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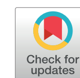
Research article

Cosmeceutical potentials of *Curcuma mangga* Val. extract in human BJ fibroblasts against MMP1, MMP3, and MMP13



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ABSTRACT

Oxidative stress, the disrupted oxidation-reduction mechanism in our body, is caused by the excessive exposure of free radicals and the impaired antioxidant defenses that can accelerate skin aging. Antioxidants can be obtained from nature, which are available widely in therapeutic-rich plants, such as white saffron (*Curcuma mangga* Val., denoted as *C. mangga*). Although many pieces of evidence reveal that *C. mangga* contains an abundance of phenolic compounds and has antioxidative effects, its cosmeceutical potentials remain unclear. The present study aimed to disclose the unexplored antiaging potentials of *C. mangga* extract (CME) in oxidative stress-induced human BJ fibroblasts with a focus on collagen protection against pro-inflammatory mediators MMP1, MMP3, and MMP13. The oxidative stress-induced cells were treated with CME and curcumin at different doses. The results showed that treatment using CME (25 μg/mL) could maintain the collagen contents up to 18.45 ± 0.68 μg/mL in H_2O_2-treated fibroblasts (only ~26.63% reduction in collagen contents), while the figure for the negative control was the lowest (12.79 μg/mL), showing a significant reduction in collagen contents by 49.13%. In addition, the gene expression of pro-inflammatory MMPs arose significantly in BJ fibroblasts after oxidative stress induction using 200 μM H_2O_2, in which the expression for MMP1, MMP3, and MMP13 increased by 7.10, 38.96, and 2.69 times, respectively. Interestingly, CME treatment (100 μg/mL) could effectively inhibit MMP1, MMP3, and MMP13 gene expression by 3.65, 34.62, and 2.02 times, respectively. In conclusion, CME showed favorable antiaging activities in H_2O_2-treated human BJ fibroblasts as confirmed by the low levels of gene expression of MPP1, MMP3, and MMP13 after treatment with CME.

1. Introduction

Skin aging happens as a consequence of the depletion of dermal structural cohesion and physiology that manifests as wrinkles and sagging. Skin aging is driven by multiple causes, either intrinsic or extrinsic (Farage et al., 2008). Several external inducers, including prolonged ultraviolet (UV) light exposure, are recognized to escalate the production of intercellular reactive oxygen species (ROS), which is the main oxidative stress inducer (Rinnerthaler et al., 2015). Oxidative stress actively influences the aging mechanism and promotes the expression of several types of enzymes in skin tissue that contribute to the reconstruction of extracellular matrix (ECM) (Brennan et al., 2003). When the presence of these enzymes getting dominant, the ECM breakdown is detectable, leading to skin texture changes (Imokawa and Ishida, 2015). The prohibition of oxidative stress or pro-inflammatory mediators can be a solution to retard skin aging process.

White saffron or *Curcuma mangga* Val. is a native plant from Indonesia and has medical benefits. Similar to the other member of genus *Curcuma*, *C. mangga* contains numerous phytochemical compounds, some of which are curcuminoids (Malek et al., 2011; Pujimulyani and Sutardi, 2003). Phytochemical compounds are often associated with a wide array of useful biological activities (Widowati et al., 2017, 2018b). According to Pujimulyani et al. (2013), *C. mangga* extract contains gallic acid, catechin, epicatechin, epigallocatechin, epigallocatechingallat, and

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gallocatechingallat of $124 \times 10^{-3} \pm 0.01$, $134 \times 10^{-3} \pm 0.05$, $442 \times 10^{-3} \pm 0.02$, $113 \times 10^{-3} \pm 0.02$, $0.37 \times 10^{-3} \pm 0.02$, and $159 \times 10^{-3} \pm 0.01$ mg/g dried extract, respectively. *C. mangga* was previously reported to have health-protection potentials, including anti-inflammation, anti-tumor, and antioxidant potentials (Kaewkroek et al., 2010; Malek et al., 2011; Pujimulyani et al., 2018). Several studies showed that antioxidants might be useful to constrain the skin aging process due to its ability to relieve oxidative stress (Masaki, 2010).

However, the antiaging activity of white saffron, especially its activity toward the expression of MMPs is poorly understood. Therefore, this present study was trying to explore the safeguarding potentials of *Curcuma mangga* Val. against collagen degradation and MMPs expression induced by oxidative stress.

