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Contributed Paper

Biological Activities of Supercritical Carbon Dioxide Fluid ($scCO_2$) Extracts from Medicinal Flowers

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ABSTRACT

The biological activities of extracts from the three medicinal flowers including *Mimusops elengi* (Sapotaceae), *Millingtonia hortensis* (Bignoniaceae) and *Gardenia jasminoides* (Rubiaceae) prepared by the two non-heated processes were investigated. The biological activities of the extracts by the supercritical carbon dioxide fluid ($scCO_2$) with the co-solvent (ethanol) at 4, 20, 33 and 50% (w/v) were compared with the extracts prepared by hexane maceration. All extracts by $scCO_2$ gave higher free radical scavenging and skin fibroblast proliferation activities than those by the hexane maceration. The $scCO_2$ extracts of *M. elengi*, *M. hortensis* and *G. jasminoides* prepared with 20, 33 and 33% (w/v) ethanol as a co-solvent which showed the high percentage yields and free radical scavenging activity, were selected to investigate for MMP-2 inhibition. The extract of *G. jasminoides* by $scCO_2$ with 33% (w/v) ethanol as a co-solvent demonstrated the highest MMP-2 inhibition activity (43.85 ± 9.61%), high percentage yield (8.78 ± 1.27 %w/w), high free radical scavenging activity (SC_{50} at 5.14 ± 0.32 mg/ml) and low toxicity on human skin fibroblast (% cell growth of 68.48 ± 5.95 %). The high MMP-2 inhibition activity of this extract indicated its beneficial to be developed as anti-aging cosmetics.

Keywords: anti-aging cosmetics, biological activities, medicinal flowers, MMP-2 inhibition, supercritical carbon dioxide extraction ($scCO_2$).

1. INTRODUCTION

Nowadays, the interest of natural occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products to replace synthetic antioxidants which are being restricted due to their carcinogenicity [1]. Aromatic and medicinal plants are the source of natural antioxidants from their main secondary metabolite constituents, such as polyphenols and essential oils [2]. Essential

oils or volatile or ethereal oils are aromatic oily liquids obtained from different plant parts and widely used as food flavours. Essential oils are complex mixtures comprising of many single compounds, including terpenes and oxygenated compounds. Essential oils have been reported to be useful in food preservation [3], aromatherapy [4] and fragrance industries. Many flowers have been used in traditional medicines to improve the quality of health care. The flower buds of *Millingtonia hortensis* (Cork Tree) were the important herbal medicines used for the treatment of asthma, sinusitis and cholagogne and tonic in Southeast Asia [5]. From the dried flowers of *M. hortensis*, nine cyclohexylethanoids, including four glucosides were isolated along with 12 related known compounds [6]. The flowers of *Mimusops elengi* L. (Bullet Wood) are white and very fragrant, because the corolla preserves fragrance even after drying. The major compounds in ethanol extract and headspace of the Bullet Wood flowers were identified as *p*-methylanisole, methyl benzoate, methyl salicylate, 2-phenyl ethyl acetate, benzyl alcohol, 2-phenyl ethyl alcohol, nerolidol, methyl cinnamate, 3-hydroxy-4-phenyl-2-butanoate, cinnamyl alcohol and 2-phenyl ethyl benzoate [7]. Preclinical studies have shown that the extracts from the Bullet Wood have antibacterial, antifungal, anticariogenic, free radical scavenging, antihyperglycemic, antineoplastic, gastroprotective, antinociceptive and diuretic effects which have supported the ethnomedicinal uses in Ayurveda [8]. *Gardenia jasminoides* J. Ellis, has been used in traditional oriental medicine for the treatment of jaundice, fever, hypertension and ulcers of the skin [9]. In the phytochemical studies, genipin, chlorogenic acid, rutin, and ursolic acid were detected in the *G. jasminoides* extracts [10].

