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ORIGINAL ARTICLE

Fimbristylis ovata and *Artemisia vulgaris* extracts inhibited AGE-mediated RAGE expression, ROS generation, and inflammation in THP-1 cells

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Abstract

Advanced glycation end products (AGEs) can induce inflammatory signaling pathways through the receptor for AGEs (RAGE). Targeting RAGE could be a therapeutic strategy for treating chronic inflammation mediated by the AGE-RAGE axis. This study aimed to investigate the effects of *Fimbristylis ovata* and *Artemisia vulgaris* extracts on AGE-RAGE signaling and AGE-mediated oxidative stress and inflammation in THP-1 cells. *F. ovata* and *A. vulgaris* were extracted by a Soxhlet extraction, and antioxidant capacity was evaluated using DPPH and ABTS assays. The human monocytic cell line THP-1 was treated with AGE (600 μg/ml) with and without *F. ovata* and *A. vulgaris* extracts (100 μg/ml). The mitochondria-targeting antioxidant MitoQ (2 μg/ml) was used as a positive control. Cell viability, ROS generation, RAGE, AGE-RAGE signaling pathway components, and inflammatory cytokine levels were analyzed. *F. ovata* and *A. vulgaris* extracts showed antioxidative effects in non-cell-based assays. Treatment of THP-1 cells with AGE significantly increased the protein levels of RAGE and significantly increased the mRNA expression of cytokines, including TNF-α, IL-1β, and IL-6. AGEs induced the generation of ROS and levels of signaling molecules downstream of RAGE, including phosphorylated and total Erk1/2, JNK, and p38 MAPK, although not significantly. *F. ovata* and *A. vulgaris* extracts significantly decreased the protein levels of RAGE and significantly decreased the mRNA levels of cytokines. In conclusion, this study revealed that *F. ovata* and *A. vulgaris* extracts exert anti-inflammatory effects through the AGE-RAGE axis. However, details on this anti-inflammatory effect through AGE-RAGE signaling should be further investigated.

Keywords AGE-RAGE signaling · Oxidative stress · Inflammation · *Fimbristylis ovata* · *Artemisia vulgaris*

Abbreviations

AGEs	Advanced glycation end products
A. vulgaris	*Artemisia vulgaris L. var. indica Maxim*
ERK1/2	Extracellular signal-regulated protein kinases 1 and 2
F. ovata	*Fimbristylis ovata* (*Burm.f.*) *Kern*
IL-1β	Interleukin1 beta
IL-6	Interleukin6
JNKs	C-Jun N-terminal kinases
MAPKs	Mitogen-activated protein kinases
NF-κB	Nuclear factor kappa B
PI3K	Phosphoinositide 3-kinase

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RAGE	Receptor for AGEs
ROS	Reactive oxygen species
TNF-α	Tumor Necrosis Factor alpha

Introduction

Advanced glycation end products (AGEs) are heterogeneous molecules formed by nonenzymatic glycation and protein, lipid and nucleic acid oxidation. In biological systems, the process of endogenous AGE formation and accumulation in various tissues begins under diabetic hyperglycemia and oxidative stress conditions. In addition to endogenously produced AGEs, AGEs also exist in heat-processed foods and cigarette smoke [1–4]. AGE-induced inflammation has been recognized as a key mechanism underlying chronic diseases (e.g., atherosclerosis), as AGEs can activate several inflammatory signaling pathways by binding to their receptor, receptor for AGEs (RAGE), and regulate the release of inflammatory molecules through oxidative stress [5, 6]. A large number of studies have reported that the AGE-RAGE interaction leads to an increase in oxidative stress and to the activation of various cell signaling pathways, including mitogen-activated protein kinases (MAPKs), nuclear factor-kappa B (NF-κB), and phosphoinositide 3-kinase (PI3K)-Akt, which lead to the expression of inflammation-related genes and promote inflammation [7–10].

Increasing evidence has suggested that reactive oxygen species (ROS) affect the biosynthesis of inflammatory modulators at the transcriptional level by modulating redox-sensitive transcription factors, including NF-κB, Nrf2, and AP-1 [11]. ROS also play a role in promoting inflammation through MAPK signaling cascades, including extracellular signal-regulated protein kinase (ERK), p38MAPK, and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), which regulate the activity of downstream transcription factors (e.g., NF-κB, ATF-2, and AP-1) and lead to the increased production of numerous inflammatory mediators, growth factors, and proinflammatory cytokines, such as TNF-α, IL-1β, and IL-6 [12, 13].

A number of natural and synthetic antioxidative compounds have been mentioned as therapeutic strategies for the treatment of many pathophysiological conditions and oxidative stress-related inflammatory diseases [14]. Phytochemicals, a group of chemicals derived from many kinds of fruits and plants, have long been highlighted due to their benefits for human health and their pharmacological activities under several pathological conditions [15]. Numerous polyphenolic compounds and extracts of polyphenolic-rich plants possess antioxidant, anticancer, and anti-inflammatory properties [14]. An in vitro study has shown that flavonoids have therapeutic effects on complications of diabetes due to their antioxidant effects against oxidative stress mediated by AGEs [16]. It has been suggested that active flavonoid derivatives in herbs exhibit potent anti-inflammatory activity [17]. Traditional Thai herbal therapies may be an alternative treatment option for inflammation-related diseases, such as type 2 diabetes [18], cardiovascular diseases [19], rheumatoid arthritis [20], chronic inflammatory lung disease [21], asthma [22], and Alzheimer's disease [23]. *Fimbristylis ovata* (*Burm.f.*) *Kern* (*F. ovata*) is a plant in the Cyperaceae family [24]. Previous studies reported that plants in the Cyperaceae family contain several antioxidant components and have anti-inflammatory properties [25–27], antipyretic effects, antinociceptive effects, and activity against *Aedes* mosquito species [28, 29]. *F. ovata* is traditionally used to treat various diseases [30, 31]. In our previous study, we have shown that *F. ovata* has antioxidant activity, anti-inflammatory properties, and neuroprotective potential. Moreover, chemical analysis of *F. ovata* extracts revealed potential active phytochemical compounds with neuroprotective substances [32, 33]. *Artemisia vulgaris L. var. indica Maxim* (*A. vulgaris*) belongs to the Compositae family. There is evidence that *A. vulgaris* contains several polyphenolic compounds [34]. Previous studies reported that *A. vulgaris* has therapeutic properties such as antimalarial, antioxidant, anti-inflammatory, and anticancer properties [35, 36]. However, no study has examined the antioxidant and anti-inflammatory effects of *F. ovata* and *A. vulgaris* extracts prepared with different conventional methods. Therefore, this study aimed to investigate whether *F. ovata* and *A. vulgaris* extracts can suppress AGE-RAGE signaling activation-induced inflammatory responses and oxidative stress in THP-1 monocytes. Discoveries from this study could help us to better understand the mechanisms of each extract separated by sequential extraction, which allows natural products to be divided according to their polarity in extraction solvents. We expect that these results will provide insights into the roles of these extracts in inflammatory conditions, particularly those caused by AGEs.