2. Materials and methods

2.1. White saffron rhizomes and extraction methods

C. mangga rhizomes were obtained from Bantul, Yogyakarta. Fresh rhizomes were cleaned, peeled, washed, cut, dried, and crushed into a fine powder and subjected to extraction. Briefly, the fine powder was soaked in 1.5 L of distilled ethanol (70%) and filtered daily to gain colorless filtrate. Ethanol was chosen as a maceration solvent because the substantial compounds of *C. mangga* are soluble in ethanol. Afterward, the filtrate was vaporized and maintained at -20 °C prior to use.

2.2. Cel culture of human skin fibroblasts and cell viability assay

The human BJ fibroblasts (ATCC® CRL-2522, USA) acquired from Aretha Medika Utama Biomolecular and Biomedical Research Center (Bandung, Indonesia) were used for *in vitro* study. For cell culture purposes, DMEM (Biowest L0416, France) supplemented with 10% of FBS (Biowest S1810, France), 1% of antibiotic-antimycotic solution (Biowest L0010, France), 1% of amphotericin B (Biowest L0009-050, France), 1% of nanomycopulitine (Biowest LX16-010, France), and 0.01% of gentamicin (Gibco 15750078, USA) was used for cell culture medium. Initially, the cell seeding (0.5×10^4 cells/well) was conducted on the 96-well plate, and the seeded cells were maintained in the cell incubator for 24 h at 37 °C and 5% of CO_2 (Thermo Fisher Scientific IH3543, USA). After reaching 80% of cell confluency, the cell medium was then supplanted with 100 μL of fresh medium containing CME and curcumin at various doses (6.25, 12.5, 25, 50, 100, and 200 μg/mL) and further maintained in the conditioned cell incubator for 24 h. After treatment, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) solution (20 μL) (Abcam ab197010, Britain) was added and placed in the incubator for 3 h. In order to measure the cell viability, the MTS-treated cells were then subjected to Multiskan™ GO Microplate Spectrophotometer (Thermo Fisher Scientific 51119300, USA) at a 490-nm wavelength for the absorbance measurement (Widowati et al., 2016; Pujimulyani et al., 2020).

2.3. Measurement of collagen contents

The collagen contents were measured after treatment with CME and curcumin, in which positive and negative controls were involved. Briefly, BJ fibroblast cell seeding (0.2×10^6 cells/well) was conducted on the 24-well plate. After reaching 80% of cell confluency, the cell medium was then supplanted with fresh medium containing CME and curcumin (25 and 100 μg/mL; 12.5 and 50 μg/mL) following incubation in the conditioned cell incubator for 2 h. Then, 200 μM H_2O_2 was added to each well to induce oxidative stress and maintained in the incubator for 1 h. After treatment, Sirius Red 1% (200 μL) (Sigma Aldrich 365548, USA) was appended, and the treated cells were preserved at 27 °C for 1 h. Then, Sirius Red was discarded and washed with HCl 0.1 N (200 μL). Afterward, NaOH 0.1 N (200 μL) was added and further incubated at 27 °C for half an hour. Then, the absorbance value was measured at a 540-nm wavelength, and the collagen contents of cells were calculated according to the collagen type I standard curve (Junqueira et al., 1979; Sweat et al., 1964; Keira et al., 2004).

2.4. MMP1, MMP3, and MMP13 gene expressions

The MMP1, MMP3, and MMP13 gene expressions were measured after treatment with CME and curcumin, in which positive and negative controls were involved. Briefly, BJ fibroblasts were seeded in a T-flask (25 m^2) with a cell density of 0.3×10^6 cells. After reaching 80% of cell confluency, 200 μM H_2O_2 was then added to induce oxidative stress and maintained in the conditioned cell incubator for 1 h. Then, the oxidative stress-induced cells were subjected to CME and curcumin treatment at various doses (100 and 25 μg/mL; 50 and 12.5 μg/mL, respectively) and further maintained in the incubator for 24 h. The RNA isolation procedure followed the protocol of RNA Isolation Kit (Bio-Rad 732-6820, USA). The samples were measured for their concentration and purity of RNA at 260/280 nm. The cDNA synthesis kit (Bio-Rad 170-8841, USA) was used to synthesize cDNA. RT-qPCR using SsoFast Evergreen Supermix (Biorad, 1725200) was performed according to the manufacturers' directions. The primers used were forward and reverse primer β-actin, MMP1, MMP3, and MMP13. Primer sequences used were presented in Table 1. The interpretation of RT-qPCR was done by the real-time PCR system (Pikoreal 96, Thermo Scientific, TCR0096) (see Table 2).