Currently, supercritical fluid has been introduced as an alternative organic solvent for plant extraction because of its environmental friendly property with one step process at low temperature. It is advantageous for heat labile bioactive compounds. The supercritical carbon dioxide fluid ($scCO_2$) which is non-toxic, non-inflammable and inexpensive [11, 12] has been used to substitute many organic solvents such as hexane, petroleum ether and ethyl acetate for the extraction of many natural thermal sensitive constituents, such as Patchouli (*Pogostemon cablin*) [13], Black Pepper (*Piper nigrum*) [14] and Marjoram (*Origanum majorana L.*) [15] essential oil.

Aging is a very complex biological process including the damage from free radicals and the dark spots of the skin from melanin overproduction. Free radicals are highly reactive molecules with unpaired electrons which can cause damage to cell membranes, lipids, proteins and DNA. Dark spots of the aged skin are from melanin overproduction which may be caused by chronic sun exposure, melasma or other hyperpigmentation diseases [16]. Tyrosinase, a copper containing monooxygenase, is a key enzyme that catalyzes melanin synthesis in melanocytes [17]. Fibroblasts which produce collagens, glycosaminoglycans, reticular and elastic fibers are found in the extracellular matrix. Collagen, the major structural component of the skin in the dermis has been suggested to be the cause of the clinical changes observed in naturally aged and photo-aged skin [18, 19]. Collagen synthesis has been studied by serial cultures of human dermal fibroblasts [19]. The effects on skin collagen synthesis for anti-aging evaluation

can be determined from the proliferation activity of the human fibroblasts by the sulforhodamine B (SRB) assay. Gelatinase A (MMP-2) digests native collagen types I, II and III in a similar manner to the collagenases [20, 21]. MMP-2 induction was mediated by phenomena accelerated in aged human skin. Increased expression of MMP-2 is involved with collagen degradation in aged human skin [22] leading to wrinkle formation and aged appearance. Thus, MMP-2 inhibitors which delay collagen degradation can be used to evaluate anti-aging activity. The MMP-2 inhibition of the three medicinal flowers including Cork Tree (*Millingtonia hortensis* L.f.), Bullet Wood (*Mimusops elengi* L.) and Gardenia (*Gardenia jasminoides* J. Ellis) have never been performed to evaluate for anti-aging cosmetic uses. In this present study, the biological activities including free radical scavenging, tyrosinase inhibition, cell proliferation and MMP-2 inhibition of human skin fibroblasts of these three flower extracts by the two non heated processes (scCO_2 extraction and hexane maceration) were investigated in order to evaluate their potential application in anti-aging cosmetics.

2. MATERIALS AND METHODS

2.1 Materials

Vitamin C (L-(+)-ascorbic acid), vitamin E, 2,2-diphenyl-1-picryhydrazyl radical (DPPH), sulforhodamine B (SRB), dimethyl sulfoxide (DMSO), gallic acid, sodium carbonate and kojic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mushroom tyrosinase (4187 U/mg) and L-tyrosine were purchased from Fluka (Buchs, Switzerland). Alpha-modified Eagle's culture medium, antibiotics penicillin and streptomycin, fetal bovine serum and trypsin were purchased from HyClone (Logan, UT, USA). Folin- Ciocalteu's reagent was purchased from Carlo Erba (Milan, Italy). All other reagents and solvents were of analytical grade.

2.2 Preparation of the Flower Extracts

2.2.1 Flower samples

Flowers of Cork Tree (*Millingtonia hortensis* L.f.), Bullet Wood (*Mimusops elengi* L.) and Gardenia (*Gardenia jasminoides* J.Ellis) were collected from Chiang Mai Province in Thailand during April to November in 2009**.** The specimen were authenticated by a botanist at Faculty of Pharmacy, Chiang Mai University, Thailand and deposited in the medicinal plant herbarium, Faculty of Pharmacy, Chiang Mai University in Thailand for authenticated specimen with voucher specimen no. 004108 for *Millingtonia hortensis* and no. 009595 for *Gardenia jasminoides*. The *Mimusops elengi* specimen was authenticated by a botanist at Natural Products Research and Development Center (NPRDC), Science and Technology Research Institute (STRI), Chiang Mai University, Thailand and deposited in the NPRDC medicinal plant herbarium, STRI, Chiang Mai University in Thailand for authenticated specimen with voucher specimen no. Manosroi 0028.