Materials and methods

Preparation of plant extracts

F. ovata and *A. vulgaris* were identified by Professor Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand (voucher No. 013431 (BCU) and A015134 (BCU), respectively). The fresh plants were cleaned with distilled water and then oven-dried at 45 °C for 5 days. Dried plants were ground to powder and extracted with petroleum ether, dichloromethane, and methanol 1:10 (w/v) by a Soxhlet extractor. The extracts were filtered, and the solvent was evaporated. Dimethyl sulfoxide (DMSO) was used to dissolve the plant crude extract

to establish the 100 mg/ml stock solution. Stock solutions were stored protected from light at − 20 °C.

DPPH assay

Antioxidant capacity was investigated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method based on electron transfer between DPPH and the antioxidants in the plant extracts. Briefly, a calibration curve of ascorbic acid was prepared. Plant extracts (20 µl) were added to DPPH reagent (180 µl) in a 96-well plate and then incubated in the dark for 30 min. The absorbance at 517 nm was measured using a microplate reader (BioTek, VT, USA). The antioxidant activity was reported as mg vitamin C equivalent antioxidant capacity (VCEAC)/g of dried plant.

ABTS assay

Antioxidant activity was analyzed by the reaction between the plant extracts and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) cation radical (ABTS•+). Briefly, a fresh ABTS•+ solution was prepared by reacting ABTS reagent with potassium persulfate. A calibration curve of ascorbic acid was prepared. The plant extracts (20 µl) were added to working reagent (180 µl) in a 96-well plate and then incubated in the dark for 45 min. The absorbance at 734 nm was measured. The antioxidant activity was reported as mg VCEAC/g of dried plant.

Cell culture

The human monocytic cell line THP-1 was grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin in a humidified incubator with 5% CO_2 at 37 °C.

MTS assay

Cell viability was investigated by a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay, which measures the mitochondrial enzyme activity that reduces MTS to a formazan product that is soluble in the tissue culture medium. Briefly, THP-1 cells were seeded in a 96-well plate at a density of 5×10^5 cells/ml and differentiated into macrophages by stimulation with 10 ng/ml phorbol 12-myristate 13-acetate. Macrophages were then treated with either plant extracts or AGE-BSA for 24 h. Thereafter, MTS was added and incubated in a humidified incubator with 5% CO_2 at 37 °C for 4 h. The absorbance at 490 nm was measured using a microplate reader. Cells without treatment were used as a negative control. Cell viability was calculated according to the following.

formula: % cell viability = [(absorbance of the treatment group – blank) × 100/(absorbance of the control group – blank)].

Determination of intracellular ROS generation

The percentage of cells undergoing oxidative stress based on the detection of intracellular superoxide radicals was analyzed using a Muse® Oxidative Stress kit (Merck, Darmstadt, Germany). The reagent is based on dihydroethidium (DHE), which is a cell-permeable fluorescent dye. Upon entering the cells, DHE and superoxide interact to form red fluorescent oxyethidium. The Muse® Cell Analyzer instrument uses microcapillary cytometry for single-cell analysis and laser-based fluorescence detection of each cell event. Briefly, THP-1 cells were seeded at a density of 5×10^5 cells/ml in 12-well plates and differentiated into macrophages by stimulation with 10 ng/ml PMA. Cells were exposed to 500 µM H_2O_2 or 600 µg/ml AGE-BSA alone or AGE-BSA in combination with 100 µg/ml plant extracts for 1 h. The mitochondria-targeted antioxidant mitoquinone mesylate (MitoQ, 2 µg/ml) was used with AGE-BSA as the positive control. MitoQ was chosen due to its protective effect against oxidative damage and inflammatory responses by inhibiting the RAGE signaling pathway [37–44]. Thereafter, the cells were incubated with working reagent for 30 min and were analyzed using the Muse® Cell Analyzer (Merck, Darmstadt, Germany).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

THP-1 cells were seeded in 6-well plates at a density of 1×10^6 cells/ml and differentiated into macrophages by treatment with 10 ng/ml PMA. Then, the cells were exposed to 1 µg/ml LPS or 600 µg/ml AGE-BSA alone or AGE-BSA in combination with 100 µg/ml plant extracts or 2 µg/ml MitoQ for 24 h. Total RNA was extracted using TRIzol reagent (Invitrogen, MA, USA) following the manufacturer's instructions. The RNA template was used for cDNA synthesis using the AccuPower® CycleScript RT PreMix Reverse Transcription System (Bioneer, Daejeon, Korea) and oligo(dT)18 primer. For the amplification reaction, qPCR was performed using the Exicycler™ 96 Real-Time Quantitative Thermal Block (Bioneer, Daejeon, Korea). mRNA expression was analyzed using the SYBR green primer pairs listed in Table 1. The specificity of the reaction products was assessed by performing melting curve analysis. The expression of each gene was normalized to the housekeeping gene β-actin. The fold change in expression was determined using the ΔΔCt method (2-ΔΔCt).

Table 1 Human Syber Green primers for qRT-PCR

Gene	Sequence	Annealing temperature (°C)	Product size (bp)
TNF-α	Forward primer 5′ TCTCGAACCCCGAGTGACAA 3′ Reverse primer 5′ TGAAGAGGACCTGGGAGTAG 3′	55	181
IL-1β	Forward primer 5′ ACCAAACCTCTTCGAGGCAC 3′ Reverse primer 5′ CATGGCCACAACAACTGACG 3′	56	300
IL-6	Forward primer 5′ GAAGAGAGCCCTCAGGCTGGACTG 3′ Reverse primer 5′ TGAACTCCTTCTCCACAAGCGC 3′	64	627
RAGE	Forward primer 5’ GTGGGGACATGTGTGTCAGAGGGAA 3’ Reverse primer 5’ TGAGGAGAGGGCTGGGCAGGGACT 3’	64	383
β-actin	Forward primer 5’ ACGGGTCACCCACACTGTGC 3’ Reverse primer 5’ CTAGAAGCATTTGCGGTGGACGATG 3’	58	656