3. Results

3.1. Effects of CME on cell viability

In order to assess the *in vitro* biocompatibility of CME in human BJ fibroblasts, CME at various doses (6.25, 12.5, 25, 50, 100, and 200 μg/mL) were introduced to the cell culture, in which curcumin was used as a control antioxidant. The results showed that both CME and curcumin did not significantly decrease the fibroblast cell viability with the highest concentration up to 50–100 μg/mL (Figure 1) as shown by the maintained cell viability as higher as 94.89% and 87.04% for CME and curcumin, respectively. Thus, CME with the highest concentrations (50–100

Table 1. The list of primers and the amplification properties.

Gene Symbols	Primer Sequence Upper strand: sense Lower strand: antisense	Product Size (bp)	Annealing (°C)	Cycle (×)	Ref.
MMP1	5′-AGAGTCTATAGGCCCACCCC-3 5′-GCTCGACGCTAGGATCTGAC-3′	97	53	40	Fong et al. (2016)
MMP3	5′-TGCTTCAGGGTTTCATCCAG-3′ 5′-GGCGGCAATCATCCTCTG-3′	169	53	40	Wang et al. (2009)
MMP13	5′-GGTCATGTGTGTGGAGAGCG-3′ 5′-GGTGCCGGTTCAGGTACTCA-3′	89	58	40	Pepper et al. (2011)
β-actin	5′-TCTGGCACCACACCTTCTACAATG-3′ 5′-AGCACAGCCTGGATAGCAACG-3′	166	53	40	Palumbo et al. (2018)

Table 2. Raw data of MMP1, MMP3, and MMP13 gene expression in oxidative stress-induced fibroblasts with different treatments.

Treat ments	Relative ratio of MMP1 gene expression				Relative ratio of MMP3 gene expression				Relative ratio of MMP13 gene expression			
	Sample 1	Sample 2	Sample 3	Mean ± SD	Sample 1	Sample 2	Sample 3	Mean ± SD	Sample 1	Sample 2	Sample 3	Mean ± SD
I	1.00	1.00	1.00	1.00 ± 0.00	1.00	1.00	1.00	1.00 ± 0.00	1.00	1.00	1.00	1.00 ± 0.00
II	8.82	6.73	8.75	8.10 ± 1.19	39.95	36.50	43.41	39.95 ± 3.45	4.16	3.65	3.26	3.69 ± 0.45
III	6.92	7.89	6.87	7.22 ± 0.58	49.18	37.79	52.71	46.56 ± 7.80	4.55	4.71	4.02	4.43 ± 0.36
IV	4.35	4.89	4.11	4.45 ± 0.40	5.21	5.78	5.03	5.34 ± 0.39	1.80	1.76	1.46	1.67 ± 0.19
V	10.20	7.89	8.00	8.70 ± 1.30	6.19	4.20	5.03	5.14 ± 1.00	2.69	2.34	2.23	2.42 ± 0.24
VI	7.52	6.96	5.13	6.54 ± 1.25	28.84	28.84	25.46	27.71 ± 1.95	2.01	2.20	1.68	1.96 ± 0.26
VII	2.00	2.83	2.16	2.33 ± 0.44	7.94	9.78	8.82	8.85 ± 0.92	1.20	1.23	1.49	1.31 ± 0.16

Note: (I) control cells (normal BJ cells), (II) BJ cells + H_2O_2 200 μM, (III) H_2O_2-treated cells + DMSO 1%, (IV) H_2O_2-treated cells + CME 100 μg/mL, (V) H_2O_2-treated cells + CME 25 μg/mL, (VI) H_2O_2-treated cells + curcumin 50 μg/mL, and (VII) H_2O_2-treated cells + curcumin 12.5 μg/mL.

μg/mL) were selected for further studies because it was less toxic at most levels of concentration.

