1.068.353	C10H18N2O3
47		D-pantothenic acid	3.5	+H	2.191.107	2.191.097	−100.525	C9H17NO5
48		Lauric acid methyl ester	13	+Na	2.141.933	2.141.938	0.542843	C13H26O2
49	Polyamine	N8-Feruloyl spermidine	2.6	+H	3.212.052	3.212.041	−110.857	C17H27N3O3
50	Stilbenoid	trans-Piceatannol	14.9	+H	2.440.736	2.440.725	−104.847	C14H12O4
51	Sugar	alpha-Methyl-D-mannopyranoside	5.2	+H	194.079	1.940.788	−0.20433	C7H14O6
52	Terpenoid	11,13-Dihydroparthenolide	14.5	+H	2.501.569	2.501.566	−0.24675	C15H22O3
53		8-Epiisolippidiol	10.8	+Na	2.661.518	2.661.509	−0.85954	C15H22O4
54		Asperilin	8.3	+Na	2.481.412	2.481.406	−0.68782	C15H20O3
55		Bakkenolide A	12.2	+H	234.162	2.341.608	−116.514	C15H22O2
56		Caryophellene	15	+Na	2.041.878	204.187	−0.7663	C15H24
57		Dihydroreynosin	14	+H	2.501.569	2.501.566	−0.31767	C15H22O3
58		Hirsutolide	8.6	+H	396.142	3.961.415	−0.55008	C16H2005
59		Inuchinenolide C	5.8	+Na	3.661.679	3.661.667	−113.932	C19H26O7
60		Ursiniolide B	14.5	+H	4.642.046	4.642.037	−0.88269	C24H32O9
61		Vachanic acid	9.9	+H	2.521.725	2.521.715	−101.991	C15H24O3

(*continued on next page*)

Table 1 (*continued*)

No	Class	Component name	Observed RT (min)	Adducts	Expected mass (Da)	Observed mass (Da)	Mass error (mDa)	Formula
62		Valerenic acid	9.9	+H	234.162	2.341.609	−112.499	C15H22O2
63	Other	Folic acid	8.1	+H	4.411.397	4.411.416	1.906.316	C19H19N7O6
64		Furaneol	6.3	+Na	1.280.473	1.280.481	0.733511	C6H8O3
65		Pangamic acid	2.4	+H	2.811.111	2.811.116	0.530119	C10H19NO8
66		Tremulacin	12.4	+H	5.281.632	5.281.622	−0.97408	C27H28O11

3. Results

3.1. Chemical profiling of PLE

In the chromatogram, peaks having the signal to noise ratio more than 3 were selected to be interpreted. The annotation of compounds was based on the molecular formula (from the exact mass of molecular ion in low energy ionization with the error threshold of 5 ppm between experimental and theoretical values) and their fragments in high energy ionization (Table 1 and Supplement Fig. 1). Overall, 66 compounds were identified in the extract, they belong to different classes including alkaloids, flavonoids, terpenoids, phenolics, lactones, and organic acids, etc.

Piperine and piperlongumine (amide alkaloid) are two well-known alkaloids found in *P. longum* as well as other species of the *Piper* genus [20]. These compounds, with the other 14 alkaloids, were characterized in PLE in this study. Notably, a wide variety of alkaloid types including isoquinoline (anhalinine, anhalonine, beta-hydrastine, canadine, emetine), pyridine (arecoline), quinolizidine (cytisine), tropane (tropacocain), indole (sempervirine, beta-colubrine, reserpine), quinolone (quinidine, chloroquine) and proto-alkaloid (colchicine) were the first time identified in the *Piper* species.

All terpenoids detected in the PLE are sesquiterpenes including vachanic acid, caryophellene, valerenic acid, bakkenolide A, 11,13-dihydroparthenolide, dihydroreynosin, 8-epiisolippidiol, asperilin. In which, caryophellene was reported earlier as main component of *P. longum* terpenoids [21]. Hirsutolide, inuchinenolide C, ursiniolide B are three sesquiterpene lactones with potential bioactivities on cancer cells reported by previous investigations [22–24].

Phenolic compounds such as flavonoids, anthranoids, stilbenoid, caffeic and hydroxylcinnamic acid derivatives were also characterized in the PLE. Of the chalcone flavonoids, kavalactones such as desmethoxyyangonin and methysticin have been well known as major compounds in *P. methysticum* [25] but they were identified in *P. longum* for the first time.

3.2. PLE inhibited LPS-induced NO, TNF-α, IL-6 secretion in LPS- murine macrophage RAW 264.7 cells

To assess the potential cytotoxicity of PLE, RAW 264.7 cells were pre-treated with different concentrations of the extract (0–100 μg/mL) for 30 min, followed by stimulation with LPS (1 μg/mL) for 24 h. Anti-inflammatory activity of cardamonin (Carda) in lypopolysaccharide (LPS)-induced inflammatory was identified in other studies [26–28]. In our study, cardamonin was used as positive control for anti-inflammatory effect stimulated LPS on RAW 264.7 cells. The viability of these cells was evaluated using the MTT assay, as shown in Fig. 1A. The results indicated that the PLE, at concentrations of 10, 25, 50, 75, and 100 μg/mL, did not induce cytotoxic effects on RAW 264.7 cells. The lowest percentage of cell viability observed was 86.43 ± 1.59%. Therefore, for further analyses, this study will focus on concentrations ranging from 10 to 100 μg/mL.

Nitric oxide (NO) is a signaling molecule with a crucial role in the development of inflammation. Moreover, proinflammatory cytokines like IL-6 and TNF-α are primarily secreted during infection and inflammation [29]. NO inhibitors and reducing cytokine secretion are considered as potential targets for therapy inflammatory. Our study aimed to investigate the effects of PLE extract on the production of NO and IL-6, TNF-α [30]. NO production was measured in RAW 264.7 cells after 24 h of treatment, using LPS to induce NO production in the cells. The results showed that PLE decreased NO levels at 25–100 μg/mL in RAW 264.7 cells (Fig. 1B). Furthermore, pretreatment with the extract at 50 μg/mL led to a remarkable reduction in NO levels, inhibiting up to 81.07 ± 6.24%. The IC_{50} values of PLE were found to be 28.5 ± 0.9 μg/mL, indicating its NO inhibition efficacy.

To assess the impact of PLE on cytokine secretion, additional experiments were conducted using ELISA (Fig. 1C and D). Treatment with the PLE extract at a concentration of 100 μg/mL significantly decreased the secretion of both IL-6 and TNF-α after 24 h. These findings provided evidence that PLE can effectively suppress the production of NO, TNF-α and IL-6 in LPS-stimulated RAW 264.7 murine macrophages.

3.3. PLE suppressed LPS-stimulated the mRNA expression of TNF-α, IL-6, COX-2 and iNOS in murine macrophage RAW 264.7 cells

Next, the effect of PLE on cytokine mRNA expression in RAW 264.7 cells were identified by qPCR experiments. It is shown that LPS-stimulated murine macrophage RAW 264.7 cells exhibited elevated mRNA expression of TNF-α, IL-6 as well as COX-2 and iNOS compared to the control group, as determined by real-time qPCR (Fig. 2). Treatment with PLE at 100 μg/mL resulted in a significant decrease in TNF-α and IL-6 mRNA expression at 6, 12, and 24 h (Fig. 2A and B). Furthermore, various concentrations of PLE reduced the mRNA expression of these cytokines by more than 20 times compared to the LPS-stimulated condition at 6 h after PLE treatment at 10–100 μg/mL (Fig. 2E and F). Fig. 2C–D and G-H showed a significant upregulation of iNOS and COX-2 mRNA expression in response