Western blotting analysis

THP-1 cells were seeded at a density of 1×10^6 cells/ml in 6-well plates and differentiated into macrophages by treatment with 10 ng/ml PMA. The cells were exposed to 1 µg/ml LPS or 600 µg/ml AGE-BSA alone or AGE-BSA in combination with 100 µg/ml plant extracts or 2 µg/ml MitoQ for 1 h or 24 h. Proteins were isolated from THP-1 cells using lysis buffer with phosphatase inhibitor. The protein concentration was measured by the Bradford protein assay (Bio-Rad, CA, USA). Proteins (15 µg) were separated by 10% SDS-PAGE and transferred to PVDF membranes. Unspecific protein-binding sites were blocked by incubating the membrane with TBS-T containing 5% nonfat dry milk for 1 h. The membranes were then incubated with primary antibodies (Table 2) overnight at 4 °C, followed by secondary antibodies (peroxidase-conjugated goat anti-mouse or anti-rabbit IgG, Cell Signaling Technology, MA, USA). The blots were incubated in ECL Select Western blotting Detection Reagent (GE Healthcare, IL, USA) and then visualized using high-performance chemiluminescence (GE Healthcare, IL, USA). The intensities of the protein bands were quantitated using ImageJ software (National Institute of Health, MD, USA), and β-actin was used as the housekeeping protein.

Statistical analysis

The results of 3 repeats are presented as the mean ± standard error of the mean (SEM) and were analyzed using one-way ANOVA with post hoc Bonferroni tests (Prism 7, GraphPad, CA, USA). A p value < 0.05 was considered statistically significant.

Results

Antioxidant capacity of *F. ovata* extracts

The results in Table 3 show that the antioxidant capacities of the dichloromethane and methanol extracts of *F. ovata* were similar and higher than that of the petroleum ether extract, as evidenced by both the ABTS and DPPH assays ($p < 0.05$).

Antioxidant capacity of *A. vulgaris* extracts

The results in Table 4 show that the antioxidant capacities of the dichloromethane and methanol extracts of *A. vulgaris* were significantly higher than that of the petroleum ether extract, as determined by the ABTS assay ($p < 0.05$). In the DPPH assay, the methanol extract of *A. vulgaris* showed the

Table 2 List of antibodies for Western blotting analysis

Primary antibody	Dilution factor	Manufacture
Phospho-Erk1/2	1:4,000	Cell signaling technology, MA, USA
Erk1/2	1:4,000	Cell signaling technology, MA, USA
Phospho-JNK	1:2,000	Cell signaling technology, MA, USA
JNK	1:2,000	Cell signaling technology, MA, USA
Phospho-p38 MAPK	1:4,000	Cell signaling technology, MA, USA
p38 MAPK	1:4,000	Cell signaling technology, MA, USA
NF-κB	1:2,000	Cell signaling technology, MA, USA
RAGE	1:1,000	Merck, Darmstadt, Germany
β-actin	1:10,000	Cell signaling technology, MA, USA

Table 3 Antioxidant capacity of *F. ovata* extracts determined by ABTS and DPPH assays

F. ovata. extracts	ABTS (mg VCEAC/g)	DPPH (mg VCEAC/g)
Petroleum ether	10.8 ± 0.4	11.87 ± 0.2
Dichloromethane	67.5 ± 2.6*	47.8 ± 2.8*
Methanol	62.9 ± 1.0*	47.1 ± 1.2*

The results are expressed as the mean ± SE, n = 3

*$p < 0.05$ vs. petroleum ether extract

Table 4 Antioxidant capacity of *A. vulgaris* extracts determined by ABTS and DPPH assays

A. vulgaris extracts	ABTS (mg VCEAC/g)	DPPH (mg VCEAC/g)
Petroleum Ether	18.0 ± 3.6	15.4 ± 0.1
Dichloromethane	90.7 ± 0.5*	40.5 ± 1.1*
Methanol	99.2 ± 0.2*	88.4 ± 0.1*#

The results are expressed as the mean ± SE, n = 3

*$p < 0.05$ vs. petroleum ether extract

#$p < 0.05$ vs. dichloromethane extract

highest antioxidant capacity ($p < 0.05$ vs. dichloromethane and petroleum ether extracts), followed by the dichloromethane extract ($p < 0.05$ vs. petroleum ether), and the petroleum ether extract showed the lowest antioxidant capacity (Table 4).

Effect of *F. ovata* and *A. vulgaris* extracts, AGE-BSA, and BSA on cell viability

Upon incubation with various concentrations (0.78–100 µg/ml) of *F. ovata* and *A. vulgaris* extracts for 24 h, no significant change in the viability of THP-1 cells was observed (Fig. 1 a-b). Upon incubation with various concentrations of AGE-BSA and BSA (25–600 µg/ml) for 24 h, there was no significant change in cell viability (Fig. 1c).

Effect of *F. ovata* and *A. vulgaris* extracts on AGE-induced intracellular superoxide radical production

Treatment with H_2O_2 and AGE-BSA for 1 h increased intracellular superoxide radical production by 30% (Fig. 2). Treatment with 100 µg/ml petroleum ether, dichloromethane and methanol extracts of *A. vulgaris* marginally reduced superoxide radical production compared with the AGE-BSA treatment. The positive control MitoQ at a concentration of 2 µg/ml did not affect the viability of THP-1 cells (Supplementary Fig. S1). Treatment with MitoQ decreased superoxide radical production to the untreated level (Fig. 2).