2.2.2 Maceration process

Briefly, 200 g of the fresh flowers were macerated with 1 L of hexane at room temperature (27±2°C) for 6 hours with continuous stirring. The extract was filtered through the paper filter (Whatman no. 1) connected with a vacuum pump. All filtrates were collected, pooled and dried by a rotary evaporator (Rotavapor R210, Buchi, Switzerland) at 40°C. The dried extracts were kept at -80°C until use.

2.2.3 Supercritical carbon dioxide fluid ($scCO_2$) extraction

Briefly, 200 g of the fresh flowers were extracted by $scCO_2$ with 4-50% (w/v) of ethanol 95% (v/v) as a co-solvent. The temperature in the collecting vessel was raised to 40°C. Carbon dioxide gas was introduced into the collecting vessel and the pressure was maintained at 300 bar. After 6 hours, the pressure was released and the extract was collected. All extracts were collected, pooled and dried by a rotary evaporator at 40°C. The dried extracts were kept at -80°C until use.

2.3 Biological Activities of the Extracts

2.3.1 DPPH radical scavenging activity

Free radical scavenging activities of the extracts and the standard antioxidants (vitamin C and E) were determined by a modified DPPH assay [23]. Briefly, 50 μl of five serial concentrations of the extracts (0.01-100 mg/ml) were dissolved in 95% v/v ethanol and 50 μl of the ethanolic solution of DPPH radical (0.25 mg/ml) were put into each well of a 96-well microplate (Nalge Nunc International, NY, USA). The reaction mixtures were allowed to stand for 30 minutes at 27±2°C, and the absorbance was measured at 515 nm by a well reader (Bio-Rad, model 680 microplate reader, USA) against the blank (95% v/v ethanol). Vitamin C and E (0.001-10 mg/ml) were used as positive controls. The experiments were done in triplicate. The percentages of radical scavenging activity were calculated as the following: Scavenging activity (%) = $[(A-B)/A]\times100$, where A was the absorbance of the control and B was the absorbance of the sample. The sample concentrations providing 50% of the scavenging (SC_{50}) were calculated from the graph plotted between the percentages of the scavenging activity and the sample concentrations.

2.3.2 Tyrosinase inhibition assay

The tyrosinase inhibition activity of the extracts was assayed by the modified dopachrome method using tyrosine as a substrate [24]. Briefly, 50 μl of five serial concentrations of the extracts at 0.01-100 mg/ml dissolved in DMSO, 50 μl of 200 units tyrosinase solution in 0.1 M phosphate buffer (pH 6.8), and 50 μl of 1 mg/ml tyrosine solution in the buffer, and 50 μl of the buffer were added into each well of 96-well plate. The mixture was incubated at 37±2°C for 60 minutes and the absorbance at 450 nm was measured. Vitamin C and kojic acid (0.001-10 mg/ml) were used as positive controls. All experiments were performed in triplicate. The percentages of tyrosinase inhibition were calculated according to the following equation: Tyrosinase inhibition activity (%) = $[(A-B)-(C-D)/(A-B)] \times 100$, where A was the absorbance of the blank after incubation, B was the absorbance of the blank before incubation, C was the absorbance of the sample after incubation, and D was the absorbance of the sample before incubation. The sample concentrations providing 50% inhibition (IC_{50}) were calculated from the graph plotted between the percentages of tyrosinase inhibition activity and the sample concentrations.