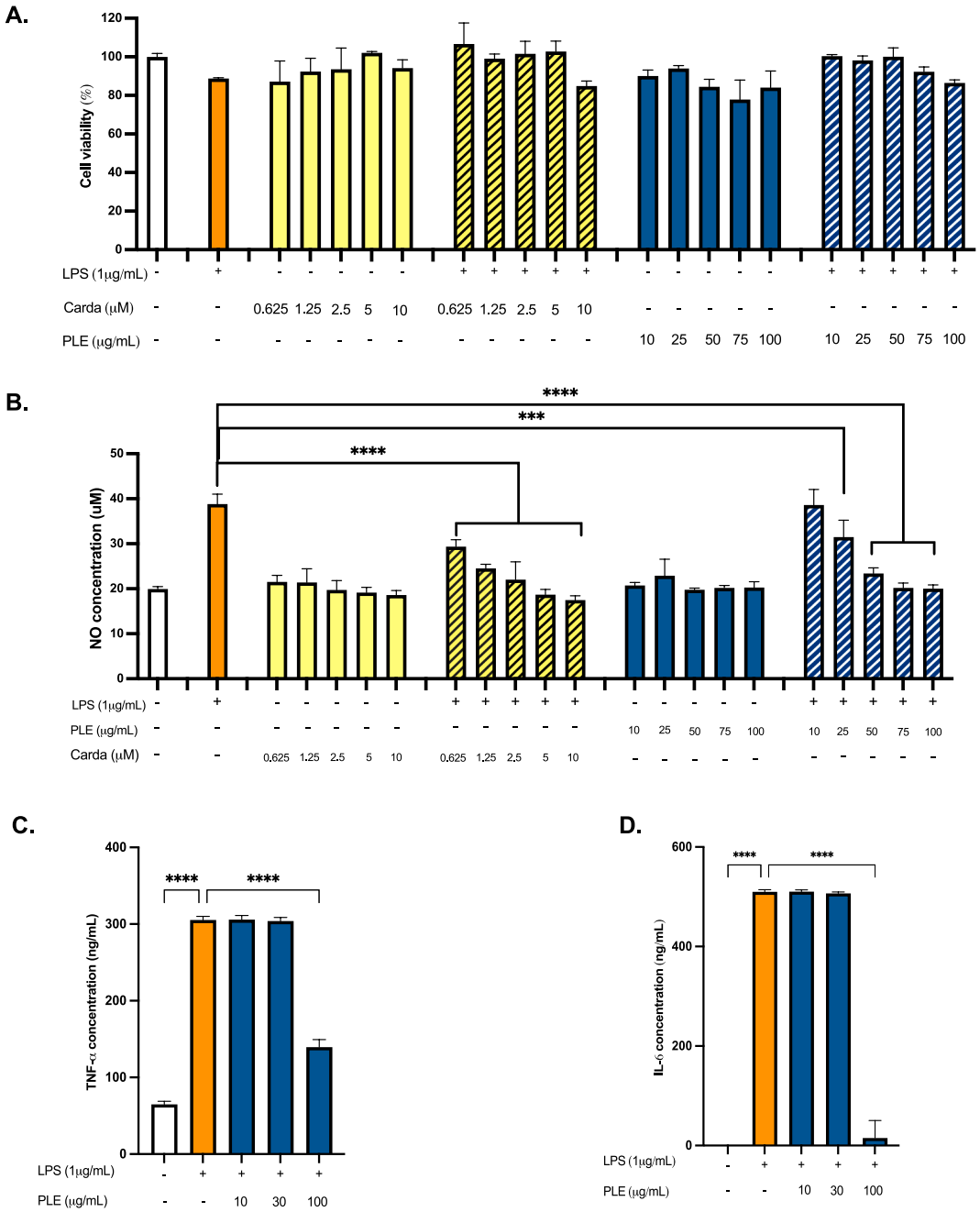

Fig. 1. PLE inhibits LPS-induced NO, TNF-α, IL-6 secretion in LPS- murine macrophage RAW 264.7 cells. Cells were pre-treated with different concentration of PLE (10, 25, 50, 75, and 100 μg/mL) in 30 min, then stimulated with LPS (1 μg/mL) for 24 h. (A) Cell viability was determined by MTT assay by using a microplate reader at 570 nm wavelength. (B) Nitric oxide level was evaluated by Griess assay. (C, D) TNF-α and IL-6 level in the supernatant were measured by ELISA. Each bar show mean ± SD of three independent experiments performed in triplicate (****$p < 0.0001$, ***$p < 0.001$ compared LPS).

to LPS compared to the control group. PLE pre-treated group displayed a considerable drop in mRNA levels of TNF-α, IL-6, iNOS and COX-2 in time course and dose dependent experiments.

3.4. PLE upregulated the expression of HO-1 in mRNA and protein levels in murine macrophage RAW 264.7 cells

HO-1 possesses significant anti-inflammatory properties promising as a therapeutic approach for chronic inflammatory and autoimmune disease treatment. However, there are challenges in translating such treatments to clinical applications. To delve further

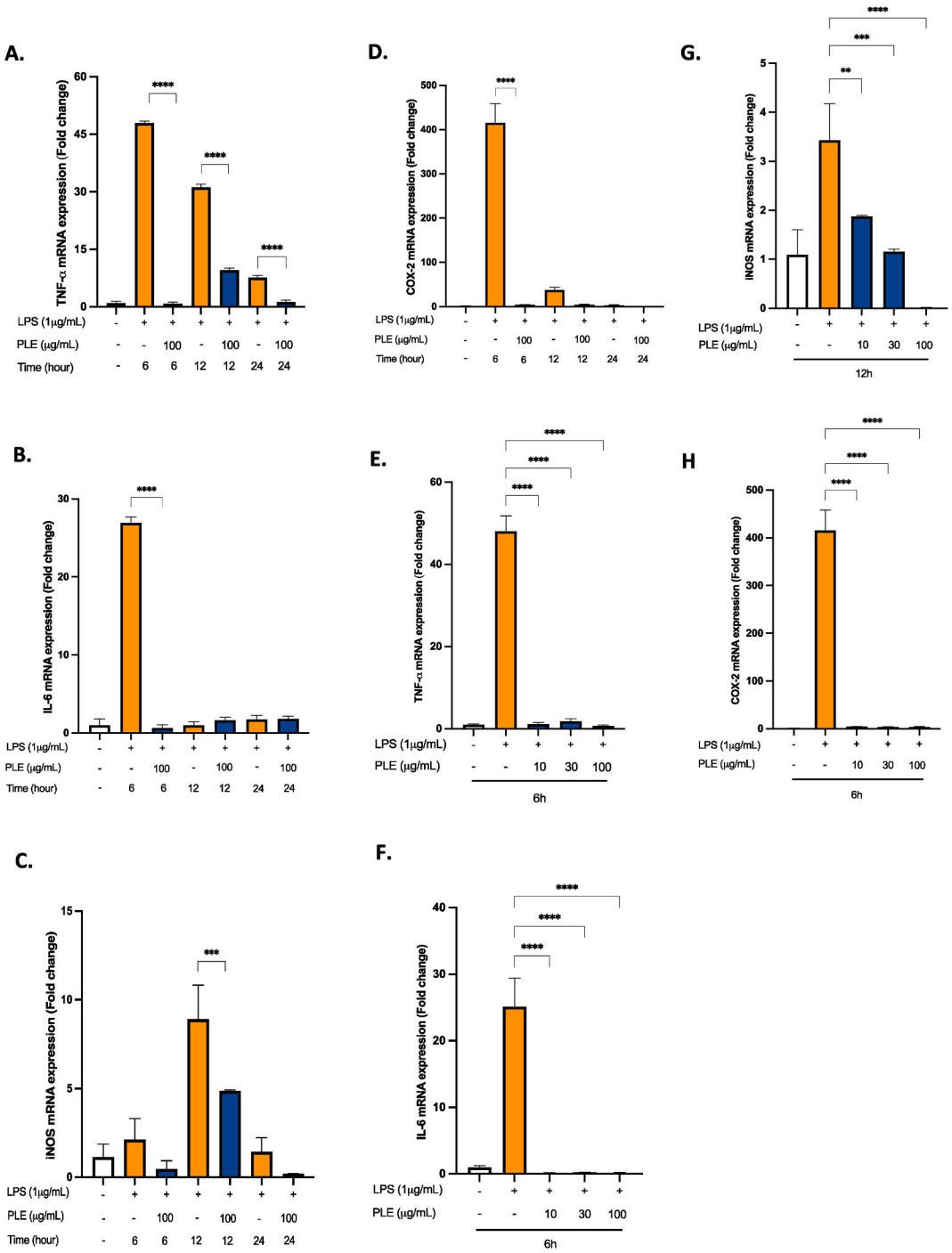

Fig. 2. PLE effects the expression of TNF-α, IL-6, COX-2 and iNOS in mRNA level in RAW 264.7 cells stimulated by LPS. Cells were pre-treated with PLE in different concentrations and stimulated by LPS in 24 h or treated with PLE and stimulated by LPS in a range of duration. Cells were harvested and total mRNA were collected for qPCR experiment with indicated primer. mRNA expression of IL-6, TNF-α, iNOS, COX-2, were relatively calculated with GAPDH. Each bar shows the mean ± SD (n = 3) of three independent experiments performed in triplicate, (****$p < 0.0001$, ***$p < 0.001$ compare with negative control).

