Biological Effects of Yeast-fermented Plant Root Extract Mixture on Skin Cells

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Running title: Biological Effect of Yeast-fermented Plant Root Extract

Abstract

Purpose: To verify the biological effects including anti-aging and anti-inflammatory effects, of fermented plant root extract mixtures that were measured *in vitro*. **Method:** To select the most effective plant root extract, the effects of DPPH free radical scavenging were measured. The mixture (MC) of selected plant roots was fermented with *Saccharomyces cerevisiae* (*S. cerevisiae*) (MF). Reverse transcription (RT)-PCR was performed on human dermal fibroblast to measure the effects of MC and MF on the mRNA expression of *COL1A1* and hyaluronic acid synthase 2 (*HAS*2) and on the anti-inflammatory-related gene expressions such as cyclooxygenase-2 (*COX-2*), interleukin-6 (*IL-6*), and tumor-necrosis factor (TNF)-*a*. Additionally, we also tested the cell renewal and proliferation for skin cell migration. Student's t-test was performed for statistical analysis, and results were expressed as mean ± standard deviation. **Results:** Among 15 root extracts, those of *Taraxacum officinale* (*T. officinale*) rhizome, *Arctium lappa* (*A. lappa*), *Anemarrhena asphodeloides* (*A. asphodeloides*), *Pueraria lobata* (*P. lobata*), and *Nelumbo nucifera* (*N. nucifera*) were selected based on the effect of DPPH free radical scavenging. The mRNA expression levels of *COL1A1* and *HAS2* were increased by MC and MF in a concentration-dependent manner. MC and MF showed cell renewal and proliferation. Inflammation-related genes were inhibited by MC or MF in a concentration-dependent manner (*TNF-a*, *IL-6*, and *COX-2*). MF showed significantly better efficacy than MC. **Conclusion:** Mixed root extracts of *T. officinale*, *A. lappa*, *A. asphodeloides*, *P. lobata*, and *N. nucifera* fermented with *S. cerevisiae* enable cell renewal, have anti-aging and anti-inflammatory effects, and can be used as an active cosmetic raw materials.

Keywords: Mixed plant root extracts, Fermentation, Anti-aging, Anti-inflammation, Cosmetic raw material

Introduction

Skin aging shows structural, and functional changes that are either intrinsic or extrinsic. Intrinsic aging is defined as the decreased skin structure and physiological activity over time, while extrinsic aging, or photoaging, is caused by external stresses such as ultraviolet and reactive oxygens (Helfrich *et al.*, 2008).

The keratinocytes from the basal layer of the skin move toward the surface of the stratum corneum. These are removed through desquamation, as the skin regenerates over time, a period called skin turnover. The life span of keratinocytes in the human skin is approximately 20–28 days (Ho & Dreesen, 2021). Skin renewal by the keratinocytes slows down as the skin becomes older (Choi *et al.*, 2017). Lifestyle habits such as insufficient sleep, alcohol drinking, and smoking retard of skin cell regeneration. Skin-related problems such as wrinkles and freckles occur due to the delay of skin turnover.

Studies on herbs and its benefit for skincare have recently increased, particularly due to the interest of cosmetic consumers in natural products (Shim, 2021; Zhang *et al.*, 2018; Moon *et al.,* 2017; Di Napoli & Zucchetti, 2021; Sivamaruthi *et al.*, 2018). The benefits of mixed plant extracts and the mixed extracts have been reported to be better than those of individual extracts (Choi, 2022; Kim *et al.*, 2019; Lee *et al.*, 2021).

By fermentation, certain common substrate components are degraded or transformed into compatible components. This process can generally increase the physiological and biochemical activities of biological substrates by modifying naturally occurring molecules and can be used to develop new pharmaceutical and cosmetic formulations (Kim, 2011; Chiba, 2007; Um *et al.*, 2017; Hussain *et al.*, 2016). The cytotoxicity of certain herbal extracts can be reduced or eliminated after treatment using various microbial species. Some probiotics, such as *Bifidobacterium bifidum* and *Saccharomyces cerevisiae*, can produce new components by fermentation or reduce the cytotoxicity of herbal extracts (Shim *et al.*, 2019; Hussain *et al.*, 2016; Wang *et al.*, 2016).

In the present study, the effects of plant roots extracts of *Taraxacum officinale*,

Arctium lappa, *Anemrrhena asphodeloides*, *Pueraria lobata*, and *Nelumbo nucifera* fermented with *S. cerevisiae* on the expression of anti-aging and inflammatory-related genes were evaluated using human keratinocytes and fibroblasts to evaluate their potential as a cosmetic raw material.

Materials and Method

1. Plant roots and extraction

Each plant root was acquired from Shenzhen Mannay Cosmetics Co. Ltd., and was dried and ground before extraction (Table 1). The resulting powder was extracted in 20 volumes of 70% alcohol at room temperature for 3 h and filtered using a filter paper (No. 2 qualitative filter papers, Whatman, England). The resulting filtrates were concentrated under a vacuum using a rotary evaporator (N-1110, EYELA, USA) until alcohol was eliminated. The concentrated extracts were stored at −20°C until further use.