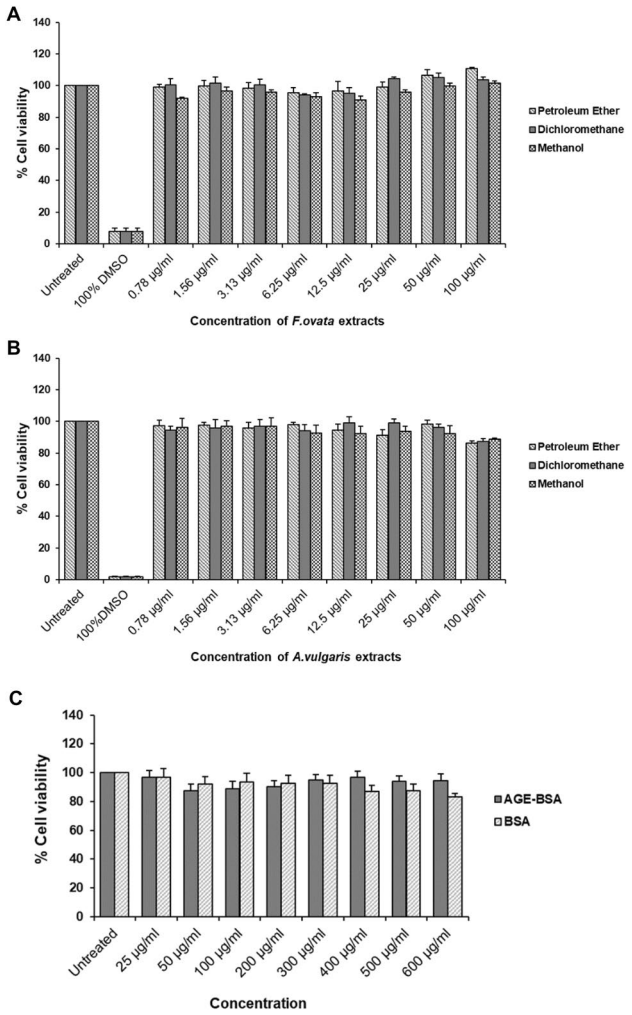

Fig. 1 Cell viability of THP-1 cells. Cells were exposed to *F. ovata* (**a**) extracts, *A. vulgaris* (**b**) extracts, AGE-BSA or BSA (**c**) for 24 h. Cell viability was analyzed by the MTS assay. The results are expressed as the mean ± SE, n = 3

Effect of *F. ovata* and *A. vulgaris* extracts on AGE-induced RAGE expression

Compared with the control condition, LPS treatment for 24 h increased RAGE protein expression by 50% without statistical significance (Fig. 3), and treatment with AGE-BSA for 24 h significantly increased RAGE protein expression ($p < 0.05$ vs. untreated; Fig. 3). In addition, treatment with the dichloromethane extracts of *F. ovata* and *A. vulgaris* and the methanol extract of *A. vulgaris* significantly attenuated RAGE protein expression compared with treatment with AGE-BSA ($p < 0.05$; Fig. 3). However, the petroleum ether extracts of *F. ovata* and *A. vulgaris* and the methanol extract of *F. ovata* did not affect RAGE protein levels. Treatment with MitoQ decreased RAGE protein expression compared with AGE-BSA treatment ($p < 0.05$; Fig. 3).

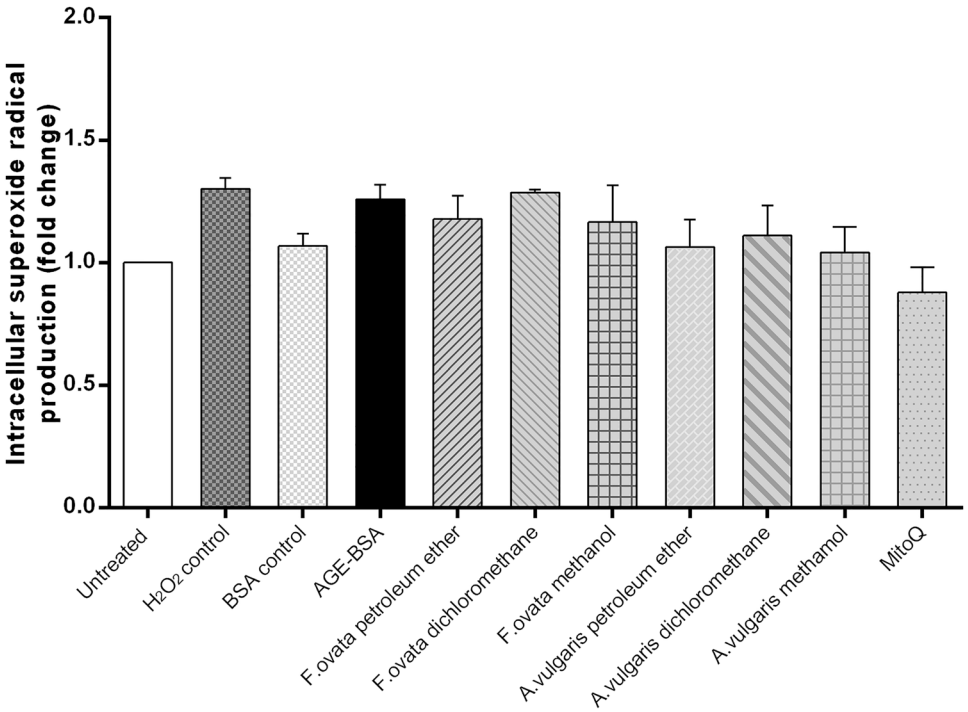

Fig. 2 Intracellular superoxide radical production in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts for 1 h. H_2O_2 was used as a negative control, and MitoQ was used as a positive control. The results are expressed as the mean ± SE, n = 3

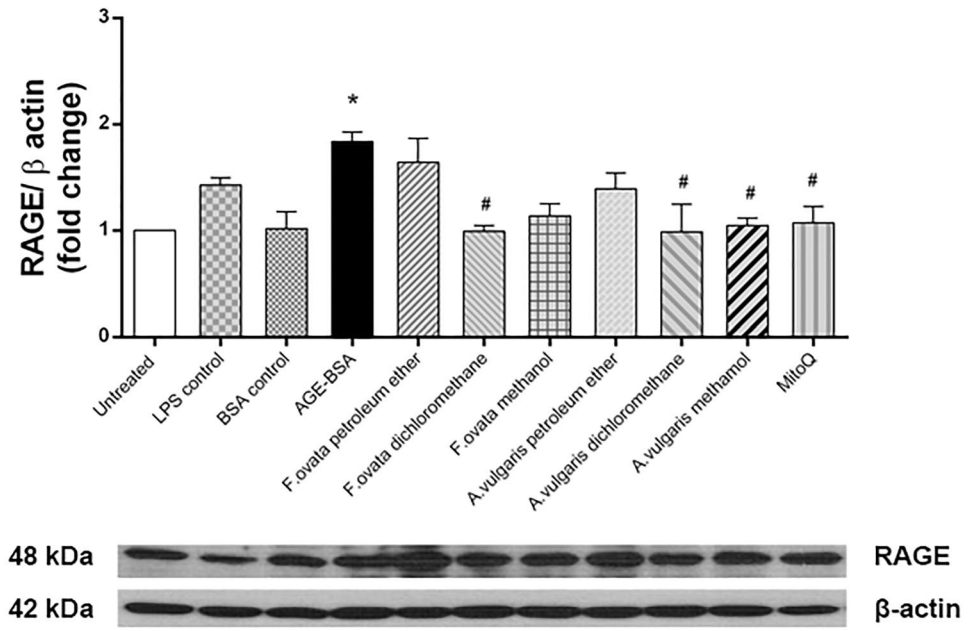

Fig. 3 Protein expression of RAGE in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts or MitoQ for 24 h. MitoQ was used as a positive control. The results are expressed as the mean ± SE, n = 3. $^{*}p < 0.05$ AGE-BSA vs. untreated, $^{\#}p < 0.05$, MitoQ and plant extracts vs. AGE-BSA