into this potential, the expression of HO-1 in both of mRNA and protein levelunder effect of PLE was examined. The mRNA expression level of HO-1 was noticeably elevated at 3 h and thereafter decreased to the basal level in a time course experiment (Fig. 3A). To explore this effect in more detail, a dose-dependent experiment was conducted to investigate the impact of PLE on HO-1 mRNA expression at 3 h after treatment. As shown in Fig. 3B, there was a 3.5-fold increase in HO-1 mRNA expression at 10 μg/mL of the

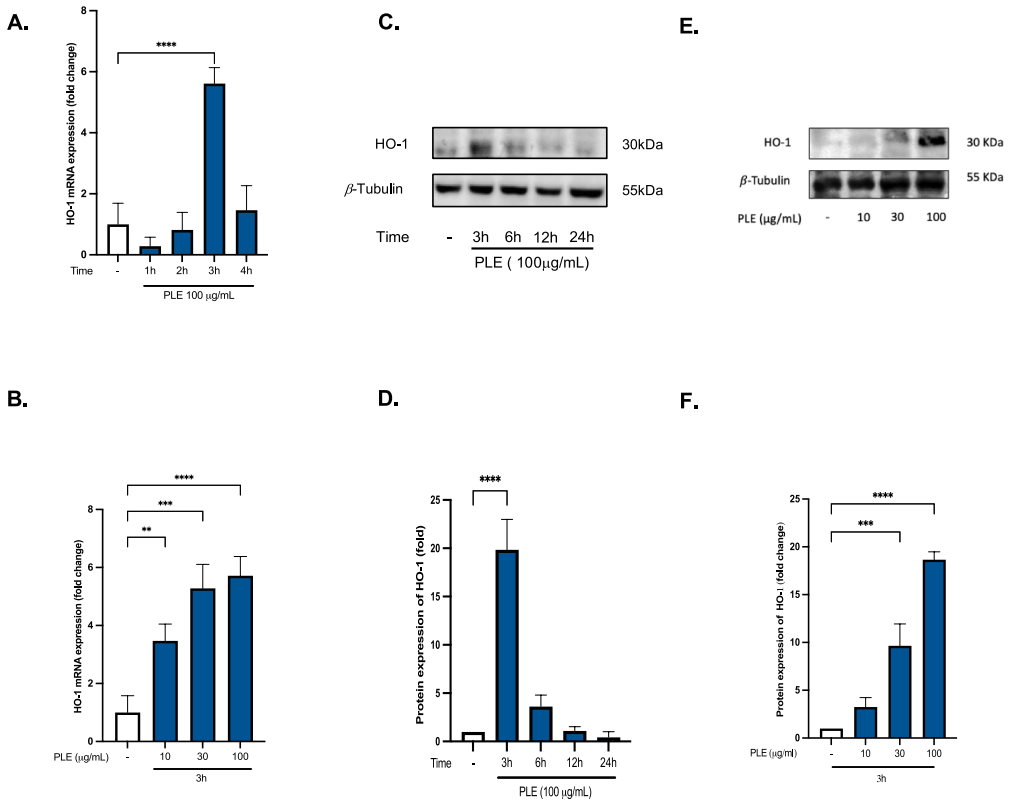

Fig. 3. Effect of PLE on the expression in mRNA and protein level of HO-1 in RAW 264.7 cells. RAW 264.7 cells were treated with PLE at different times or treated with different concentration in a specific period. Total cells were harvested and isolated mRNA or protein for qPCR or Western Blotting experiment. The expression of mRNA level **(A, B)** was relatively calculated with GAPDH and the expression of HO-1 in protein level was relatively calculated with their housekeeping protein β-Tubulin (C–F). Each bar shows the mean ± SD (n = 3) of three independent experiments performed in triplicate, (****p < 0.0001, ***p < 0.001 and **p < 0.01 compare with control). Uncropped image of blots were the availability of blot source data in the supplementary material.

extract, which further rose to 6-fold at 100 μg/mL.

To further investigate the effect of PLE on HO-1 protein expression, RAW 264.7 cells were treated with different concentrations of the extract at 3 h and time course at 100 μg/mL (Fig. 3C–E). The data revealed an increase in the HO-1 protein expression after 3 h, which then returned to basal levels after 6 h. Furthermore, the protein levels of HO-1 exhibited a significant increase when treated with PLE in a dose dependent manner (Fig. 3D–F). These findings strongly suggested that PLE may play a role in HO-1 up-regulation on RAW 264.7 murine macrophages.

3.5. PLE reduced LPS-stimulated MAPK pathway in RAW 264.7 murine macrophages

iNOS and COX-2 are crucial inflammatory molecules that exhibit several similarities in their pathophysiological aspects. The product of iNOS, nitric oxide (NO), has been shown to influence COX-2 expression and prostaglandin production in both inflammatory and tumor experimental models [31]. Inflammatory stimuli can activate many intracellular signaling pathways, including MAPK pathways, which regulating COX-2 expression [32]. The treatment of PLE also led to a notable reduction in iNOS and COX-2 protein levels (Fig. 4A–C), displaying a similar trend as the mRNA levels. At a concentration of 100 μg/mL, the extract significantly reduced COX-2 and iNOS protein expression by 6 and 30 times, respectively, compared to the group stimulated with LPS ($p < 0.001$). Therefore, PLE extract demonstrated anti-inflammatory effects by inhibiting iNOS and COX-2 at both mRNA and protein expression, suggesting its potential as a promising therapeutic approach for treating inflammatory diseases.

Fig. 5 showed the expression of p-JNK/JNK, and p-p38/p38 protein level at different time points. It was observed that PLE extract effectively inhibited the phosphorylation of p38, JNK, and AMPK activated by LPS at 5 min and 15 min (Fig. 5A–C). Treating RAW 264.7 cells with LPS for 15 min resulted in a concentration-dependent increase in p38 MAPK phosphorylation, which was significantly reduced by PLE (Fig. 5D–F). These results clearly demonstrate that PLE diminishes the levels of *p*-JNK and p-p38 induced by LPS in RAW 264.7 cells, indicating its potential anti-inflammatory effect through the inhibition of MAPKs family stimulated by LPS treatment.