3.2. Effects of CME on collagen contents

The effects of CME on collagen contents were assessed in oxidative stress-induced fibroblasts. In order to render oxidative stress, cells were treated with 200 μM H_2O_2, resulting in a significant decrease of collagen contents in BJ cell culture (Figure 2). Overall, treatment using CME (25 μg/mL) could maintain the collagen contents up to 18.45 ± 0.68 μg/mL, suppressing the degradation of collagen by 26.63%. In comparison, CME (100 μg/mL) and curcumin (12.5 and 50 μg/mL) were not significantly different in protecting collagen against degradation, showing collagen contents of 15.95 ± 1.67, 15.48 ± 1.48, and 15.56 ± 0.61 μg/mL, respectively. Meanwhile, the figure for the negative control, H_2O_2-treated fibroblasts, was the lowest (12.79 μg/mL), showing a significant reduction in collagen contents by 49.13%.

3.3. Effects of CME on MMP1, MMP3, and MMP13 gene expressions

The gene expressions of pro-inflammatory MMPs arose significantly in BJ fibroblasts after oxidative stress induction using 200 μM H_2O_2, in which the expression for MMP1, MMP3, and MMP13 increased by 7.10, 38.96, and 2.69 times, respectively (Figure 3A-C). Then, CME was assessed for its inhibitory potentials against those pro-inflammatory MMPs. The results showed that CME (100 μg/mL) could effectively inhibit MMP1 gene expression by 3.65 times compared to the negative control (Figure 3A). Interestingly, CME treatments (25 and 100 μg/mL) were superior in suppressing the MMP3 gene expression, showing a significant reduction in the MMP3 gene expression by 34.82 and 34.62 times, respectively, as compared to the negative control (Figure 3B). Meanwhile, CME (100 μg/mL) exhibited favorable inhibitory effects against MMP13 gene expression as shown by the reduction of MMP13 gene expression by 2.02 times compared to the negative control (Figure 3C).

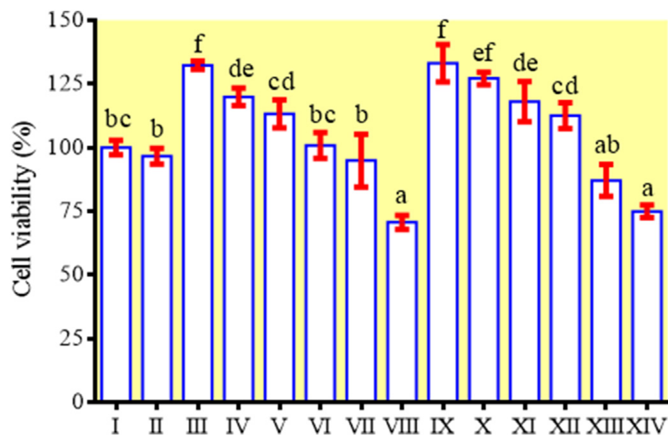

Figure 1. Effects of CME and curcumin on fibroblast cell viability. (I) control cells (normal BJ cells), (II) normal cells + DMSO 1%, (III) CME 6.25 μg/mL, (IV) CME 12.5 μg/mL, (V) CME 25 μg/mL, (VI) CME 50 μg/mL, (VII) CME 100 μg/mL, (VIII) CME 200 μg/mL, (IX) curcumin 6.25 μg/mL, (X) curcumin 12.5 μg/mL, (XI) curcumin 25 μg/mL, (XII) curcumin 50 μg/mL, (XIII) curcumin 100 μg/mL, and (XIV) curcumin 200 μg/mL. The data are presented as mean ± *SD*. Different notations (a, ab, b, bc, cd, de, ef, and f) indicate a significant difference ($p < 0.5$) based on Tukey HSD Post Hoc test ($n = 3$).

4. Discussion

Plants serve as an essential source of antiaging agents due to the presence of several phytochemical compounds that can modulate the skin aging process. Primary plant pigments are recognized to minimize damages due to UV light exposure (Heinrich et al., 2003), while common dietary phytochemicals ameliorate oxidative stress (Tanigawa et al., 2014). Agricultural products have many potentials for human healths, such as antioxidant, antibacterial, and antiviral properties (Mongalo et al., 2020). The present study examined the effects of CME and curcumin in protecting collagen from degradation and suppressing the pro-inflammatory enzymes MMP1, MMP3, and MMP13 in free radicals-induced human skin fibroblast cells as aging cells model (Figure 4).