Effect of *F. ovata* and *A. vulgaris* extracts on MAPK signaling

Upon treatment with LPS and AGE-BSA for 1 h, the levels of phospho-Erk1/2 (Fig. 4a), total Erk1/2 (Fig. 4b), and phospho-JNK (Fig. 5a) were increased by 50%, although without statistical significance. The total JNK levels were increased marginally by LPS treatment and by 50% in the AGE-BSA treatment (Fig. 5b). Compared with the control condition, both the LPS and AGE-BSA treatments increased phospho-p38 MAPK levels by 70% but only marginally increased p38 MAPK levels without statistical significance (Fig. 6a, b). There was no change in the Erk1/2, JNK, and p38 MAPK protein levels between the AGE-BSA group and the plant extract groups (Figs. 4, 5, 6). MitoQ treatment marginally reduced phosphorylated and total Erk1/2, JNK, and p38 MAPK levels, although without statistical significance when compared with AGE-BSA treatment (Figs. 4,

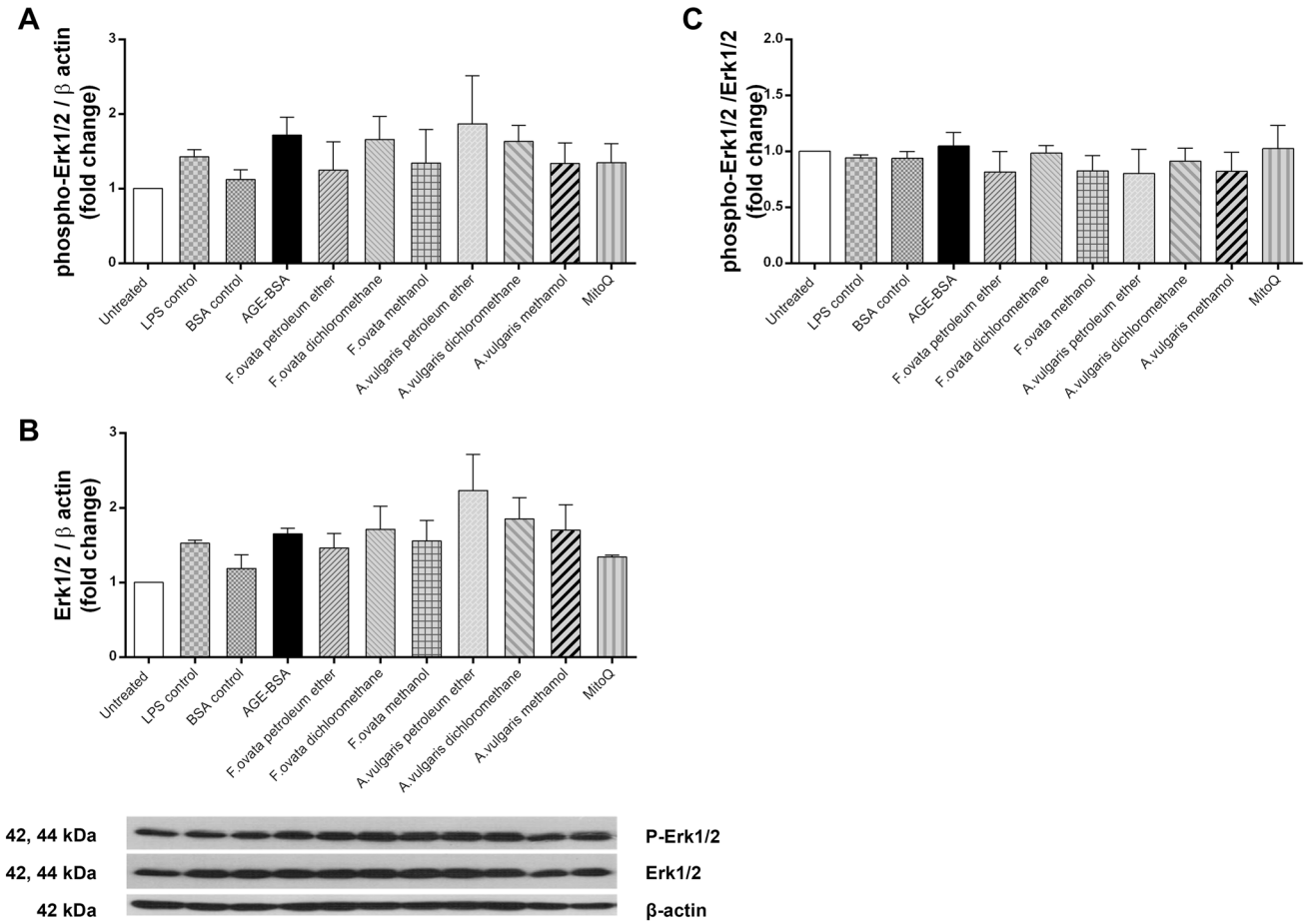

Fig. 4 Phospho-Erk1/2 (**a**), total Erk1/2 (**b**), and phospho-Erk1/2/total Erk1/2 (**c**) protein levels in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts or MitoQ for 1 h. The results are expressed as the mean ± SE, n = 3. *$p < 0.05$ AGE-BSA vs. untreated

5, 6). There was no change in phospho-Erk1/2/total Erk1/2, phospho-JNK/total JNK, or phospho-p38 MAPK/total p38 MAPK between all groups.

Effect of *F. ovata* and *A. vulgaris* extracts on NF-κB expression

The protein levels of the transcription factor NF-κB were increased by 40% and 60% by LPS and AGE-BSA treatments, respectively, compared with the control condition (Fig. 7). There was no difference in NF-κB levels between the AGE-BSA group and the plant extract groups, whereas MitoQ normalized NF-κB to the control level.

Effect of *F. ovata* and *A. vulgaris* extracts on AGE-induced inflammatory cytokine expression

Our data revealed that LPS and AGE-BSA significantly induced TNF-α, IL-1β, and IL-6 mRNA expression ($p < 0.05$ vs. untreated; Fig. 8a–c). Treatment with all extracts of *F. ovata* and *A. vulgaris* normalized TNF-α mRNA expression to the control level ($p < 0.05$ vs. AGE-BSA; Fig. 8a). IL-1β mRNA expression was significantly decreased ($p < 0.05$ vs. AGE-BSA; Fig. 8b) by treatment with the methanol extract of *F. ovata* and all extracts of *A. vulgaris* compared with treatment with AGE-BSA. Moreover, all the extracts of *F. ovata* and the petroleum ether extract of *A. vulgaris* normalized IL-6 mRNA expression to the control level ($p < 0.05$ vs. AGE-BSA; Fig. 8c). MitoQ treatment significantly inhibited TNF-α, IL-1β, and IL-6 mRNA expression compared with AGE-BSA treatment ($p < 0.05$; Fig. 8a–c).