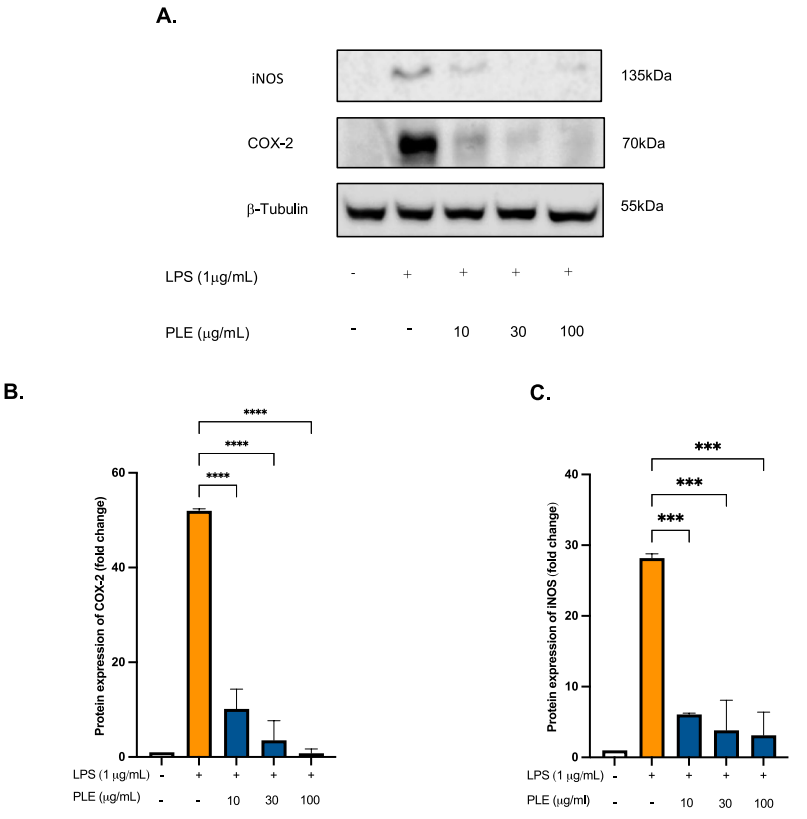

Fig. 4. PLE inhibits protein expression level of iNOS and COX-2 in RAW 264.7 cells. RAW 264.7 cells were incubated for 30 min with PLE then stimulated with LPS (1 μg/mL) for 24 h. Total cell lysates were prepared and then performed Western blot analysis with the indicated antibodies (**A**). The expression of COX-2 and iNOS was relatively calculated with their housekeeping protein β-Tubulin **(B, C)**. Each bar shows the mean ± SD (n = 3) of three independent experiments performed in triplicate, (****$p < 0.0001$, ***$p < 0.001$ compare with LPS). Uncropped image of blots were provided the supplementary material.

4. Discusion

Piper longum L. is a well-known plant of traditional medicine, possessing a wide range of biological activities, making it a promising candidate for both functional food and pharmaceutical industries. It was reported beneficial effects such as analgesic, immunomodulatory, and antitumor activities. Our study focused on evaluating the anti-inflammatory potential effect of the PLE extract on LPS-stimulated RAW 264.7 murine macrophages. The results revealed that the PLE reduced the expression of COX-2 and iNOS at both mRNA and protein levels, as well as inhibited the MAPKs signaling pathway via decreasing p38 and JNK phosphorylation. This extract also suppressed the production of NO and pro-inflammatory cytokines such as TNF-α and IL-6 and upregulated both mRNA and protein expression of HO-1. Induction of HO-1 is widely recognized for its effectiveness in cellular processes. These findings highlight the potential of this extract as an anti-inflammatory agent, with promising applications in various fields, including food and pharmaceutical industries (Fig. 6).

Heying Pei et al. found 47 known compounds from EtOH fractions of *Piper nigrum* L. Among of them, they demonstrated that pipernigramide E, F, G exhibit inflammatory activity in RAW 264.7 cell by inhibit NF-κB activation [33]. In our study, we used MeOH fraction and among 66 compounds identified by LC-HRMS analysis, PLE contains many alkaloid and phenolic compounds which showed anti-inflammatory effects. A several *anti*-inflammtory alkaloid is named canadine [34] chloroquine [35], colchicine [36], cytisine [37], emetine [38], piperine, piperlongumine [39], quinidine, reserpine, sempervirine [40]. In those, some alkaloids were the first time identified in the *Piper* species such as cytisine, quinidine, chloroquine, and colchicine. Piperlongumine was shown as an inhibitor of Nod-like receptor family pyrin domain-containing protein-3 (NLRP3) inflammasome for anti-inflammatory activity [39]. Moreover, some phenolic compounds including flavonoids, anthranoids, stilbenoids, caffeic and hydroxylcinnamic acid derivatives were also exhibiteded their anti-inflammatory effect such as aloe emodin [42], rhein [43] dillaplole [44], *para*-hydroxylphenylacetat acid [45], salldroside [46], veratric acid [47], cichoric acid, curcumin [48], bergenin [41], rutin [49], kaempferol [50]. The previous evidence proved anti-inflammatory capacity of the other candidates of kavalactone, organic acid and terpenoid groups as acid folic [51], valerenic acid [52]. Veratric acid inhibited NO production on the inactivation of MAPKs, including p38, JNK, and ERK1/2 [52]. Another study shown that bergenin decrease the expression of NO and proinflammatory cytokines by inhibiting the activation of the NF-κB and MAPKs signaling pathways [41]. 42/66 compounds performed anti-inflammatory activity explaining its potential

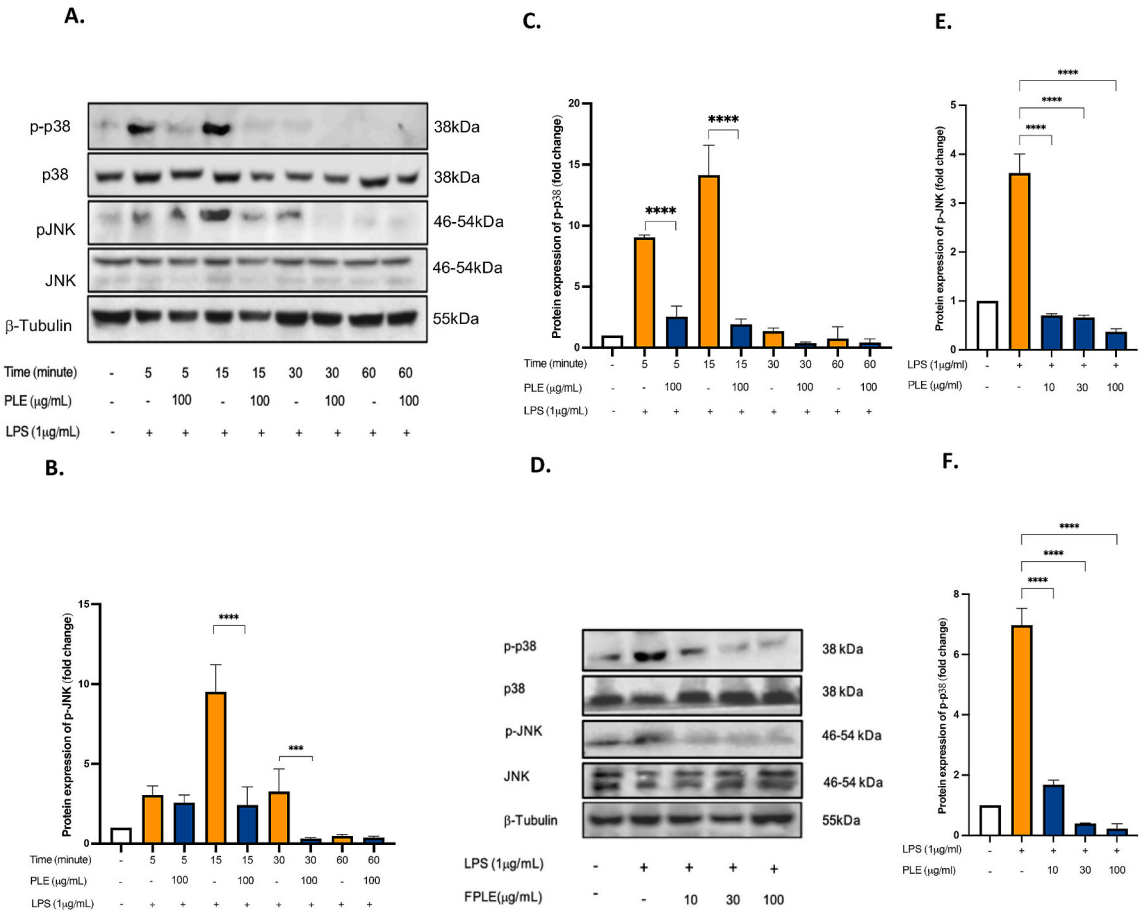

Fig. 5. Effect of PLE on the expression of p38, JNK, p-p38, and *p*-JNK in RAW 264.7 cells stimulated by LPS. RAW 264.7 cells were incubated for 30 min with PLE (100 μg/mL) and then stimulated with LPS (1 μg/mL) for 5, 15, 30, and 60 min **(A, B, C)**. RAW 264.7 cells were incubated for 30 min with PLE (10,30,100 μg/mL) and then stimulated with LPS (1 μg/mL) for 15 min **(D, E, F)** Total cell lysates were prepared and then performed Western blot analysis with the indicated antibodies. The expression of p38, p-p38, JNK, and *p*-JNK was relatively calculated with their housekeeping protein β-Tubulin. Each bar shows the mean ± SD (n = 3) of three independent experiments performed in triplicate (****$p < 0.0001$, ***$p < 0.001$ compare with LPS). An uncropped image of each blot were provided in the supplementary material.