The select root powders were mixed at equal amounts and extracted as described above. To prepare the fermented root mixture extracts, pre-cultured *S. cerevisiae* (1×10^6/mL) was inoculated on the root mixture extracts and fermented at 28°C with 30 rpm for 3 days. To terminate the fermentation, the cultures were boiled at 100°C for 30 min and filtered using a 0.45-μm filter.

The international nomenclature cosmetic ingredient (INCI) of each plant root extract used in this study is described in Table 1.

2. DPPH free radical-scavenging activity assay

The free radical-scavenging activity of plant root extracts was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma Aldrich, USA) assay. 50 μL of the diluted extracts were mixed with 100 μL of 0.1 mM DPPH solution. The DPPH solution without the test sample was used as a control. The mixture was incubated for 30 min at room temperature and subsequently measured for its absorbance at

515 nm. The antioxidative activity was calculated using the formula below and expressed as the percentage of DPPH radical elimination:

[(Ablank-Asample)/Ablank]×100 (%)

where Ablank is the absorbance of the blank DPPH solution and Asample is the absorbance of the DPPH solution after the addition of test sample.

3. Cell lines

Human dermal fibroblasts (HDF; Thermo Fisher Scientific, USA) were cultured in Fibroblast Basal Medium (FBM 106; Gibco, USA) supplemented with low serum growth supplement (LSGS; Gibco, USA), 100 IU/mL penicillin, and 100 μg/mL streptomycin. Human epidermal keratinocytes (HEKa; Invitrogen, USA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, USA) supplemented with antibiotics (100 IU/mL penicillin and 100 μg/mL streptomycin) and 10% fetal bovine serum (FBS; Gibco BRL, USA). The cells were maintained at 5% CO_2 at 37°C. Confluent monolayer cultures of HDF and HEKa were trypsinized with 0.25% trypsin-EDTA.

4. Cell viability

Cell viability was determined by measuring the conversion of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) into formazan via mitochondrial oxidation according to a modified Mosmann's method (Mosmann, 1983). HEKa and HDF were seeded at 1×10^5 cells/well in a 96-well plate and cultured for 18 h. The prepared sample was added in 0, 1, 2, and 5% concentrations. After incubating for 24 h, the medium was gently removed, the cells were washed twice in phosphate-buffered saline (PBS), and then the wells were filled with 5 mg/mL of solution MTT (Sigma Aldrich) as the new medium. Afterward, formazan crystals produced from MTT were solubilized in 150 μL dimethyl sulfoxide (DMSO; Sigma Aldrich). The absorbance of each well was then measured at 570 nm using a microplate reader (Epoch2C, USA). The optical density of formazan formed in

control cells without sample was considered as 100% viability.

5. Wound-healing (cell renewal) test

To examine cell renewal, HEKa was subjected to a wound-healing assay. The cells were inserted on a 12-well plate and then incubated for 4 h under cell culture conditions. On the removal of the culture medium, a "wound gap" in a cell monolayer was created by scratching, and then 2% of test samples along with a new culture medium were added to the cells; the plate was incubated again for 18 h. Four hours after incubation, cell migration was analyzed to measure the cell renewal effect.

6. Cell proliferation

To examine the cell proliferation of the test sample, CytoSelect™ BrdU Cell Proliferation ELISA Kit (Cell Biolabs Inc., USA) was used. The cells were inserted on a 96-well plate and then incubated for 18 h under cell culture conditions. Upon removal of the culture medium, the cells were washed with PBS, and then test samples along with a new culture medium were added to the cells; the plate was again incubated for 24 h. Afterward, a BrdU Solution was added to the wells, and the plate was again incubated at 37°C and 5% CO_2 in a humidified incubator for 4 h. In each tested well, 100 µL of diluted anti-BrdU antibody and secondary antibody HRP conjugate were added. After incubation for 30 min, absorbance was measured according to the manufacturer's instructions. The cell proliferation rate of each sample treated was calculated based on the blank.

7. RNA isolation and revers transcription (RT)-PCR

To determine the mRNA expression of *COL1A1*, hyaluronic acid synthase2 (*HAS2*), tumor-necrosis factor-α (*TNF-a*), interleukin-6 *(IL-6),* and cyclooxygenase-2 (*COX-2*), cells were cultured in Medium 106 supplemented with 1× LSGS, 100

IU/mL penicillin, and 100 μg/mL streptomycin at 5% CO_2 at 37°C for 24 h. The medium was gently removed, and the cells were treated with 0.1%, 0.5%, 1%, and 2% concentrations of the sample and then cultured for 48 h. Total RNAs from each well were isolated using the TransZol reagent and obtained to generate the cDNA using the PrimeScript 1st cDNA synthesis kit, as recommended by the manufacturer's instructions. Amplifications were performed in a thermal cycler. The experiment consisted of 35 cycles: denaturation at 95°C for 1 min, annealing at 50–60°C for 30 s, and extension at 72°C for 1 min. The sequences of primer pairs, and further details of PCR, are given in Table 2. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a control gene. PCR products were electrophoresed on a 1% agarose gel and stained with ethidium bromide to analyze the relative ratio of changes in the target gene in comparison to those of the control.

8. Statistical analysis

The results obtained were expressed as mean±standard deviation (SD) from at least three independent experiments. The student's *t*-test with Microsoft Excel (Microsoft, USA) was used to analyze the data. $p<0.05$ was considered to indicate statistical significance.