2.3.3 Cell proliferation activity on aged human skin fibroblasts

2.3.3.1 Cell culture

The normal human skin fibroblasts were provided by Dr. Natthanej Luplertlop at the Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. Cells were cultured under the standard conditions in the complete culture medium containing

α-modified Eagles culture medium (MEM-Alpha) supplemented with 10% v/v fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 mg/ml). Cells were incubated in a temperature-controlled and humidified incubator (Shel Lab, model 2123TC, USA) with 5% CO_2 at 37°C. Cells were used at the 30th passage.

2.3.3.2 Cell proliferation by the SRB assay

The extracts were tested for cell proliferation of the aged normal human skin fibroblasts (15th passage) by the SRB assay as previously described [25]. Vitamin C (0.00001 - 1 mg/ml) was used as a positive control. The cells were plated at a density of 1.0×10^5 cells per well in 96-well plates and left overnight for cell attachment on the plate in 5% CO_2 atmosphere at 37°C. Cells were then exposed to five serial concentrations of the extracts (0.00001-1 mg/ml) for 24 hours. After incubation, the adherent cells were fixed *in situ*, washed and dyed with SRB. The bound dye was solubilized and the absorbance was measured at 540 nm by a well reader. The experiments were done in triplicate. The percentages of cell proliferation were calculated according to the following equation: Cell growth (%) = (A-B / C-B) × 100, where A was the absorbance of the sample, B was the absorbance of the blank and C was the absorbance of the control.

2.3.4 Gelatinolytic activity (Zymography) of MMP-2 inhibition on human skin fibroblast

The flower extracts which gave high anti-oxidative activity especially the free radical scavenging (SC_{50}) and the proper percentage yields were selected to investigate for the gelatinolytic activity of MMP-2 inhibition in comparing to vitamins C and E. A monolayer of 5×10^5 cells at the 15th passage of normal aged human skin fibroblasts was maintained in the culture medium without FBS for 24 h, treated with the flower extracts, the standard vitamin C and E at 0.1 mg/ml and incubated for 48 h. The culture supernatants were collected. To assess the gelatinolytic activities of MMP-2 in the culture media, SDS-PAGE zymography using gelatin as a substrate was performed. Briefly, 20 μl of the cell culture supernatant were suspended in the loading buffer, 0.125 M Tris (pH 6.8), 4% SDS and 0.04% bromophenol blue, without prior denaturation and run on 10% SDS polyacrylamide gel containing 1 mg/ml gelatin. After electrophoresis, gels were washed to remove SDS and incubated for 20 min in the renaturing buffer (50 mM Tris, 5 mM $CaCl_2$, 0.02% NaN_3, 2.5% Triton X-100). The gels were then incubated for 24 h at 37°C in developing buffer of 50 mM Tris (pH 7.5), 5 mM $CaCl_2$, 0.02% NaN_3 and 1% Triton X-100. Gels were subsequently stained with 0.5% Coomassie brilliant blue G-250 and de-stained in 30% methanol and 10% acetic acid (v/v) to detect gelatinolytic activity [26]. The gel was documented by a gel documentation system (Bio-Rad Laboratories, Hertfordshire, UK) and analyzed by Quantity 1-D analysis® software. The area (mm^2) multiplied by density (intensity/mm^2) of the bands on the gel was determined as the relative MMP-2 content (intensity unit) [27]. The density value of this software is the total intensity of all the pixels in the volume divided by the area of the volume. The percentages of MMP-2 inhibition in comparing to the control (the untreated systems) were calculated by the following equation: MMP-2 inhibition (%) = 100 - [(MMP-2 content $_{sample}$ / MMP-2 content $_{control}$)×100]. The assays were done in three independent

separate experiments. The potency of MMP-2 inhibition of the extracts was compared with the positive control (vitamin C and E).

2.3.5 Total phenolic contents (TPC)

The total phenolic contents in the form of gallic acid in the dried powder of the selected flower extracts were determined using the Folin-Ciocalteu reagent [28]. Briefly, 50 μl of the extract (10 mg/ml) were mixed with 100 μl of Folin-Ciocalteu reagent and 50 μl of 20% (w/v) sodium carbonate (Na_2CO_3) at 25±2°C. After incubation for 30 min, the absorbance of the blue color developed in each assay mixture was recorded at 760 nm by a well reader. The TPC of the extracts were expressed in milligram of gallic acid equivalents (GAE) per gram of the extract.