Dermal matrixes consist of several types of proteins and collagen that maintain tensile strength (Farage et al., 2008). The existence of

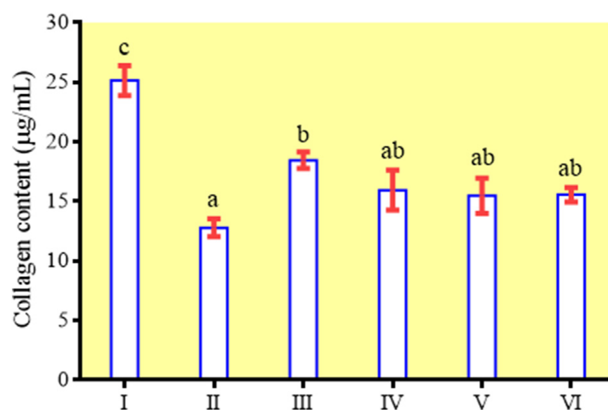

Figure 2. Effects of CME and curcumin toward collagen contents in oxidative stress-induced fibroblasts. (I) control cells (normal BJ cells), (II) normal cells + H_2O_2 200 μM, (III) H_2O_2-treated cells + CME 25 μg/mL, (IV) H_2O_2-treated cells + CME 100 μg/mL, (V) H_2O_2-treated cells + curcumin 12.5 μg/mL, and (VI) H_2O_2-treated cells + curcumin 50 μg/mL. The data are presented as mean ± *SD*. Different notations (a, ab, b, and c) indicate a significant difference ($p < 0.5$) based on Tukey HSD Post Hoc test ($n = 3$).

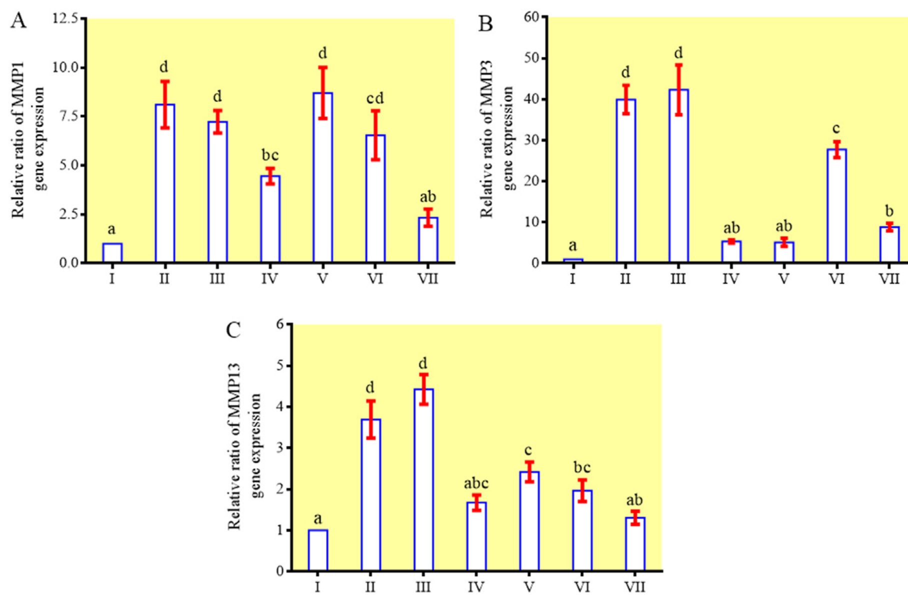

Figure 3. Effects of CME and curcumin on (A) MMP1, (B) MMP3, and (C) MMP13 gene expressions in oxidative stress-induced fibroblasts. (I) control cells (normal BJ cells), (II) BJ cells + H_2O_2 200 μM, (III) H_2O_2-treated cells + DMSO 1%, (IV) H_2O_2-treated cells + CME 100 μg/mL, (V) H_2O_2-treated cells + CME 25 μg/mL, (VI) H_2O_2-treated cells + curcumin 50 μg/mL, and (VII) H_2O_2-treated cells + curcumin 12.5 μg/mL. The data are presented as mean ± *SD*. Different notations (a, ab, abc, bc, c, cd, and d) indicate a significant difference ($p < 0.5$) based on Tukey HSD Post Hoc test ($n = 3$).