Results and discussion

In the cosmetic industry, natural ingredients have been increasingly a point of concern. In this study, we focused on plant roots due to their medicinal use in Asian cultures (Bark *et al.*, 2010). We prepared 15 plant root extracts (Table 1) and evaluated the antioxidant effect using DPPH free radical scavenging assay. The tested plant root extracts show an antioxidant effect at 10% treatment (Figure 1). Among them, only five show more than 80% of DPPH free radical-scavenging activity: *Taraxacum offininale*, *Arctium lappa*, *Anemrrhena asphodeloides*, *Pueraria lobata*, and *Nelumbo nucifera*.

The synergic effect of a mixture of plant extract and the fermented mixture of plant

extract have been reported (Kim *et al.*, 2019; Um *et al.*, 2017). Five roots were selected to be mixed and extracted (MC). In addition, MC was fermented using *S. cerevisiae* (MF). Fermentation is a strategy to improve efficacy and reduced toxicity product and is frequently used to manufacture cosmetic raw materials.

The results showed that MC and MF treated on HDF and HEKa had more than 80% of cell viability at 2% concentration and following works were carried out in this range (Figure 2).

The effects of each root extract, MC, and MF on cell renewal were measured in HEKa cells: 34–63% by each root extract and 67% of cell renewal activity by MC-treated HEKa cells, which is similar to that by positive controls. MF-treated HEKa cells recovered the wound gap by approximately 77%, which was approximately 20% higher than that of MC. Thus, MF promotes skin cell regeneration and activates the proliferation of the skin cells (Figure 3).

Our results also confirmed that MC and MF affect cell proliferation in HEKa cells. Figure 4 shows that MC and MF have cell renewal and cell proliferation in a dose-dependent manner. Results indicated that cell proliferation was significantly increased by MF-treated cells compared to that by MC-treated cells ($p<0.05$). Cell proliferation in HDF cells treated with 2% MF and 2% MC was significantly higher than that in untreated cells (234.06% and 195.38%, respectively); MF treatment results in approximately 38.68% higher than that of MC treatment at 2% treatments. In terms of efficacy, our result was similar with that of Hsu and Chiang when they compared the effect of fermented *Radix astragali* with *Bacillus subtilis* natto and non-fermented *R. astragali*, which is well-known for its medicinal effect. The fermented *R. astragali* has a better anti-aging effect despite the reduction in concentration of main isoflavonoids such as galycosin, formononetin, and ononin (Hsu & Chiang, 2009).

Collagen is composed of three polypeptide α-chains that form a triple helical structure. *COL1A1* is a gene-encoding type I collagen that is one of fibril-forming collagen (Yue, 2014; Ricard-Blum, 2011). The effects of MC and MF on *COL1A1* mRNA expression were evaluated in HDF cells using RT-PCR. The mRNA

expression levels of *COL1A1* in MC- and MF-treated HDF cells increased in a dose-dependent manner (Figure 2). Similar to the cell proliferation test result, the effect of MF on *COL1A1* mRNA expression was higher than that of MC with a significantly different ($p<0.05$). The mRNA expression levels of *COL1A1* in HDF cells treated with 2% MC and MF were 157.37% and 195.11%, respectively, while that of a positive control (50-μM retinyl palmitate treatment) was 220.57% (Figure 5A). *COL1A1* mRNA expression in MF-treated cells was approximately 24% higher than that of MC-treated cells at 2% ($p<0.05$).

In the skin, gene expression of *HAS*2 is stimulated by keratinocyte growth factor, which activates keratinocyte migration and stimulates wound healing (Papakonstantinou *et al.*, 2012). We measured the effect of MC and MF on *HAS2* mRNA expression. *HAS2* mRNA expression was increased in a dose-dependent manner, with MF-treated cells having significantly higher results than those of MC-treated cells ($p<0.05$). HAS2 expression levels of HDF cells treated with 2% MC and MF were 221.71% and 256.64%, respectively, and that of 50 μg/mL hyaluronic acid was 267.39% (Figure 5B).

Inflammation of the skin was caused by TNF-α, ILs, and other cytokines that could be upregulated by environmental or physiological stresses, thereby restricting the growth of skin cells while inhibiting skin inflammation and aging. Inflammation is one of the intrinsic reasons for aging, and to maintain a healthy skin, inflammation control is important (Baylis *et al.*, 2013). The effect of MC and MF on inflammation-related gene expressions such as *TNF-a*, *IL6*, and *COX2* was measured in HEKa cells (Figure 6). *TNF-a* mRNA expression in HDF cells after treatment of 2% of MC was measured as 157.37% of UV-irradiated control, whereas the same concentration of MF treatment was measured as 195.11%. A positive control treatment with 50 μM retinyl palmitate resulted in a *TNF-a* expression level of 220.57% (Figure 6A).

At 2% treatment of MC, *IL6* mRNA expression in HDF cells was measured as 157.37% of UVB-irradiated control, whereas the same concentration of MF treatment was measured as 195.11%. A positive control treatment with 50 μM retinyl palmitate resulted in an *IL6* expression level of 220.57% (Figure 6B).