2.4 Statistical Analysis

The results were presented as the mean of three independent experiments and analyzed by SPSS (version 16.0). ANOVA was used for the analysis of the test results (LSD test) at the significance level of p-value < 0.05.

3. Results and Discussion

3.1 Physical Characteristics of the Extracts

The flower extracts from *M. hortensis* by $scCO_2$ with 4, 20, 33 and 50% (w/v) of 95% (v/v) ethanol showed the highest percentage yields at 8.57±1.33, 11.77±1.28, 14.40±1.25 and 13.00±1.62% (w/w), respectively (Table 1). The percentage yields of *M. elengi* and *G. jasminoides* by $scCO_2$ with various concentrations of ethanol were not significantly different ($p < 0.05$). The *M. hortensis* flower extract had the darkest color appearance. The color of the extracts was deeper when higher ethanol concentrations were used in the $scCO_2$ extraction process. Most extracts prepared by $scCO_2$ were viscous liquid, while those by hexane maceration were semi-solid. The $scCO_2$ extracts gave higher percentage yields than the hexane extracts. This may be due to the more solvation property of $scCO_2$ than the hexane that may destroy the plant cellular structure leading to higher extraction and solubility of essential oil in the vacuoles of the flower cells [29].

3.2 Biological Activities of the Extracts

3.2.1 DPPH radical scavenging activity

The *M. elengi* extract prepared by $scCO_2$ with 20% (w/v) ethanol (SC_{50} value of 4.28±0.45 mg/ml) gave the highest DPPH scavenging activity, which was higher than the extract from *M. hortensis* prepared by $scCO_2$ with 20 and 50% ethanol (SC_{50} value of 4.33±0.44 and 4.51±0.32 mg/ml) of 1.01 and 1.05 times, respectively, but lower activity than the standard vitamin C (SC_{50} value of 0.036± 0.005 mg/ml), vitamin E (SC_{50} value of 0.049±0.009 mg/ml) of 119 and 87 times, respectively. Different ethanol concentrations as the co-solvent in the extraction of *M. elengi* and *M. hortensis* by $scCO_2$ did not show any significant differences in free radical scavenging activity. The *G. jasminosides*, *M. elengi* and *M. hortensis* extracts prepared by hexane maceration showed lower scavenging activities than those by the $scCO_2$ extraction. This agreed with the previous study that the extracts prepared by $scCO_2$ showed higher antioxidant activity than by the organic solvent [30]. The high antioxidant activity of the *M. elengi* flower extract might be from its contents of *p*-methylanisole, methyl benzoate, methyl salicylate, 2-phenyl ethyl acetate, benzyl alcohol, 2-phenyl ethyl alcohol, nerolidol, methyl-E-cinnamate,

3-hydroxy-4-phenyl-2-butanoate, E-cinnamyl alcohol and 2-phenyl ethyl benzoate, respectively [7]. These compounds have been previously reported to play an important role in scavenging activity instead of free phenolic acids, soluble phenolic esters and insoluble phenolic acid esters [31, 32]. Some triterpenes (taraxerol, β-amyrin, ursolic acid and oleanolic acid) and flavonoids (rutin, quercetin, kaempferol, isorhamnetin) were obtained from the flower extract of *Philadelphus coronarius* L. which also showed high free radical scavenging activity [33]. The significant negative relationship between the scavenging activity and the total phenolic contents of the three selected flower extracts have been observed at r=-0.998 (p-value$<$0.05). The total phenolic contents in the selected three flowers extracts were shown in Table 1. The *G. jasminoides* (33% of ethanol in $scCO_2$) showed the highest contents of total phenolic compounds at 4.18±0.28 mg of gallic acid equivalents (GAE) per gram of the extract, which was higher than the extracts of *M. hortensis* (20% of ethanol in $scCO_2$) and *M. elengi* (33% of ethanol in $scCO_2$) at 3.05±0.41 and 2.53±0.32 (GAE) mg/g of the extract. However, the *M. elengi* (33% of ethanol in $scCO_2$) gave the highest free radical scavenging activity.