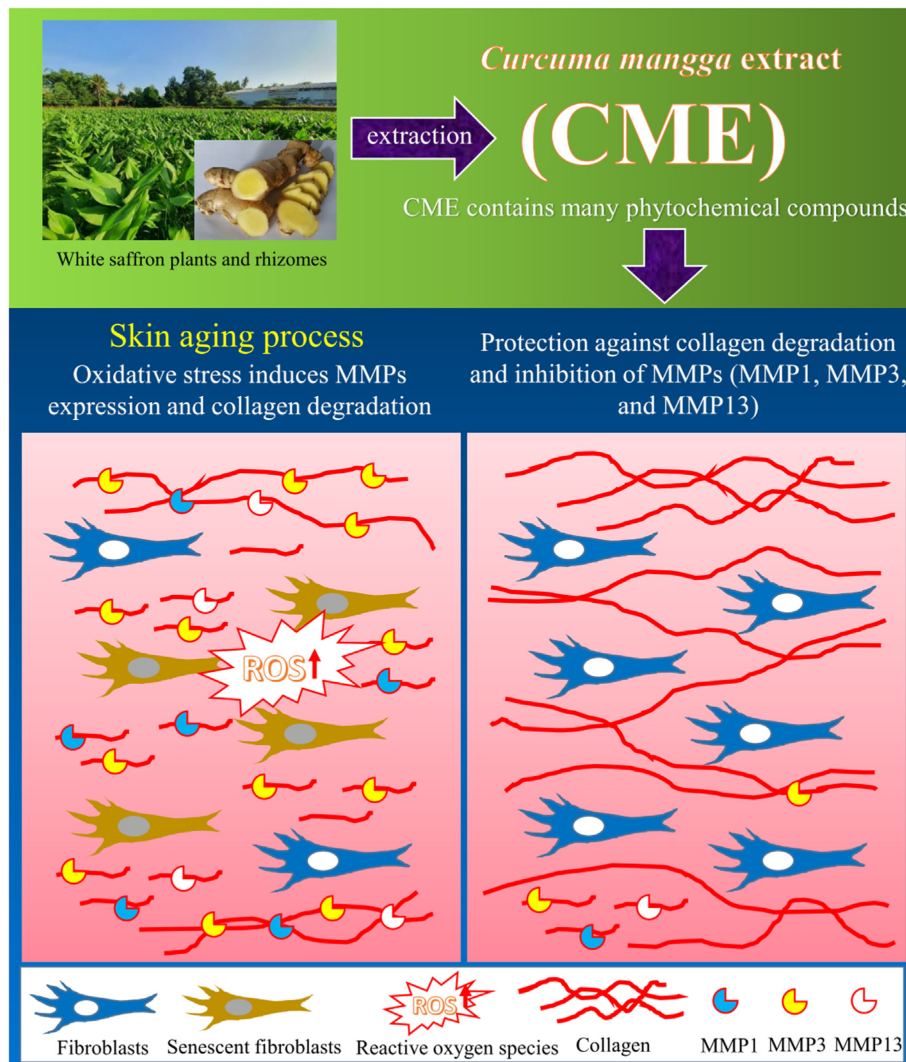

Figure 4. A schematic diagram of the skin aging process due to ROS-induced oxidative stress, causing the upregulation of MMP enzymes that degrade the collagen fibers. CME can protect collagen against degradation by suppressing the activity of MMP1, MMP3, and MMP13, resulting in collagen stabilization and maintained fibroblast cell viability.

undamaged collagen is related to the firmness and the overall shape of the skin. Thus, the degradation of collagen significantly reduces the morphological cohesion and stability of the collagen fibers (Farage et al., 2013). Oxidative stress promotes skin ECM remodeling through the activation of several factors, including protease family known as MMPs (Brennan et al., 2003; Davalli et al., 2016). MMPs, such as MMP1, MMP3, and MMP13, are the major enzymes that are involved in collagen degradation, resulting in ECM breakdown (Girsang et al., 2019). MMPs can alter the ECM and may augment skin wrinkling, which is the main feature of untimely skin aging (Pittayapruek et al., 2016). It was verified that ROS induced transcriptional activation and MMPs gene expression regulation mediated by NF-κB (Pittayapruek et al., 2016). The NF-κB upregulated MMPs, including MMP1 and MMP3 in dermal skin cells (Vicentini et al., 2011; Lee et al., 2012).