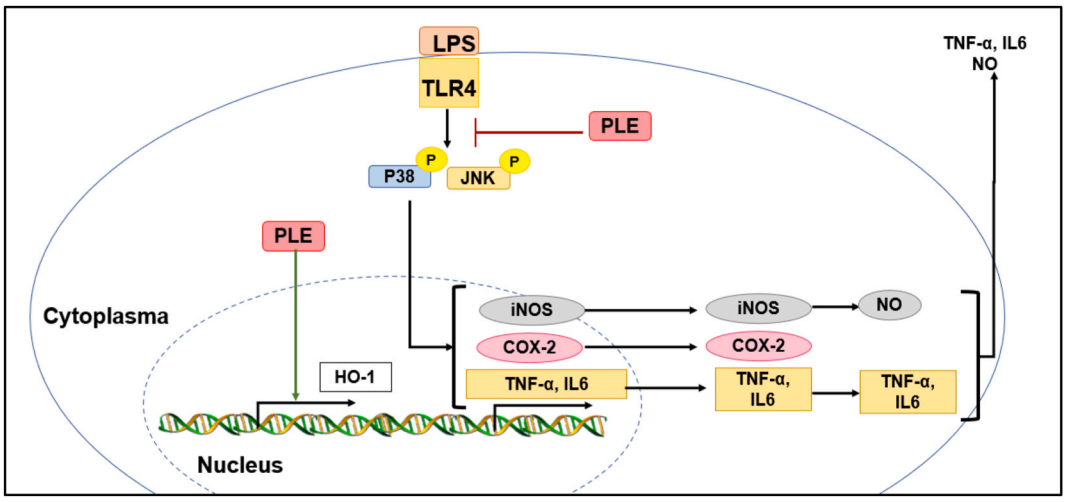

Fig. 6. Schematic diagram of anti-inflammatory effect of PLE on RAW 264.7 cells stimulated by LPS.

anti-inflammatory property.

HO-1 plays a crucial role in maintaining cellular homeostasis and provides significant tissue protection by reducing oxidative injury, countering cellular damage, and attenuating the inflammatory response [53–55]. Its induction efficiently suppresses the inflammatory response by inhibiting the production of various inflammatory cytokines [54–56]. Several plant-derived components, such as curcumin, resveratrol, and 2′-hydroxychalcone, have also been reported to induce HO-1 and exert anti-inflammatory effects in different cell types [54,57–61]. Our data also demonstrate that PLE induces the expression of HO-1 mRNA and protein, which effectively counteracts the inflammatory activities triggered by LPS in RAW 264.7 cells. These findings suggest that the increase of HO-1 by PLE is at least partly responsible for its anti-inflammatory effects. In our study, the exact mechanisms underlying the anti-inflammatory effects of HO-1 have not been fully elucidated. Therefore, it is important to evaluate more enzymatic HO-1 as potential factors that could inhibit macrophage-mediated inflammation.

It has been hypothesized that HO-1 exerts a protective antioxidant response through degrading heme, a pro-oxidant factor, and replacing it with bilirubin, a potent antioxidant [62]. The anti-inflammatory actions of HO-1 are attributed to several factors, including degradation of the pro-oxidant heme, formation of the antioxidants biliverdin/bilirubin, and release of anti-inflammatory/anti-apoptotic carbon monoxide [53,63,64]. PLE also contains many alkaloid and phenolic compounds which have antioxidant properties. Anti-oxidant property of these compounds, on the other hand was provened in curcumin [65,66], sempervirine [40], kaempferitrin [67], narcissoside [68], nicotiflorin [69], rutin [70], ferulic acid [71], caryophellene [72], chloroquine [73], tremulacin [74], colchichin [75], ursiniolide B [76], acid folic [51]. These data showed the potent antioxidant activity *in vitro* of PLE. Therefore, to display the connection between the chemical profile with this effect, total phenolic and flavonoid contents were further measured. PLE extract in our study contains a high level of phenolic and flavonoid with the total phenolic contents (TPC) of PLE were 370.35 ± 18.19 mg GAE/g extract while total flavonoid contents (TFC) of PLE was 398,76 ± 17,872 mg QE/g extract. (Supplemental Fig. 2). Moreover, PLE were elucidated potential antioxidant properties by DPPH and ABTS with the $IC_{50} = 21.84 \pm 0.11$ μg/mL and 17 ± 0.04 μg/mL, respectively (Supplemental Table 1).

Under the action of stimuli such as LPS - a common component on bacterial membranes, macrophages will secrete a series of inflammatory mediators such as NO, prostaglandin E2 (PGE2), nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) and inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin IL-1β, and IL-6. Nuclear factor-kappa B (NF-κB) is a classic and central transcription factor, which directly controls the expression of pro-inflammatory genes. The transcriptional activation of NF-κB represents the translocation of its dimers (mostly p50/p65 and p50/c-Rel) from the cytosols into the nuclei of macrophages. Moreover, TLR-mediated responses to LPS also involve mitogen-activated protein kinases (MAPKs) which are p38, ERK, and the c-Jun, JNK. This study investigated the PLE decrease iNOS and COX-2 mRNA and protein level via reduced MAPK signaling. The limitation of the study was not conduct in NF-κB and it should have further investigation.

Qian Wan et al. demonstrated the enhanced anti-inflammatory effects of *Piper longum* L. extract, highlighting its potential as a promising natural remedy [77]. It was revealed that specific bioactive components in the extract displayed significant capabilities in hindering nitric oxide (NO). Expanding upon these discoveries, our ongoing investigation is committed to elucidating the intricate mechanism through which *Piper longum* L. extract achieves its anti-inflammatory effectiveness. By examining the molecular pathways and signaling cascades implicated, our goal is to offer a thorough comprehension of how this plant extract brings about its therapeutic effects [78].

PLE reduced the level of nitric oxyed with IC_{50} was 28.46 ± 0.91 μg/mL. Our study illustrated an increase of HO-1 in mRNA and protein level in RAW 264.7 cells treated with *Piper longum* fruits extract. Recent research suggested that heme is a substance with significant proinflammatory qualities and the HO products, namely CO, bilirubin and free iron have strong anti-inflammatory effects. Although the mechanism of action of HO-1 did not fully understand, some animal models illustrated that HO-1 is useful to prevent small bowel damage in the stomach which is brought on by NSAIDs. The upregulation of HO-1 enzymatic activity could be a potential target for inflammation illness therapy. These evidences demonstrated effect of *Piper longum* L. fruits extract in the upregulation of HO-1 as well as downregulation of the phosphorylation of p38 and JNK pathways which shown good anti-inflammatory properties for drug development.

5. Conclusions

The study has evaluated chemical profile and the anti-inflammatory property fruits methanolic extract of *Piper longum* L in LPS-triggered RAW 264.7 cells. PLE has identified 66 compounds including alkaloid and phenolic which play a role in potential anti-inflammatory and antioxidants effect, and some compounds were identified for the first time in *Piper* species. Our result showed that PLE inhibited nitric oxide products and induced HO-1 protein expression. PLE displayed a prominent potential in reducing the production of nitric and the expression of iNOS, COX-2 in a concentration-dependent as well as the pro-inflammation cytokines TNF-α and IL-6. Moreover, at the protein level by down-regulating the phosphorylation of p38 and JNK- MAPKs. This study suggested that *Piper longum* L. is a potential candidate for anti-inflammatory drug production.