Discussion

In this study, we assessed the antioxidant and anti-inflammatory capacity of *F. ovata* and *A. vulgaris* extracts. Different extraction methods were examined. The results from our study showed that the dichloromethane and methanol extracts of *F. ovata* had the same high antioxidant activity,

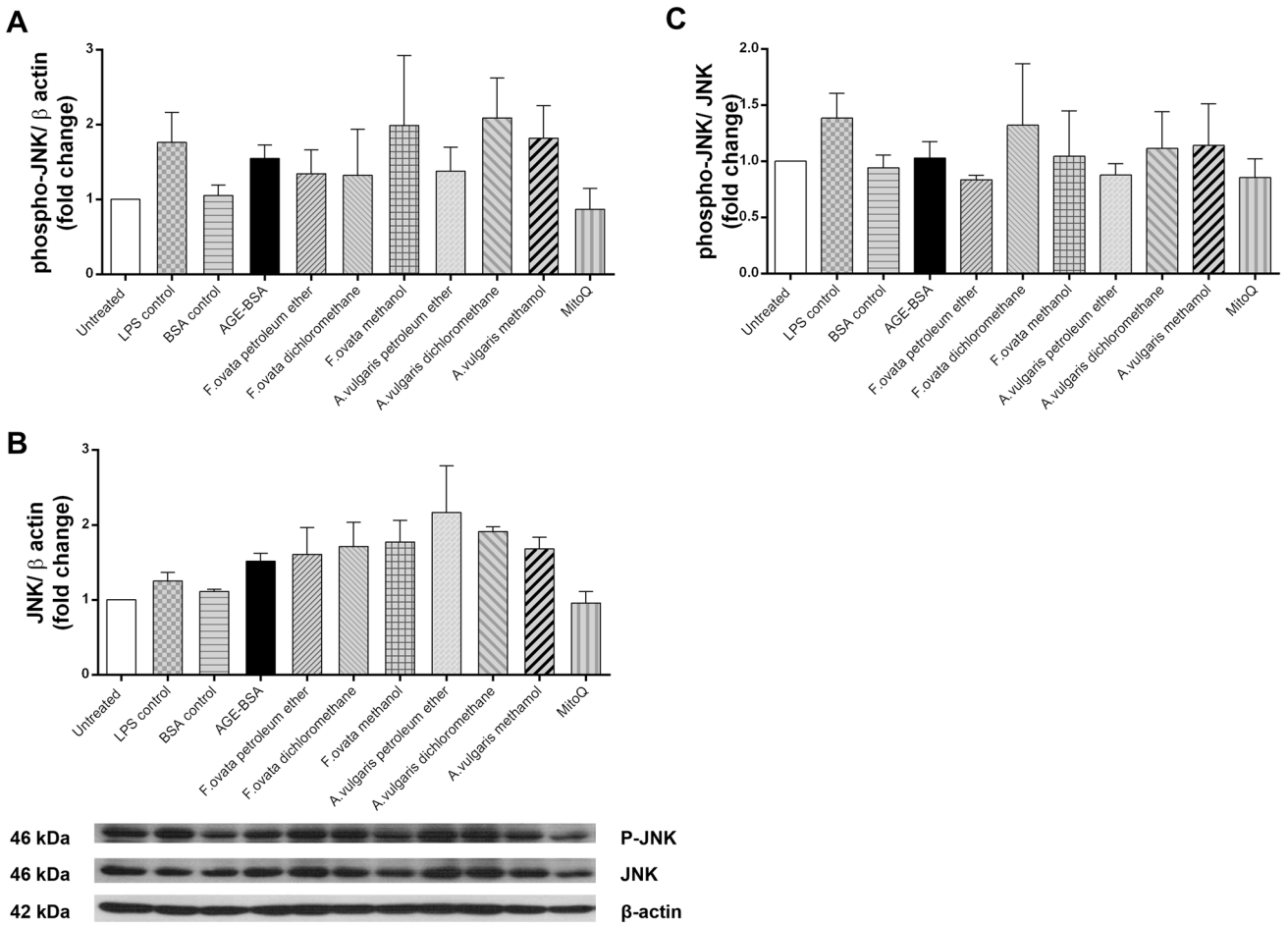

Fig. 5 Phospho-JNK (**a**), total JNK (**b**), and phospho-JNK/total JNK (**c**) protein levels in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts or MitoQ for 1 h. The results are expressed as the mean ± SE, n = 3. *$p < 0.05$ AGE-BSA vs. untreated

while the methanol extract of *A. vulgaris* had the highest antioxidant activity. All extracts of *F. ovata* and *A. vulgaris* showed potent anti-inflammatory effects, which were associated with the inhibition of AGE-induced RAGE expression. The radical-scavenging capacity of plant extracts was dependent on solvent polarity, which is related to the polar nature of the active compounds in each plant. Among the major classes of phytochemicals, phenolic compounds are the most extensively studied, especially their health benefits due to potential protection against oxidative damage [45]. Polar solvents are efficiently used to recover phenolic compounds from plants [46]. Our results indicated that the natural antioxidants in *F. ovata* and *A. vulgaris* may mainly be preserved in the polar solvent extracts. Notably, there were some differences in the radical-scavenging capacity of the plant extracts detected by the DPPH and ABTS assays. The ABTS assay is superior to the DPPH assay and reveals antioxidant activity in a more sensitive manner since it has faster reaction kinetics. The ABTS assay is also useful for assessing the antioxidant capacity of samples extracted with acidic solvents and of samples containing hydrophilic, lipophilic, and pigment compounds [47, 48]. However, while none of the *F. ovata* and *A. vulgaris* extracts showed marked antioxidant activity in cells, the ROS production induced by AGE was inhibited by the known mitochondria-targeting antioxidant MitoQ. This may be due to the dose of *F. ovata* and *A. vulgaris* extracts used in this study. The inconsistency between our findings of antioxidative effects of plant extracts evaluated using non-cell-based assays and ROS generation in THP-1 cells may be due to the principle of oxidative stress kit in this study which is based on the detection of only superoxide radical production in cells. The production of other radicals, such as hydroxyl radicals, peroxyl radicals, and alkoxyl radicals, should be further investigated.