The effect of MC and MF on *COX2* mRNA expression in HDF cells also similar with other inflammatory-related biomarkers (157.37% and 195.11%, respectively, of UVB-irradiated control). A positive control treatment with 50 μM retinyl palmitate resulted in a *COX2* expression level of 220.57% (Figure 6C). The expression of *TNF-α*, *IL6*, and *COX2* mRNA in MC- or MF-treated HEKa cells was inhibited in a concentration-dependent manner.

NN root contains betulinic acid, rutin, isoquercetin, and other flavonoids that have antioxidant, anti-inflammatory, and cytoprotective activities (Sruthi *et al.*, 2019). Petkova *et al.* reported the phytochemical composition of AL root extracts and their antioxidant effects. AL roots contain phenolic compounds such as chlorogenic acid, caffeic acid, and p-coumaric acid (Petkova *et al.*, 2022), and these compounds are well-known antioxidants (Predes *et al.*, 2011; Rosalia *et al.*, 2010). Phytochemicals such as carotenoids, flavonoids, and phenolic compounds have a number of medicinal properties such as antioxidant, antiviral, antifungal, and antibacterial effects, (Di Napoli & Zucchetti, 2021). Wang *et al.* isolated saponins from AA roots and investigated the isolated compounds that inhibit LPS-induced NO production (Wang *et al.*, 2018). PL root, known as Kuzu, has been used as a traditional medicine for treating cardiovascular diseases and type 2 diabetes mellitus in China (Luo *et al.*, 2007). PL root contains antioxidants, isoflavones, triterpene, and their glycosides and have anti-inflammatory and anti-apoptosis properties (Zhang *et al.*, 2018; Wong *et al.*, 2011). Each root contains various active compounds that possess biological activities.

In this study, we prepared a mixture of extracts using five roots selected from 15 roots based on DPPH radical scavenging activity, and the effect of MC and MF were compared on the mRNA expression of *COLA1A1* and *HAS2* and cell proliferation, as well as their effect of MC and MF on the expression of inflammation-related mRNA such as *TNF-α*, *IL-6*, and *COX-2*. Based on our results, the treatment of MC and MF could enhance anti-aging properties by promoting *COL1A1* and *HAS2* mRNA expression and inducing a positive effect on cell migration. Fermentation of the mixture of the selected roots extracts significantly contributed to the increased anti-aging capabilities.

Conclusion

Of 15 plant root extracts, only five root extracts were selected based on the result of DPPH free radical scavenging activity: *T. officinale*, *A. lappa*, *A. asphodeloides*, *P. lobata*, and *N. nucifera*. The mixture of selected root extracts (MC) and their fermented products (MF) with *S. cerevisiae* improved *COL1A1* and *HAS2* mRNA expressions and cell proliferation efficacy in HDF. MC and MF also showed anti-inflammatory effects in human keratinocytes and inhibited the mRNA expression of *TNF-a, IL6*, and *COX2*. Furthermore, MF had better efficacies for anti-aging and anti-inflammation than MC. Based on these results, MF has better efficacy than MC and can be used as an active material for cosmetics with anti-aging and anti-inflammation effects.

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Author's contribution

HL and YJK designed the study and performed the extraction and biochemical assays. HL and WHC managed the project. HL wrote the manuscript with assistance from YJK. All authors read and approved the final manuscript.

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국문초록

효모 발효 식물 뿌리 혼합 추출물의 피부세포에 대한 생물학적 활성

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목적: 혼합 식물 뿌리 추출물 및 발효 혼합 식물뿌리 추출물의 생물학적 활성을 확인하기 위하여 체외 실험을 통하여 항노화 및 항염증 효과를 확인하였다. **방법:** DPPH 라디칼 소거 활성 평가를 통하여 항산화 활성이 우수한 식물 뿌리추출물을 선별하였으며, 선별된 식물 뿌리의 혼합 추출물 (MC) 및 S. cerevisia를 이용하여 식물 뿌리 혼합추출물의 발효물을 (MF) 제조하였다. 인체 피부 섬유아세포에서 MC 및 MF의 *COL1A1* 및 *HAS2* mRNA 발현에 대한 영향을 확인하였으며, 세포 증식 실험을 통하여 세포 이동능을 평가하였다. MC 및 MF의 항염증 효과를 확인하기 위하여 인체 각질 세포에서 RT-PCR을 통하여 COX2 *TNF-a* IL6의 발현을 확인하였다. 실험 결과는 평균치와 표준편차로 나타내었고, 결과는 *t*-test를 사용하여 분석하였다. **결과:** DPPH 라디칼 소거 활성 평가를 통하여 15종의 뿌리 추출물 중 5 종을 선별하였다. MC 및 MF는 *COL1A1* 및 *HAS2*의 발현을 농도의존적으로 증가시켰으며, 세포 증식효과를 보여주었다. 또한 MC 및 MF는 염증 관련 유전자인 *TNF-a*, *IL6*, *COX2*의 발현을 농도 의존적으로 저해하였다. 모든 실험에서 MF는 MC와 비교하여 더 좋은 효과를 보여주었다. **결론:** 서양민들레뿌리 줄기/뿌리추출물, 우엉뿌리추출물, 지모뿌리추출물, 칡추출물 및 연꽃뿌리추출물의 혼합 발효물은 항노화 및 항염효과를 갖고 있어 기능성 화장품 원료로 적용 가능하다.