Data availability statement

The authors confirmed that data will be made available on request.

Funding statement

This research was funded by Vietnam Academy of Science and Technology (VAST) under grant number THTEXS.05/22-25.

CRediT authorship contribution statement

Uyen Thi Tu Phan: Writing – review & editing, Writing – original draft, Methodology, Investigation. **Hai Dang Nguyen:** Writing – review & editing, Project administration, Funding acquisition. **Thi Kieu Oanh Nguyen:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Tuan Hiep Tran:** Visualization, Methodology. **Thanh Huong Le:** Methodology, Data curation, Conceptualization. **Thi Thu Phuong Tran:** Writing – review & editing, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e26174.

References

[1] R. Medzhitov, Inflammation 2010: new adventures of an old flame, Cell 140 (6) (2010) 771–776, https://doi.org/10.1016/j.cell.2010.03.006.
[2] L. Silva, A literature review of inflammation and its relationship with the oral cavity, Glob. J. Infect. Dis. Clin. Res. 2 (2015) 1–7, https://doi.org/10.17352/2455-5363.000006.
[3] M. Mittal, M.R. Siddiqui, K. Tran, S.P. Reddy, A.B. Malik, Reactive oxygen species in inflammation and tissue injury, Antioxidants Redox Signal. 20 (7) (2014) 1126–1167, https://doi.org/10.1089/ars.2012.5149.
[4] E.B. Byun, et al., The procyanidin trimer C1 inhibits LPS-induced MAPK and NF-κB signaling through TLR4 in macrophages, Int. Immunopharm. 15 (2) (2013) 450–456, https://doi.org/10.1016/j.intimp.2012.11.021.
[5] J.B. Soares, P. Pimentel-Nunes, R. Roncon-Albuquerque, A. Leite-Moreira, The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases, Hepatol. Int. 4 (4) (2010) 659–672, https://doi.org/10.1007/s12072-010-9219-x.
[6] P. Coulombe, S. Meloche, Atypical mitogen-activated protein kinases: structure, regulation and functions, Biochim. Biophys. Acta Mol. Cell Res. 1773 (8) (2007) 1376–1387, https://doi.org/10.1016/j.bbamcr.2006.11.001.
[7] Z. Chen, et al., "MAP kinases,", Chem. Rev. 101 (2001) 2449–2476, https://doi.org/10.1016/B978-008055232-3.62901-0.
[8] M. Cargnello, P.P. Roux, Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases, Microbiol. Mol. Biol. Rev. 75 (1) (2011) 50–83, https://doi.org/10.1128/mmbr.00031-10.
[9] J. Wang, et al., An immunomodulatory polysaccharide from blackberry seeds and its action on RAW 264.7 cells via activation of NF-κB/MAPK pathways, Food Agric. Immunol. 31 (1) (2020) 575–586, https://doi.org/10.1080/09540105.2020.1747407.
[10] S. Feng, et al., " Astragalus polysaccharide enhances the immune function of RAW264.7 macrophages via the NF-κB p65/MAPK signaling pathway ,", Exp. Ther. Med. 21 (1) (2020) https://doi.org/10.3892/etm.2020.9452.
[11] Y. Yu, et al., Sulfated Cyclocarya paliurus polysaccharides exert immunomodulatory potential on macrophages via Toll-like receptor 4 mediated MAPK/NF-κB signaling pathways, Food Sci. Hum. Wellness 13 (1) (2024) 115–123, https://doi.org/10.26599/FSHW.2022.9250009.
[12] F.F. Dutra, M.T. Bozza, Heme on innate immunity and inflammation, Front. Pharmacol. 5 (May) (2014), https://doi.org/10.3389/fphar.2014.00115. MAY.
[13] L.E. Otterbein, R. Foresti, R. Motterlini, Heme oxygenase-1 and carbon monoxide in the heart: the balancing act between danger signaling and pro-survival, Circ. Res. 118 (12) (2016) 1940–1959, https://doi.org/10.1161/CIRCRESAHA.116.306588.
[14] C.S.T. Origassa, N.O.S. Câmara, Cytoprotective role of heme oxygenase-1 and heme degradation derived end products in liver injury, World J. Hepatol. 5 (10) (2013) 541–549, https://doi.org/10.4254/wjh.v5.i10.541.
[15] C. Nathan, A. Ding, Nonresolving Inflammation," Cell 140 (6) (2010) 871–882, https://doi.org/10.1016/j.cell.2010.02.029.
[16] Z. Zhang, M. Bao, N. Lu, L. Weng, B. Yuan, Y.-J. Liu, The E3 ubiquitin ligase TRIM21 negatively regulates the innate immune response to intracellular double-stranded DNA, Nat. Immunol. 14 (2) (Feb. 2013) 172–178, https://doi.org/10.1038/ni.2492.
[17] C. R, R. Medzhitov, Stress, inflammation, and defense of homeostasis, Mol. Cell 54 (2) (2014) 281–288, https://doi.org/10.1016/j.molcel.2014.03.030.Stress.
[18] F. Hu, et al., Phenolic compounds from Chaenomeles speciosa alleviate inflammation in lipopolysaccharide-treated RAW264.7 macrophages via the NF-κB and MAPK pathways, Food Sci. Hum. Wellness 12 (4) (2023) 1071–1080, https://doi.org/10.1016/j.fshw.2022.10.025.
[19] A. Kumar, S. Panghal, S.S. Mallapur, V. Ram, B.K. Singh, Anti-inflammatory activity of piper longum fruit oil A, Indian J. Pharmaceut. Sci. 71 (4) (2009) 454–456.
[20] P. Chandra, V. Bajpai, M. Srivastva, K.B.R. Kumar, B. Kumar, Metabolic profiling of Piper species by direct analysis using real time mass spectrometry combined with principal component analysis, Anal. Methods 6 (12) (2014) 4234–4239, https://doi.org/10.1039/c4ay00246f.
[21] N.S. Dosoky, P. Satyal, L.M. Barata, J.K.R. Da Silva, W.N. Setzer, Volatiles of black pepper fruits (Piper nigrum L.), Molecules 24 (23) (2019) 1–13, https://doi.org/10.3390/molecules24234244.
[22] D. Piao, et al., DNA topoisomerase inhibitory activity of constituents from the flowers of inula japonica, Chem. Pharm. Bull. 64 (3) (2016) 276–281, https://doi.org/10.1248/cpb.c15-00780.
[23] J. Huo, et al., Cytotoxic sesquiterpenoids from Vernonia bockiana, J. Asian Nat. Prod. Res. 10 (6) (2008) 571–575, https://doi.org/10.1080/10286020801966906.
[24] S. Wagner, R. Arce, R. Murillo, L. Terfloth, J. Gasteiger, I. Merfort, Neural networks as valuable tools to differentiate between sesquiterpene lactones' inhibitory activity on serotonin release and on NF-κB, J. Med. Chem. 51 (5) (2008) 1324–1332, https://doi.org/10.1021/jm701318x.
[25] C.P. Xiang, et al., A survey of the chemical compounds of piper spp. (piperaceae) and their biological activities, Nat. Prod. Commun. 11 (9) (2016) 1403–1408, https://doi.org/10.1177/1934578x1601100948.

[26] A. Takahashi, N. Yamamoto, A. Murakami, Cardamonin suppresses nitric oxide production via blocking the IFN-γ/STAT pathway in endotoxin-challenged peritoneal macrophages of ICR mice, Life Sci. 89 (9–10) (2011) 337–342, https://doi.org/10.1016/j.lfs.2011.06.027.