Several plants are known to inhibit MMPs activities through direct inhibition or pathways modulation (Widowati et al., 2018a). The present study showed that CME protects fibroblasts from collagen degradation induced by oxidative stress. CME exhibited potent inhibition against MMPs, especially the MMP3 overexpression. This protective property might be related to the antioxidant properties of CME (Pujimulyani et al., 2018), which maintain collagen contents and fibroblast cell viability due to the attenuation of MMPs expression.

Curcumin is the main constituent derived from *C. longa*. According to Retnosekar (2014), the curcuminoid content in *C. longa* is much higher than in *C. mangga* and *C. zedoaria*, namely 38.27 versus 9.75 and 3.58 mg/100 mg dried extract, respectively. Klawitter et al. (2012) studied the effects of *C. longa* extract and curcumin derived from *C. longa* on gene expression of matrix-degrading enzymes (MMP1, MMP3, and MM13) in IL-1β-treated human intervertebral disc cells. The result showed that *C. longa* extract (using EtOH) with a dose of 100 μg/mL could suppress the gene expression of MMP1, MMP3, and MMP13 by approximately 40, 120, and 28 times, respectively. In addition, curcumin treatment (10 μM) could inhibit the gene expression of MMP1, MMP3, and MMP13 by approximately 120, 220, and 100 times, respectively. The results were linear with ours, where CME treatment (100 μg/mL) could effectively inhibit MMP1, MMP3, and MMP13 gene expressions in oxidative stress-induced BJ fibroblasts by 3.65, 34.62, and 2.02 times, respectively.

Curcumin is known to mediate MMP1 and MMP3 activities by inhibiting the signaling pathways of PKCδ and JNK/c-Jun (Mun et al., 2009). Another phytochemical compound, such as epigallocatechin gallate (EGCG), modulates MMPs synthesis through the MAPK signaling interference (Bae et al., 2008). A coffee extract was also demonstrated to inhibit MMPs expression in a similar manner (Chiang et al., 2011). Besides, brazilin, which is the major constituent of *Caesalpinia sappan* L., impedes the expression and secretion of MMP1 and MMP3 mediated by the UVB by inactivating the NF-κB signaling in the cells, leading to the prevention of skin photoaging (Lee et al., 2012).

Curcumin is water-insoluble, thus limiting their potential for biomedical applications. In order to improve the solubility and accessibility of curcumin, scientists formulate curcumin into nanosuspension or encapsulation in nanoparticles. Ahmad et al. (2013, 2016), for instance, developed curcumin-loaded nanoparticles to relieve oxidative stress-associated brain injury in rat models. The results showed that curcumin-loaded nanoparticles had good physical properties, including size, zeta potential, and drug release profiles and could reduce pro-inflammatory cytokines and normalize the cerebral ischemia in rats. Interestingly, curcumin nanoemulsion is promising for wound healing and anti-inflammation. The nanoemulsion can be prepared through simple ultrasonication. Curcumin nanoemulsion had good skin permeation and could heal the wounds in rats more effectively than free curcumin (Ahmad et al., 2019a, 2019b). Curcumin has anti-inflammatory as well as antioxidant properties, which can mitigate symptoms of aging, indicating the possible antiaging role of curcumin (Sikora et al., 2010). Several compounds that are present in CME, such as curcumin, quercetin, EGCG, and gallic acid (Pujimulyani and Sutardi, 2003; Pujimulyani et al., 2012, 2013), may modulate multiple pathways compared to curcumin alone.

5. Conclusion

In conclusion, CME and its phytochemical protected fibroblasts against collagen degradation due to oxidative stress through the suppression of pro-inflammatory MMPs (MMP1, MMP3, and MMP13), thus disclosing unexplored cosmeceutical potentials of white saffron extract.

Declarations

Author contribution statement

Dwiyati Pujimulyani: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Lilis Suryani, Astuti Setyowati: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Anisa S. Handayani, Seila Arumwardana: Performed the experiments.

Wahyu Widowati, Ali Maruf: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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