핵심어: 식물뿌리추출물, 발효, 항염, 항노화, 화장품원료

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中文摘要

酵母发酵植物根提取物混合物对皮肤细胞的生物学作用

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目的：为了确认混合植物根提取物和发酵混合植物根提取物的生物活性，通过体外实验证实了抗衰老和抗炎作用。**方法：**通过对 DPPH 自由基清除活性的评价，筛选出具有优异抗氧化活性的植物根提取物，并利用所选择的植物根混合提取物(MC)和酿酒酵母制备了混合植物根提取物(MF)的发酵产物。确认 MC 和 MF 对人皮肤成纤维细胞 COL1A1 和 HAS2 mRNA 表达的影响，并通过细胞增殖实验评价了细胞迁移能力。为了证实 MC 和 MF 的抗炎作用，通过 RT-PCR 证实了 COX2、TNF-α、IL6 在人角质形成细胞中的表达。实验结果用平均值和标准差表示，结果采用 t 检验分析。**结果：**通过对 DPPH 自由基清除活性的评价，选择了 15 种根提取物中的 5 种。MC 和 MF 以浓度依赖性方式增加 COL1A1 和 HAS2 的表达，并表现出细胞增殖作用。此外，MC 和 MF 以剂量依赖性方式抑制炎症相关基因 TNF-α、IL6、COX2 的表达。在所有实验中，与 MC 相比，MF 显示出更好的效果。**结论：** 蒲公英根茎/根提取物、牛蒡根提取物、即墨根提取物、葛根提取物和莲藕提取物的混合发酵产物具有抗衰老和抗炎作用，可作为功能性化妆品原料应用。

关键词：混合植物根提取物，发酵，抗衰老，消炎，化妆品原料

TABLES

Table 1. INCI of plant root extracts used in this study.

INCI	Korean name	Chinese name	Abbreviations
Althaea rosea root extract	접시꽃뿌리추출물	蜀葵花提取物	AR
Anemarrhena asphodeloides root extract	지모뿌리추출물	知母根提取物	AA
Arctium lappa root extract	우엉뿌리추출물	牛蒡根提取物	AL
Lepidium meyenii root extract	마카뿌리추출물	迈因葶苈根提取物	LM
Nelumbo nucifera root extract	연꽃뿌리추출물	莲根提取物	NN
Oenothera biennis (Evening Primrose) root extract	달맞이꽃뿌리추출물	月见草根提取物	OB
Pachyrhizus erosus root extract	얌빈뿌리추출물	豆薯根提取物	PE
Paeonia suffruticosa root extract	모란뿌리추출물	牡丹根提取物	PS
Panax notoginseng root extract	삼칠뿌리추출물	三七根提取物	PN
Polygonum cuspidatum root extract	호장근뿌리추출물	虎杖根提取物	PC
Polygonum multiflorum root extract	하수오뿌리추출물	何首乌根提取物	PM
Pueraria lobata root extract	칡뿌리추출 물	野葛根提取物	PL
Sciadopitys verticillata root extract/	금송뿌리추출물	金松根提取物	SV
Sedum rosea root extract	돌꽃뿌리추출물	玫瑰红景天根提取物	SR
Taraxacum officinale rhizome/root extract	서양민들레뿌리줄기/뿌리추출물	欧蒲公英根茎/根提取物	TO

Table 2. Primer sequences

Primer	Forward sequence	Reverse sequences
GAPDH[*1]	ATT GTT GCC ATC AAT GAC CC	AGT AGA GGC AGG GAT GAT GT
GAPDH[*2]	CTG GCA CCC AGC ACA ATG AAG	ACC GAC TGC TGT CAC CTT CA
HAS2	GCT ACC AGT TTA TCC AAA CG	GTG ACT CAT CTG TCT CAC CG
COL1A1	GGC CCA GAA GAA CTG GTA CA	CGC TGT TCT TGC AGT GGT AG
IL6	ATG AAC TCC TTC TCC ACA AGC GC	GAA GAG CCC TCA GGC TGG ACT G
COX2	GAA GAG CCC TCA GGC TGG ACT GT	AGA TCA TCT CTG CCT GAG TAT CTT
TNF-a	CAT TCT GGG AGG GGT CTT CC	GGT TGA GGG TGT CTG AAG GA
AQP3	TGC AAT CTG GCA CTT CGC	GCC AGC ACA CAC ACG ATA A

*1 for HDF; *2 for HEKa; *GAPDH*, glyceraldehyde-3-phosphate dehydrogenase; *HAS 2*, hyaluronic acid synthase 2; *COL1A1*, collagen type 1 alpha 1 chain; *IL 6*, interleukin 6; *COX2*, cyclooxygenase 2; *TNF*-α, tumor-necrosis factor-α.