[27] Y.L. Chow, et al., Cardamonin from Alpinia rafflesiana inhibits inflammatory responses in IFN-γ/LPS-stimulated BV2 microglia via NF-κB signalling pathway, Int. Immunopharm. 12 (4) (2012) 657–665, https://doi.org/10.1016/j.intimp.2012.01.009.

[28] S. Hatziieremia, A.I. Gray, V.A. Ferro, A. Paul, R. Plevin, The effects of cardamonin on lipopolysaccharide-induced inflammatory protein production and MAP kinase and NFκB signalling pathways in monocytes/macrophages, Br. J. Pharmacol. 149 (2) (2006) 188–198, https://doi.org/10.1038/sj.bjp.0706856.

[29] K. Popko, et al., Proinflammatory cytokines IL-6 and TNF-α and the development of inflammation in obese subjects, Eur. J. Med. Res. 15 (Suppl. II) (2010) 120–122 [Online]. Available: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4360270/pdf/2047-783X-15-S2-120.pdf.

[30] J.N. Sharma, A. Al-Omran, S.S. Parvathy, Role of nitric oxide in inflammatory diseases, Inflammopharmacology 15 (6) (2007) 252–259, https://doi.org/10.1007/s10787-007-0013-x.

[31] A. Murakami, H. Ohigashi, Targeting NOX, INOS and COX-2 in inflammatory cells: chemoprevention using food phytochemicals, Int. J. Cancer 121 (11) (2007) 2357–2363, https://doi.org/10.1002/ijc.23161.

[32] W. Yang, E. Tiffany-Castiglioni, M.Y. Lee, I.H. Son, Paraquat induces cyclooxygenase-2 (COX-2) implicated toxicity in human neuroblastoma SH-SY5Y cells, Toxicol. Lett. 199 (3) (2010) 239–246, https://doi.org/10.1016/j.toxlet.2010.09.005.

[33] Heying Pei, Linlin Xue, Minghai Tang, Huan Tang, Shuang Kuang, Lun Wang, Ma Xu, Xiaoying Cai, Yan Li, Min Zhao, Aihua Peng, Haoyu Ye, Lijuan Chen Heying Pei, Linlin Xue, Minghai Tang, Huan Tang, Shuang Kuang, Lun Wang, Ma Xu, Xiaoying Cai, Yan Li, Alkaloids from black pepper (piper nigrum L.) exhibit anti- inflammatory activity in murine macrophages by inhibiting activation of NF-κB pathway, J. Agric. Food Chem. 68 (8) (2020) 2406–2417, https://doi.org/10.1021/acs.jafc.9b07754, 2020. d.

[34] A. Rostom, P. Moayyedi, R. Hunt, Canadian consensus guidelines on long-term nonsteroidal anti-inflammatory drug therapy and the need for gastroprotection: benefits versus risks, Aliment. Pharmacol. Ther. 29 (5) (2009) 481–496, https://doi.org/10.1111/j.1365-2036.2008.03905.x.

[35] S. Oh, et al., Anti-inflammatory activity of chloroquine and amodiaquine through p21-mediated suppression of T cell proliferation and Th1 cell differentiation, Biochem. Biophys. Res. Commun. 474 (2) (2016) 345–350, https://doi.org/10.1016/j.bbrc.2016.04.105.

[36] A.Y. Gasparyan, L. Ayvazyan, M. Yessirkepov, G.D. Kitas, Colchicine as an anti-inflammatory and cardioprotective agent, Expet Opin. Drug Metabol. Toxicol. 11 (11) (2015) 1781–1794, https://doi.org/10.1517/17425255.2015.1076391.

[37] C. Gotti, F. Clementi, Cytisine and cytisine derivatives. More than smoking cessation aids, Pharmacol. Res. 170 (May) (2021) 105700, https://doi.org/10.1016/j.phrs.2021.105700.

[38] M.D. Bleasel, G.M. Peterson, Emetine is not ipecac: considerations for its use as treatment forSAR-CoV2, Pharmaceuticals 13 (12) (2020) 1–20, https://doi.org/10.3390/ph13120428.

[39] J. Shi, Y. Xia, H. Wang, Z. Yi, R. Zhang, X. Zhang, Piperlongumine is an NLRP3 inhibitor with anti-inflammatory activity, Front. Pharmacol. 12 (January) (2022) 1–10, https://doi.org/10.3389/fphar.2021.818326.

[40] I. Moualek, et al., Assessment of the antioxidant and anti-inflammatory properties of aqueous extract of rosa sempervirens leaves, Cell. Mol. Biol. 69 (3) (2023) 214–222, https://doi.org/10.14715/cmb/2023.69.3.31.

[41] X. jiao Gao, M. yao Guo, Z. cai Zhang, T. cheng Wang, Y. guo Cao, N. sheng Zhang, Bergenin plays an anti-inflammatory role via the modulation of MAPK and NF-κB signaling pathways in a mouse model of LPS-induced mastitis, Inflammation 38 (3) (2015) 1142–1150, https://doi.org/10.1007/s10753-014-0079-8.

[42] M.Y. Park, H.J. Kwon, M.K. Sung, Evaluation of aloin and aloe-emodin as anti-inflammatory agents in aloe by using murine macrophages, Biosci. Biotechnol. Biochem. 73 (4) (2009) 828–832, https://doi.org/10.1271/bbb.80714.

[43] H. Wang, D. Yang, L. Li, S. Yang, G. Du, Y. Lu, Anti-inflammatory effects and mechanisms of rhein, an anthraquinone compound, and its applications in treating arthritis: a review, Nat. Products Bioprospect. 10 (6) (2020) 445–452, https://doi.org/10.1007/s13659-020-00272-y.

[44] R. Parise-Filho, et al., The anti-inflammatory activity of dillapiole and some semisynthetic analogues, Pharm. Biol. 49 (11) (2011) 1173–1179, https://doi.org/10.3109/13880209.2011.575793.

[45] T. Liu, et al., Glucose transporter 1-mediated glucose uptake is limiting for B-cell acute lymphoblastic leukemia anabolic metabolism and resistance to apoptosis, Cell Death Dis. 5 (10) (2014) 1–13, https://doi.org/10.1038/cddis.2014.431.

[46] Z. Qi, S. Qi, L. Ling, J. Lv, Z. Feng, Salidroside attenuates inflammatory response via suppressing JAK2-STAT3 pathway activation and preventing STAT3 transfer into nucleus, Int. Immunopharm. 35 (2016) 265–271, https://doi.org/10.1016/j.intimp.2016.04.004.

[47] W.S. Choi, P.G. Shin, J.H. Lee, G. Do Kim, The regulatory effect of veratric acid on NO production in LPS-stimulated RAW264.7 macrophage cells, Cell. Immunol. 280 (2) (2012) 164–170, https://doi.org/10.1016/j.cellimm.2012.12.007.

[48] Q. Wang, B. Lin, Z. Li, J. Su, Y. Feng, Cichoric acid ameliorates monosodium urate-induced inflammatory response by reducing NLRP3 inflammasome activation via inhibition of NF- k B signaling pathway, Evid. base Compl. Alternative Med. 2021 (2021), https://doi.org/10.1155/2021/8868527.

[49] C. Tian, X. Liu, Y. Chang, R. Wang, M. Yang, M. Liu, Rutin prevents inflammation induced by lipopolysaccharide in RAW 264.7 cells via conquering the TLR4-MyD88-TRAF6-NF-κB signalling pathway, J. Pharm. Pharmacol. 73 (1) (2021) 110–117, https://doi.org/10.1093/jpp/rgaa015.

[50] G.O. De Melo, et al., Antinociceptive and anti-inflammatory kaempferol glycosides from Sedum dendroideum, J. Ethnopharmacol. 124 (2) (2009) 228–232, https://doi.org/10.1016/j.jep.2009.04.024.

[51] O. Asbaghi, et al., The effect of conjugated linoleic acid supplementation on oxidative stress markers: a systematic review and meta-analysis of randomized controlled trials, Antioxidants 10 (2022) 871, https://doi.org/10.1016/j.clnesp.2022.04.004.