3.2.2 Tyrosinase inhibition activity

At all concentration of ethanol as a co-solvent used in the $scCO_2$ extraction, the extracts gave almost no tyrosinase inhibition activity (IC_{50} > 200 mg/ml) in comparing to kojic acid which gave the IC_{50} at 0.049± 0006 mg/ml. The *M. elengi* extract prepared by hexane maceration gave the highest tyrosinase inhibition activity (IC_{50} value of 120.03±31.21 mg/ml) from the graphical estimation, but lower activity than the standard vitamin C (IC_{50} value of 0.24±0.02 mg/ml) and kojic acid (IC_{50} value of 0.049± 0.006 mg/ml) of 500 and 2,450 times, respectively. Other flower extracts which gave the IC_{50} value of tyrosinase inhibition activity more than 200 mg/ml were classified as "no activity" due to their low activities. These extracts may contain no constituent which can bind with the tyrosinase enzyme, a copper-containing monooxygenase, which is the key enzyme in melanin synthesis [17]. Thus, no inhibition of melanogenesis in skin of these flower extracts can be anticipated.

3.2.3 Cell proliferation activity

At 0.1 mg/ml, the *G. jasminoides* extract prepared by $scCO_2$ with 4% (w/v) ethanol (% cell growth at 77.20±1.72%) showed the highest cell proliferation activity which was higher than the *G. jasminoides* extract prepared by $scCO_2$ with 20% (w/v) ethanol (% cell growth at 76.21±6.31%) and the *M. elengi* extract prepared by $scCO_2$ with 4% (w/v) ethanol (% cell growth at 75.22±7.87%), but lower activity than the standard vitamin C (% cell growth at 209.05±75.06%) of 2.9 times. The flower extracts prepared by hexane maceration showed higher toxicity to the normal human skin fibroblast than those prepared by the $scCO_2$. This might due to not only the toxicity of the remaining residue of hexane in the extracts, but also some cytotoxic compounds which can be soluble in hexane. Hexane had been listed as a hazardous solvent by the U.S. Environmental Protection Agency which may be toxic to the normal cell [34]. In each group of the flower extract, the hexane extract showed the highest toxicity within each group. As known, different solvents can extract different compounds. Hexane can extract the non-polar compound more

Table 1. Comparison of appearance, percentage yields, antioxidative, tyrosinase inhibition activities, the percentages of cell growth on human skin fibroblasts (15th passage) and the total phenolic contents of the three flower extracts prepared by the two non-heated methods ($scCO_2$ and hexane maceration).