FIGURES

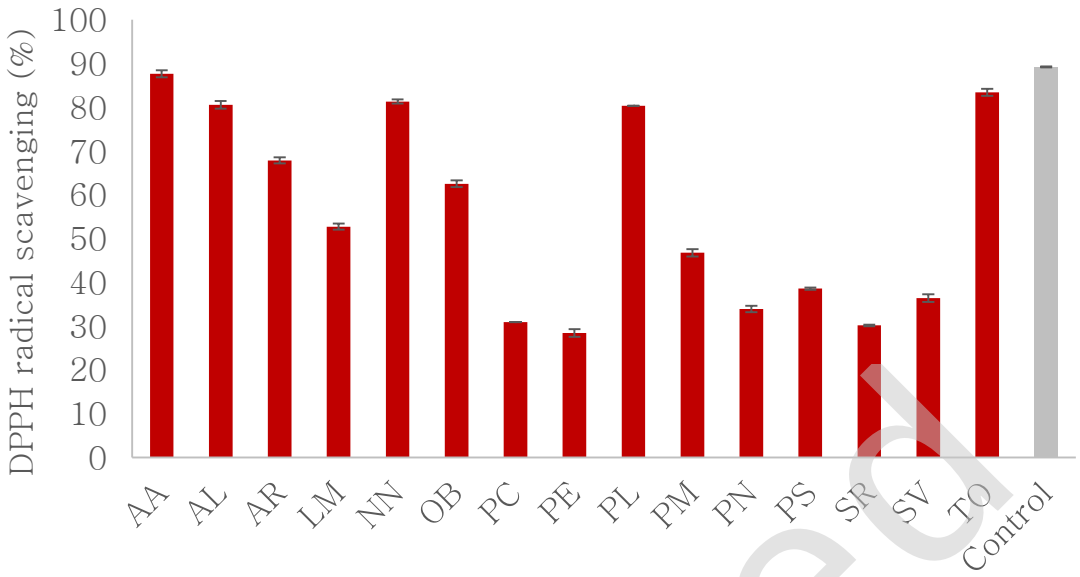


Figure 1 DPPH radical scavenging activity of plant root extracts.

DPPH free radical-scavenging activity of plant root extracts (10%). Quercetin (1000 μg/mL) was used as a positive control The results are expressed as the mean ± SD of three independent experiments. AA, *Anemarrhena asphodeloides* root extract; AL, *Arctium lappa* root extract; AR, *Althaea rosea* root extract; LM, *Lepidium meyenii* root extract; NN, *Nelumbo nucifera* root extract; OB, *Oenothera biennis* root extract; PC, *Polygonum cuspidatum* root extract; PE, *Pachyrrhizus erosus* root extract; PL, *Pueraria lobata* root extract; PM, *Polygonum multiflorum* root extract; PN, *Panax notoginseng* root extract; PS, *Paeonia suffruticosa* root extract; SR, *Sedum rosea* root extract; SV, *Sciadopitys verticillata* root extract; TO, *Taraxacum officinale* rhizome/root extract.