[52] F.A. Egbewande, N. Nilsson, J.M. White, M.J. Coster, R.A. Davis, The design, synthesis, and anti-inflammatory evaluation of a drug-like library based on the natural product valerenic acid, Bioorg. Med. Chem. Lett 27 (14) (2017) 3185–3189, https://doi.org/10.1016/j.bmcl.2017.05.021.

[53] N.G. Abraham, A. Kappas, Pharmacological and clinical aspects of heme oxygenase, Pharmacol. Rev. 60 (1) (2008) 79–127, https://doi.org/10.1124/pr.107.07104.

[54] C. Hwangbo, H.S. Lee, J. Park, J. Choe, J.H. Lee, The anti-inflammatory effect of tussilagone, from Tussilago farfara, is mediated by the induction of heme oxygenase-1 in murine macrophages, Int. Immunopharm. 9 (13–14) (2009) 1578–1584, https://doi.org/10.1016/j.intimp.2009.09.016.

[55] R. Motterlini, R. Foresti, Heme oxygenase-1 as a target for drug discovery, Antioxidants Redox Signal. 20 (11) (2014) 1810–1826, https://doi.org/10.1089/ars.2013.5658.

[56] A. Boutten, D. Goven, E. Artaud-Macari, J. Boczkowski, M. Bonay, NRF2 targeting: a promising therapeutic strategy in chronic obstructive pulmonary disease, Trends Mol. Med. 17 (7) (2011) 363–371, https://doi.org/10.1016/j.molmed.2011.02.006.

[57] R. Motterlini, R. Foresti, R. Bassi, C.J. Green, Curcumin, an antioxidant and anti-inflammatory agent, induces heme oxygenase-1 and protects endothelial cells against oxidative stress, Free Radic. Biol. Med. 28 (8) (2000) 1303–1312, https://doi.org/10.1016/S0891-5849(00)00294-X.

[58] S.H. Juan, T.H. Cheng, H.C. Lin, Y.L. Chu, W. Sen Lee, Mechanism of concentration-dependent induction of heme oxygenase-1 by resveratrol in human aortic smooth muscle cells, Biochem. Pharmacol. 69 (1) (2005) 41–48, https://doi.org/10.1016/j.bcp.2004.09.015.

[59] H. Abuarqoub, R. Foresti, C.J. Green, R. Motterlini, Heme oxygenase-1 mediates the anti-inflammatory actions of 2′-hydroxychalcone in RAW 264.7 murine macrophages, Am. J. Physiol. Cell Physiol. 290 (4) (2006) 1092–1099, https://doi.org/10.1152/ajpcell.00380.2005.

[60] H.Y. Hsu, L.C. Chu, K.F. Hua, L.K. Chao, Heme oxygenase-1 mediates the anti-inflammatory effect of curcumin within LPS-stimulated human monocytes, J. Cell. Physiol. 215 (3) (2008) 603–612, https://doi.org/10.1002/jcp.21206.

[61] J. Rios, M. Recio, J. Escandell, I. Andujar, Inhibition of transcription factors by plant-derived compounds and their implications in inflammation and cancer, Curr. Pharmaceut. Des. 15 (11) (2009) 1212–1237, https://doi.org/10.2174/138161209787846874.

[62] M.L. Angelotti, E. Lazzeri, P. Romagnani, Localization of Injury and Repair Pathways, Third Edit, 2019, https://doi.org/10.1016/B978-0-323-44942-7.00032-7.

[63] Y. Choo, et al., Caffeoylglycolic acid methyl ester, a major constituent of sorghum, exhibits anti-inflammatory activity via the Nrf2/heme oxygenase-1 pathway, RSC Adv. 5 (2015) 17786–17796, https://doi.org/10.1039/C4RA13847C.

[64] S.W. Ryter, J. Alam, A.M.K. Choi, Heme oxygenase-1/carbon monoxide: from basic science to therapeutic applications, Physiol. Rev. 86 (2) (2006) 583–650, https://doi.org/10.1152/physrev.00011.2005.

[65] K. Jakubczyk, A. Drużga, J. Katarzyna, K. Skonieczna-żydecka, Antioxidant potential of curcumin—a meta-analysis of randomized clinical trials, Antioxidants 9 (11) (2020) 1–13, https://doi.org/10.3390/antiox9111092.
[66] V.P. Menon, A.R. Sudheer, Antioxidant and anti-inflammatory properties of curcumin, Adv. Exp. Med. Biol. 595 (September 2001) (2007) 105–125, https://doi.org/10.1007/978-0-387-46401-5_3.
[67] W. Jiang, et al., Protective effects of kaempferitrin on advanced glycation end products induce mesangial cell apoptosis and oxidative stress, Int. J. Mol. Sci. 19 (11) (2018), https://doi.org/10.3390/ijms19113334.
[68] R.H. Fu, et al., Neuroprotective Capability of Narcissoside in 6-OHDA-Exposed Parkinson's Disease Models through Enhancing the MiR200a/Nrf-2/GSH Axis and Mediating MAPK/Akt Associated Signaling Pathway 11 (11) (2022), https://doi.org/10.3390/antiox11112089.
[69] K. Szewczyk, S. Sezai Cicek, C. Zidorn, S. Granica, Phenolic constituents of the aerial parts of Impatiens glandulifera Royle (Balsaminaceae) and their antioxidant activities, Nat. Prod. Res. 33 (19) (2019) 2851–2855, https://doi.org/10.1080/14786419.2018.1499644.
[70] A.B. Enogieru, W. Haylett, D.C. Hiss, S. Bardien, O.E. Ekpo, Rutin as a potent antioxidant: implications for neurodegenerative disorders, Oxid. Med. Cell. Longev. 2018 (2018), https://doi.org/10.1155/2018/6241017.
[71] K. Zduńska, A. Dana, A. Kolodziejczak, H. Rotsztejn, Antioxidant properties of ferulic acid and its possible application, Skin Pharmacol. Physiol. 31 (6) (2018) 332–336, https://doi.org/10.1159/000491755.
[72] S.S. Dahham, et al., The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of Aquilaria crassna, Molecules 20 (7) (2015) 11808–11829, https://doi.org/10.3390/molecules200711808.
[73] T. Magwere, Y.S. Naik, J.A. Hasler, Effects of chloroquine treatment on antioxidant enzymes in rat liver and kidney, Free Radic. Biol. Med. 22 (1–2) (1997) 321–327, https://doi.org/10.1016/S0891-5849(96)00285-7.
[74] C.L. Si, J. Xu, J.K. Kim, Y.S. Bae, P.T. Liu, Z. Liu, Antioxidant properties and structural analysis of phenolic glucosides from bark of Populus ussuriensis Kom, Wood Sci. Technol. 45 (1) (2011) 5–13, https://doi.org/10.1007/s00226-009-0286-x.
[75] D. Das, et al., Antioxidant properties of colchicine in acute carbon tetrachloride induced rat liver injury and its role in the resolution of established cirrhosis, Biochim. Biophys. Acta, Mol. Basis Dis. 1502 (3) (2000) 351–362, https://doi.org/10.1016/S0925-4439(00)00059-4.
[76] G. Lee, J.S. Park, E.J. Lee, J.H. Ahn, H.S. Kim, Anti-inflammatory and antioxidant mechanisms of urolithin B in activated microglia, Phytomedicine 55 (2019) 50–57, https://doi.org/10.1016/j.phymed.2018.06.032.
[77] Q. Wan, et al., Alkaloids from piper longum exhibit anti-inflammatory activity and synergistic effects with chemotherapeutic agents against cervical cancer cells, J. Agric. Food Chem. 71 (27) (Jul. 2023) 10349–10360, https://doi.org/10.1021/acs.jafc.3c01667.
[78] Z. Guo, J. Xu, J. Xia, Z. Wu, J. Lei, J. Yu, Anti-inflammatory and antitumour activity of various extracts and compounds from the fruits of Piper longum L, J. Pharm. Pharmacol. 71 (7) (2019) 1162–1171, https://doi.org/10.1111/jphp.13099.