plant		extraction method		appearance	percentage yields (%w/w)	SC_{50} (mg/ml)	IC_{50} (mg/ml)	% cell growth on human skin fibroblast	TPC (GAE (mg/g) of the extract)
scientific name	common name		Percentages of 95% ethanol (%w/v)						
Millingtonia hortensis	Cork Tree	$scCO_2$	4	dark brown viscous liquid	8.57±1.33	5.62±1.01	NA	72.24±14.09	-
			20	dark brown viscous liquid	11.77±1.28	4.33±0.44	NA	67.29±3.43	-
			33	dark brown viscous liquid	14.40±1.25	4.83±0.38	NA	65.30±4.54	3.05±0.41
			50	dark brown viscous liquid	13.00±1.62	4.51±0.32	NA	73.73±14.72	-
		hexane		green semi-solid	0.87±0.12	16.53±2.45	NA	64.31±61.98	-
Mimusops elengi	Bullet Wood	$scCO_2$	4	light brown viscous liquid	7.05±1.23	4.65±0.34	NA	75.22±7.87	-
			20	brown viscous liquid	9.61±1.08	4.28±0.45	NA	72.24±5.95	2.53±0.32
			33	brown viscous liquid	7.58±1.25	4.83±0.28	NA	67.29±4.54	-
			50	dark brown viscous liquid	8.58±1.54	4.73±0.56	NA	73.23±16.91	-
		hexane		yellow semi-solid	0.31±0.10	6.60±0.54	120.03±31.21	57.37±8.41	-
Gardenia jasminoides	Gardenia	$scCO_2$	4	light brown viscous liquid	8.03±1.31	7.64±1.75	NA	77.20±1.72	-
			20	dark brown viscous liquid	7.52±1.45	11.23±1.89	NA	76.21±6.31	-
			33	dark brown viscous liquid	8.78±1.27	5.14±0.32	NA	68.48±5.95	4.18±0.28
			50	dark brown semi-solid	6.95±1.01	8.08±0.43	NA	69.27±6.25	-
		hexane		greenish-yellow semi-solid	0.87±0.21	21.39±3.61	NA	66.29±4.20	-
vitamin C					-	0.036±0.005	0.24±0.02	209.05±75.06	-
vitamin E					-	0.049±0.009	-	-	-
kojic acid					-	-	0.049±0.006	-	-

Note: - represented "not applicable"
NA represented "no activity at the concentration < 200 mg/ml
SC_{50} value (mg/ml) was the concentration of the sample that scavenged 50 % of the DPPH radicals.
IC_{50} value (mg/ml) was the concentration of the sample that inhibited 50 % of the tyrosinase enzyme.
% cell growth was the percentages of cell proliferation of the treated sample (at 0.1 mg/ml) in comparing to the control (no treatment).
Total phenolic contents were presented as gallic acid equivalent (GAE) mg/g of the extracts.

than the $scCO_2$ fluid [35]. Moreover, the non polar compound in the hexane extract can also penetrate the cell membrane better than the more polar compound existing in the $scCO_2$ with ethanol extracts. However, *G. jasminoides* hexane extract gave % cell growth more than *M.hortensis* $scCO_2$ 33%. This might be from the different constituent in each flower extract. The flavonoids in the *M.hortensis* $scCO_2$ extract such as hispidulin and hortensin may be toxic to the cells [36]. In addition, different flowers also contain different compounds which may have different effects on the cells.

3.2.4 Zymography of MMP-2 inhibition

In the $scCO_2$, the flower extracts of *M. elengi*, *M. hortensis* and *G. jasminoides* prepared by $scCO_2$ with 33, 20 and 33% (w/v) ethanol were selected to test for gelatinolytic activity (Zymography) of MMP-2 inhibition. Figure 1 showed the inhibition of MMP-2 expression by zymography of the extracts, standard vitamin E and C. The *G. jasminoides* extracts prepared by $scCO_2$ with 33% (w/v) ethanol showed the highest MMP-2 inhibition at 43.85±9.61%, but significant lower than vitamin C (72.15±5.65%) and vitamin E (62.78±4.93%) of 1.65 and 1.43 times, respectively ($p < 0.05$). The high free radical scavenging abilities of the extracts may lead to the inhibition of the expression levels of MMP-2 and -9 in gelatin zymography [37]. Moreover, the *G. jasminoides* extract (33% of ethanol in $scCO_2$) which showed the highest MMP-2 inhibition, also has the highest contents of total phenolic compounds. The phenolic compounds including, sesquiterpene lactones, triterpenes, phytosterols and cinnamic acid derivatives have been reported to inhibit MMP-2 and -9 [38, 39]. As known, MMPs can be activated by ROS