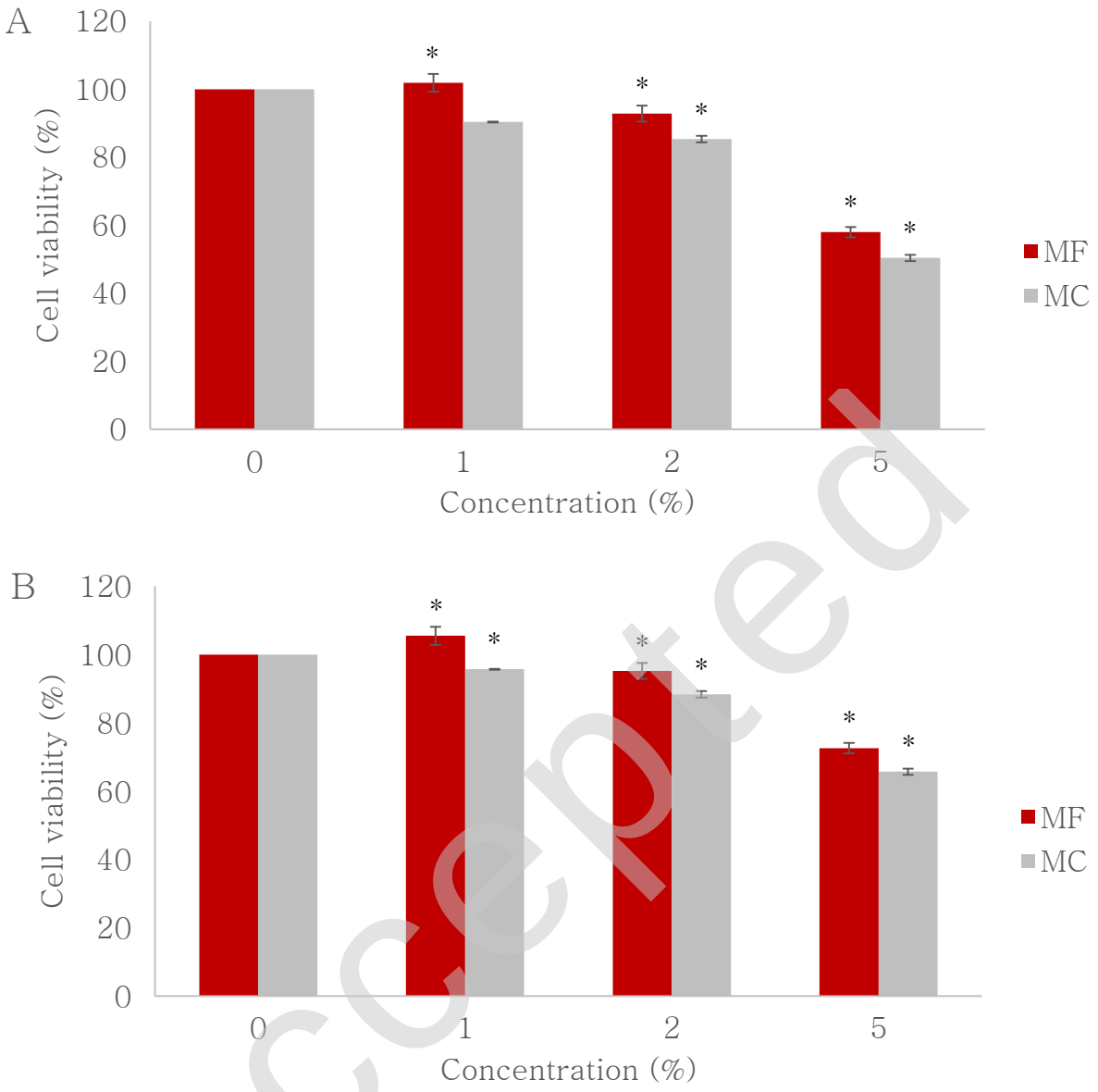


Figure 2 Cell viability of root extracts treated cells.

Cells are treated with each root extract 1, 2, and 5% concentrations for 24 h. Cytotoxicity was measured using the MTT assay. The results are expressed as the mean±SD of three independent experiments. A, HDF cells; B, HEKa cells. *Statistically significant ($p<0.05$).

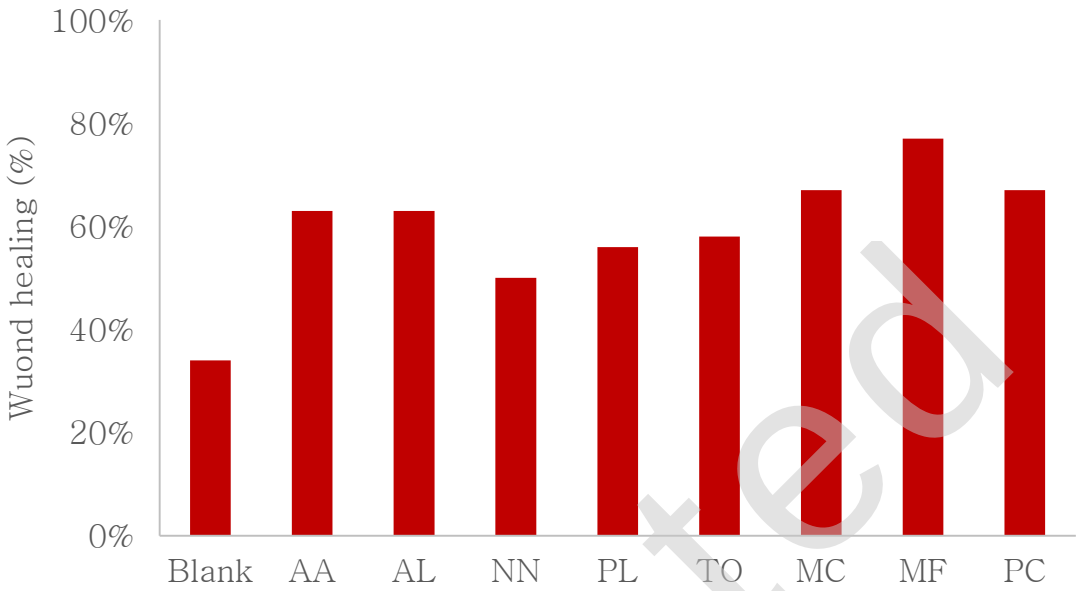


Figure 3. cell regeneration effect of plants roots extract, MC, and MF in HEKa cells.

"Wounds gap" in a cell monolayer is created by scratching and test samples. Cells are treated with 2% of each sample for 18 h. AA, *Anemarrhena asphodeloides* root extract; AL, *Arctium lappa* root extract; NN, *Nelumbo nucifera* root extract; PL, *Pueraria lobata* root extract; TO, *Taraxacum officinale* rhizome/root extract; MC, mixed plant root extract; MF, fermented mixed roots extract. 0.1% of madecassoside is used for a positive control (PC).