The AGE-RAGE interaction leads to an increase in oxidative stress and to the activation of various cell signaling pathways, including MAPKs, PI3K-Akt, and NF-κB, which causes the expression of a variety of inflammation-related

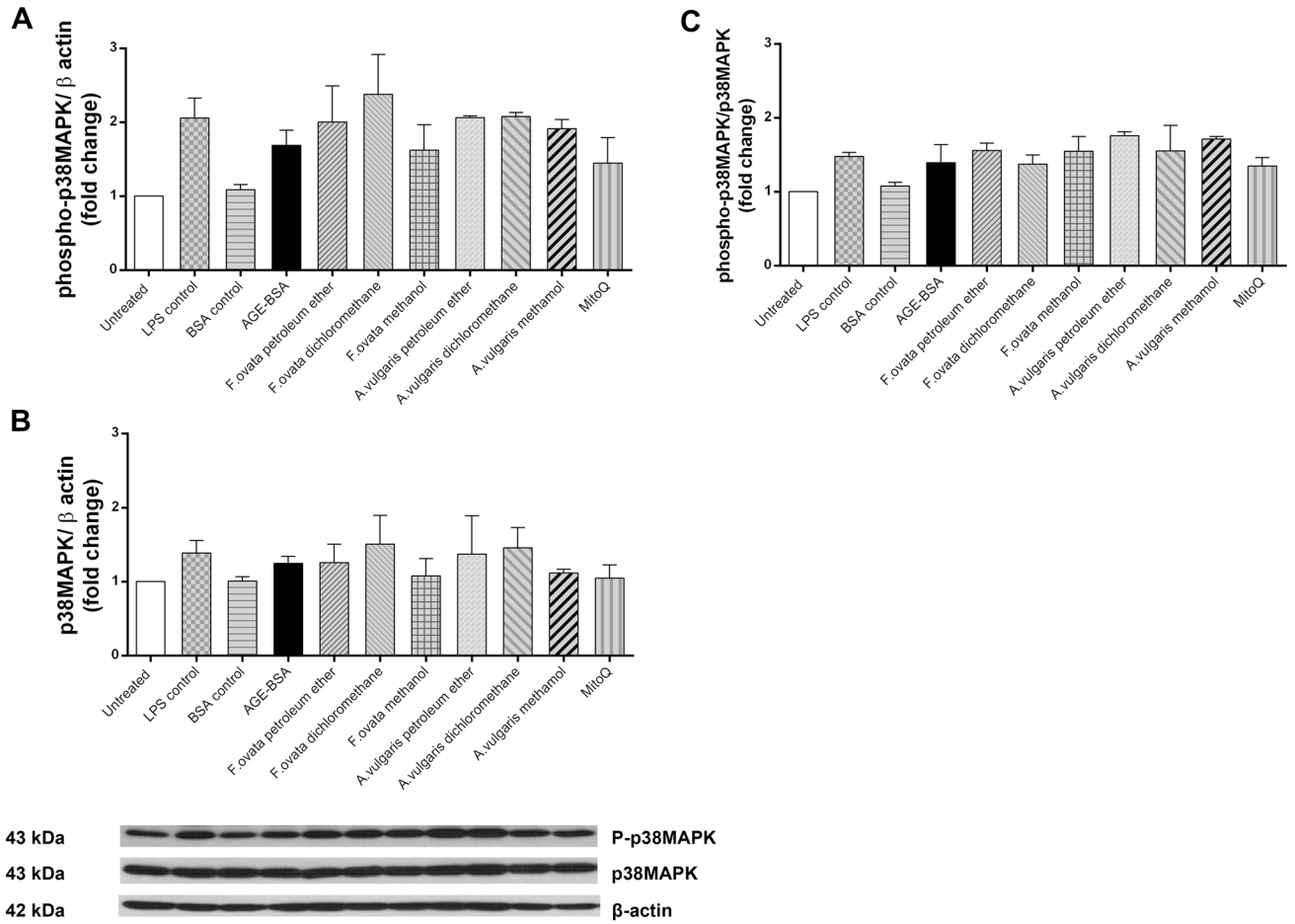

Fig. 6 Phospho-p38 MAPK (**a**), total p38 MAPK (**b**), and phospho-p38 MAPK/total p38 MAPK (**c**) protein levels in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts for 1 h. MitoQ was used as a positive control. The results are expressed as the mean ± SE, n = 3. *$p < 0.05$ AGE-BSA vs. untreated

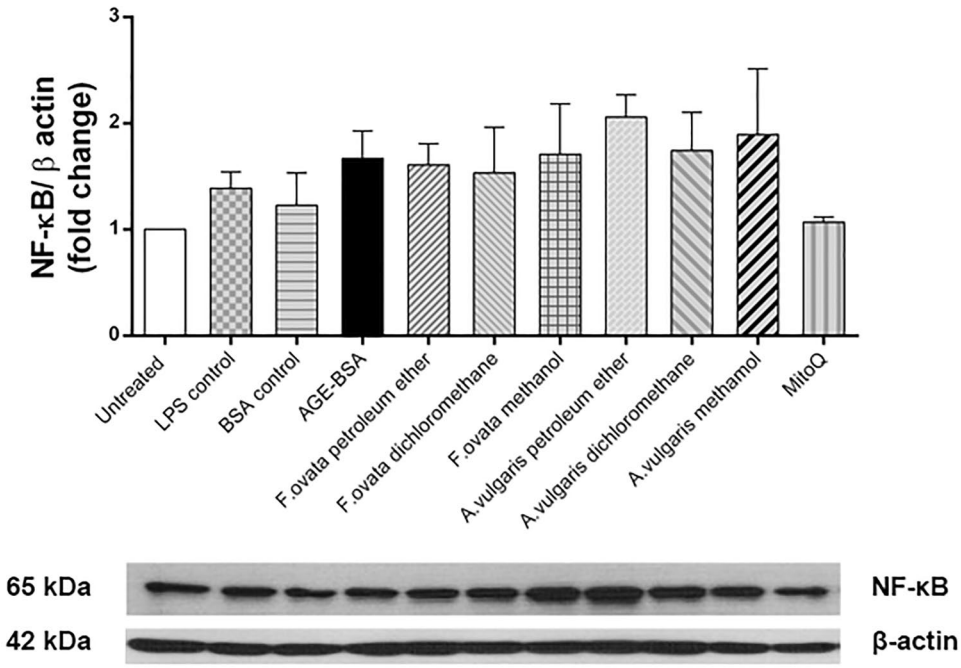

Fig. 7 NF-κB protein levels in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts for 1 h. MitoQ was used as a positive control. The results are expressed as the mean ± SE, n = 3