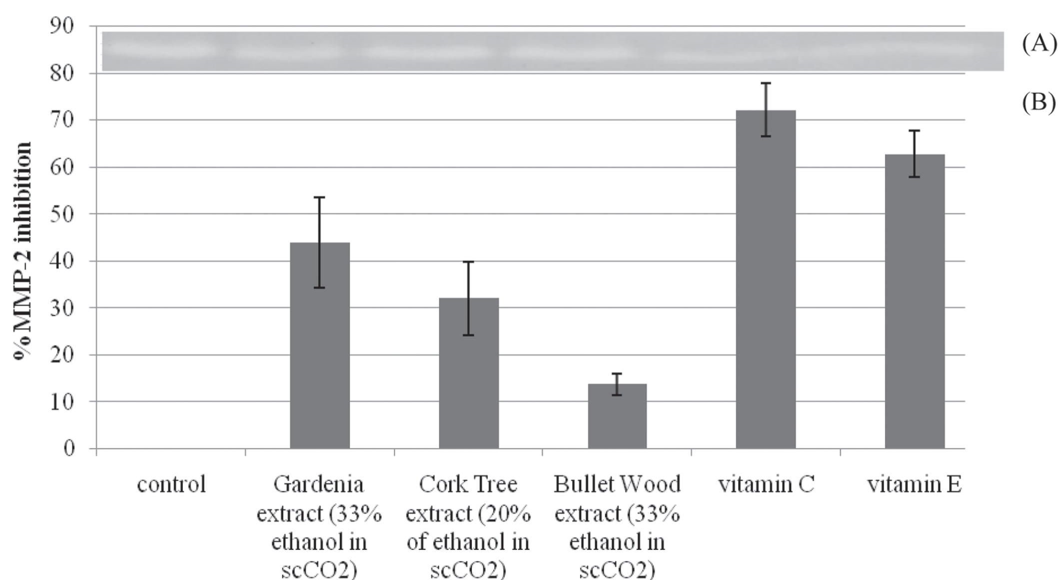

Figure 1. MMP-2 inhibition activity of the flower extracts from *M. elengi* (Bullet Wood) prepared by $scCO_2$ with 33% (w/v) ethanol, *M. hortensis* (Cork Tree) by $scCO_2$ with 20% (w/v) ethanol and *G. jasminoides* (Gardenia) by $scCO_2$ with 33% (w/v) ethanol compared to vitamin C and E. (A) zymograms and (B) the percentages of MMP-2 inhibition.

generated by UV causing photoaging. In fact, ROS can affect the gene expression including the induction of MMPs through the signal transduction pathway [40, 41]. Several studies have explained that antioxidant activity of some plant extracts can inhibit ROS by their reducing power which break the free radical chain by donating a hydrogen atom [42]. The flower extract of *Calendula officinalis* which is the rich sources of carotenoids including lutein, lycopene and β-carotene gave high antioxidant activity and also the expression of MMP-2 and MMP-9 inhibition activity in B16F-10 melanoma [43]. Therefore, the flower extracts prepared by the scCO2 which gave high antioxidative activity and also high MMP-2 inhibition activity have indicated their beneficial to be developed as anti-aging cosmetics.

4. Conclusion

This study has investigated the biological activities of the medicinal flower extracts prepared by the two non-heated processes (scCO_2 and hexane maceration). The extracts from the scCO_2 method showed higher free radical scavenging and normal human skin fibroblast proliferation activities than those by the hexane maceration. The extracts of *M. elengi* prepared by scCO_2 with 33% (w/v) ethanol, *M. hortensis* by scCO_2 with 20% (w/v) ethanol and *G. jasminoides* by scCO_2 with 33% (w/v) ethanol gave the high percentage yields and scavenging activity. The *G. jasminoides* extract by scCO_2 with 33% (w/v) ethanol gave the highest MMP-2 inhibition activity (43.85±9.61%) with the high percentage yield (8.78±1.27 % w/w), high free radical scavenging (SC_{50} at 5.14±0.32 mg/ml) and low cytotoxicity on human skin fibroblast. Hence, the high biological activities, especially the MMP-2 inhibition have suggested the potential of this flower extract to be developed as anti-aging cosmetics.

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6. References

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