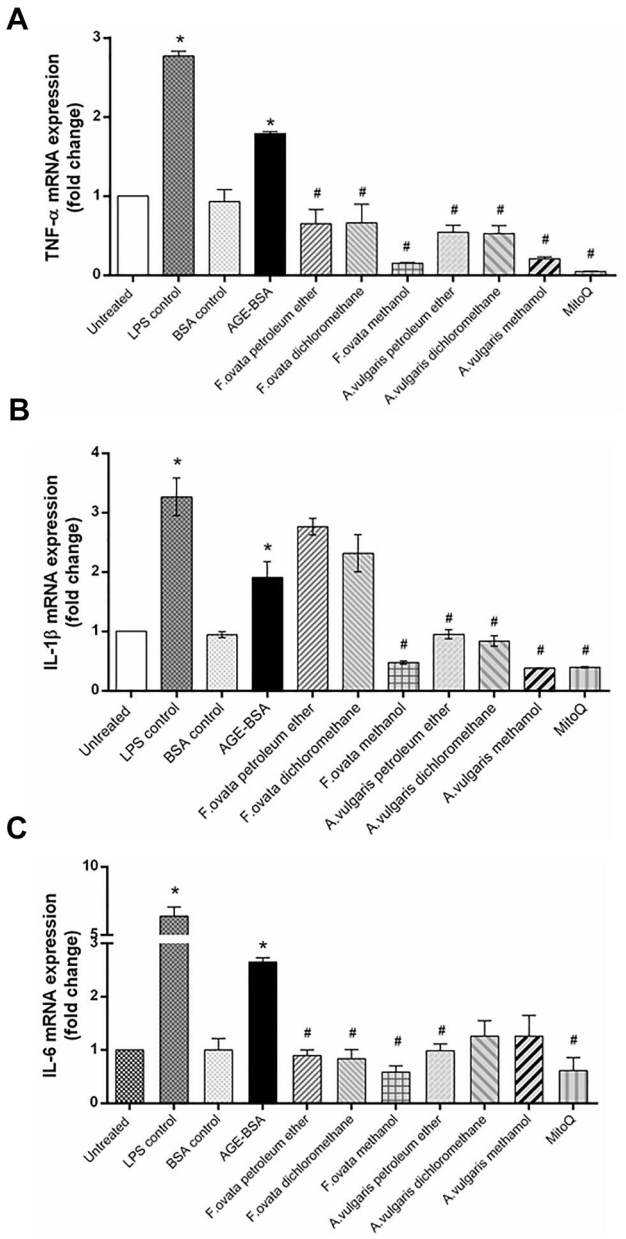

Fig. 8 TNF-α (**a**), IL-1β (**b**), and IL-6 (**c**) mRNA expression in THP-1 cells. Cells were exposed to AGE-BSA alone or AGE-BSA in combination with plant extracts or MitoQ for 24 h. The results are expressed as the mean ± SE, n = 3. $^{*}p < 0.05$ AGE-BSA vs. untreated, $^{\#}p < 0.05$ MitoQ and plant extracts vs. AGE-BSA

genes and promotes inflammation [7, 8]. Increasing evidence suggests that the AGE-RAGE axis is a therapeutic target for chronic inflammation-related conditions. AGE inhibitors can prevent oxidative stress and have protective effects against inflammation [49]. In addition, clinical research revealed that sRAGE, acting as a RAGE competitor, could suppress vascular inflammation [50]. Moreover, knockout of the RAGE gene resulted in reduced atherosclerosis and vascular inflammation [51]. Therefore, targeting RAGE could be a therapeutic strategy for the treatment of conditions caused by AGE-RAGE axis-mediated oxidative stress and chronic inflammation.

In this study, AGEs induced the expression of RAGE, signaling molecules downstream of RAGE, including Erk1/2, JNK, p38 MAPK, and NF-κB, and inflammation-related genes, including TNF-α, IL-1β, and IL-6. Most *F. ovata* and *A. vulgaris* extracts suppressed RAGE expression and inflammatory cytokine production, except for the petroleum ether extracts. This anti-inflammatory effect was independent of their antioxidant capacity, at least at these experimental doses. The role of *F. ovata* and *A. vulgaris* extracts in the inhibition of AGEs is, at least, mediated by attenuating the increase in RAGE but without impacting Erk1/2, JNK, p38 MAPK, and NF-κB.

It has also been reported that AGE-mediated stimulation of Toll-like receptor-4 (TLR-4) signaling could induce the inflammatory cytokines IL-1β and IL-6 in THP-1 cells [52, 53]. RAGE and TLRs share common ligands and signaling pathways, suggesting a cooperative interaction in immune response stimulation. Incorporation of the RAGE signal into the TLR pathways markedly amplifies inflammatory signaling [54]. In this study, *F. ovata* and *A. vulgaris* extracts exerted a suppressive effect on RAGE and inflammatory cytokine levels but did not affect Erk1/2, JNK, p38 MAPK, or NF-κB; thus, AGE-mediated stimulation of TLRs should also be considered in our results. The effects of *F. ovata* and *A. vulgaris* extracts on the incorporation of RAGE and TLRs should be further investigated.

The PI3K/Akt [55, 56], protein kinase C [57], and JAK/STAT pathways [58] are among the known signaling cascades that can be activated by RAGE. Additional studies are needed to discover the alternative pathway that mediates the anti-inflammatory effects of *F. ovata* and *A. vulgaris* extracts. It would be interesting to further clarify the effect of *F. ovata* and *A. vulgaris* extracts on signaling cascades, including the PI3K/Akt, protein kinase C, and JAK/STAT pathways.

In addition to the transcription factor NF-κB, the AGE-RAGE axis could activate several proinflammatory transcription factors, including cAMP-response-element-binding protein (CREB) [59, 60], early growth response-1 (EGR-1) [61], and activator protein-1 (AP-1) [62]. We did not precisely determine the mechanisms of AGE-induced proinflammatory transcription factors; therefore, these issues should also be considered. In this study, AGEs induced the protein levels of NF-κB. However, *F. ovata* and *A. vulgaris* extracts did not affect NF-κB levels. Therefore, the effects of *F. ovata* and *A. vulgaris* extracts on NF-κB activities, such as the active form of NF-κB, IkappaB-alpha degradation, and NF-κB nuclear translocation, need to be further investigated.

In summary, *F. ovata* and *A. vulgaris* extracts have an inhibitory effect on AGE-mediated RAGE overexpression and inflammatory responses and showed antioxidative effects in non-cell-based assays. Therefore, *F. ovata* and *A. vulgaris* might be useful as alternative options to prevent AGE-RAGE signaling-mediated inflammatory conditions. Future studies are needed to elucidate the active compounds in various extracts and to confirm the effect of *F. ovata* and *A. vulgaris* extracts on more AGE-RAGE cascade components.

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Declarations

Conflict of interest The authors declare no conflict of interest.

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