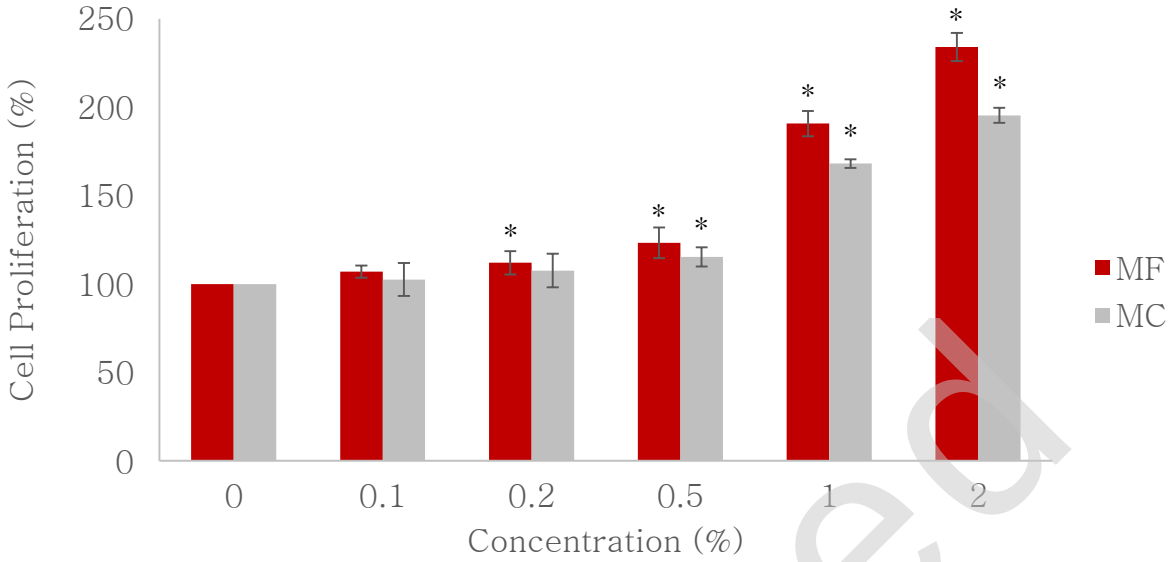


Figure 4 Effect of MC and MF on cell proliferation in HEKa cells.

Various concentration of MC and MF (0.1, 0.5, 1, and 2 %) were treated on HEKa cells and measured the cell proliferation activity using CytoSelect Brd U Cell proliferation ELISA Kit. Data are expressed as mean±SD of three independent experiments. *Statistically significant (p<0.05).

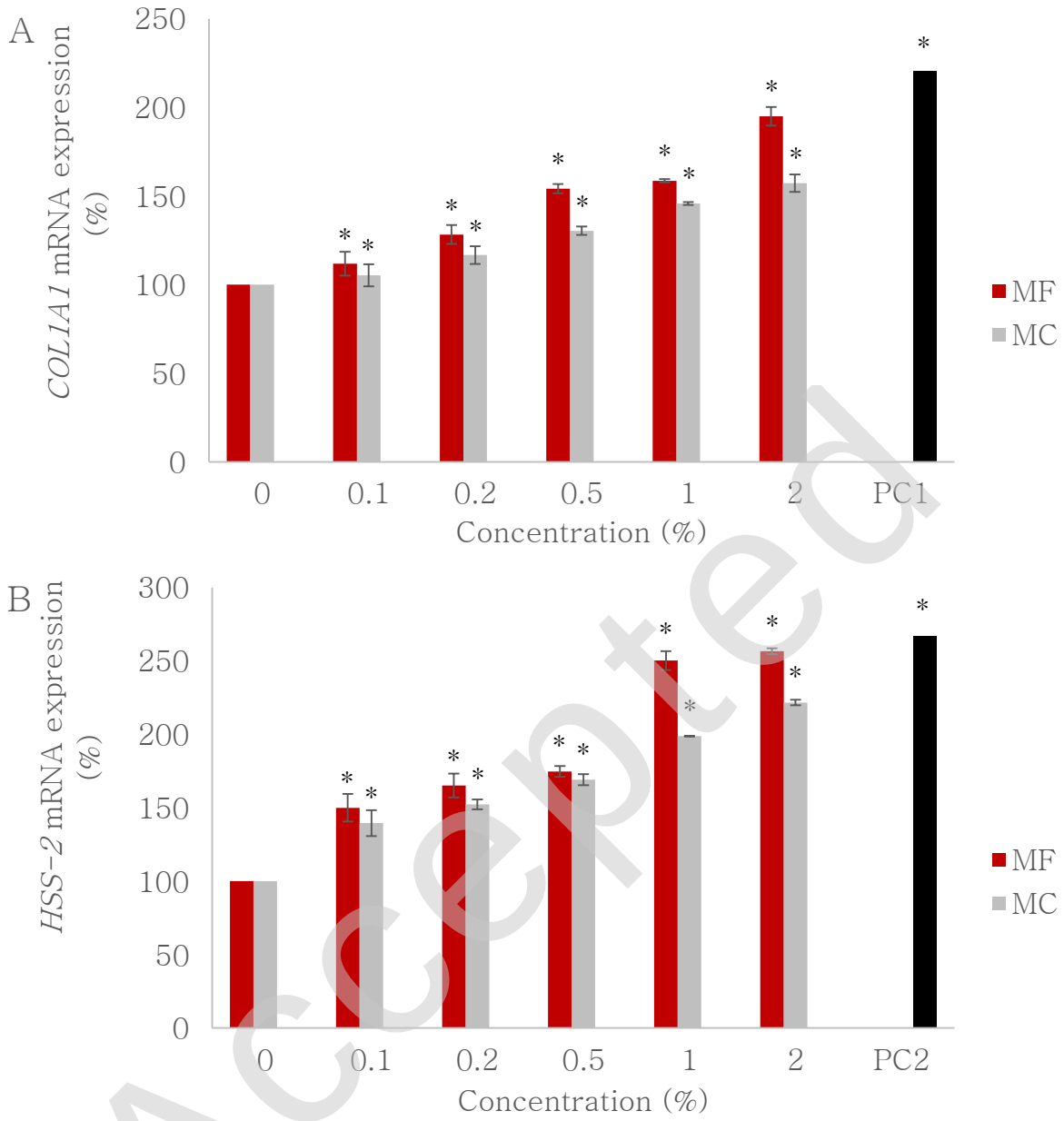
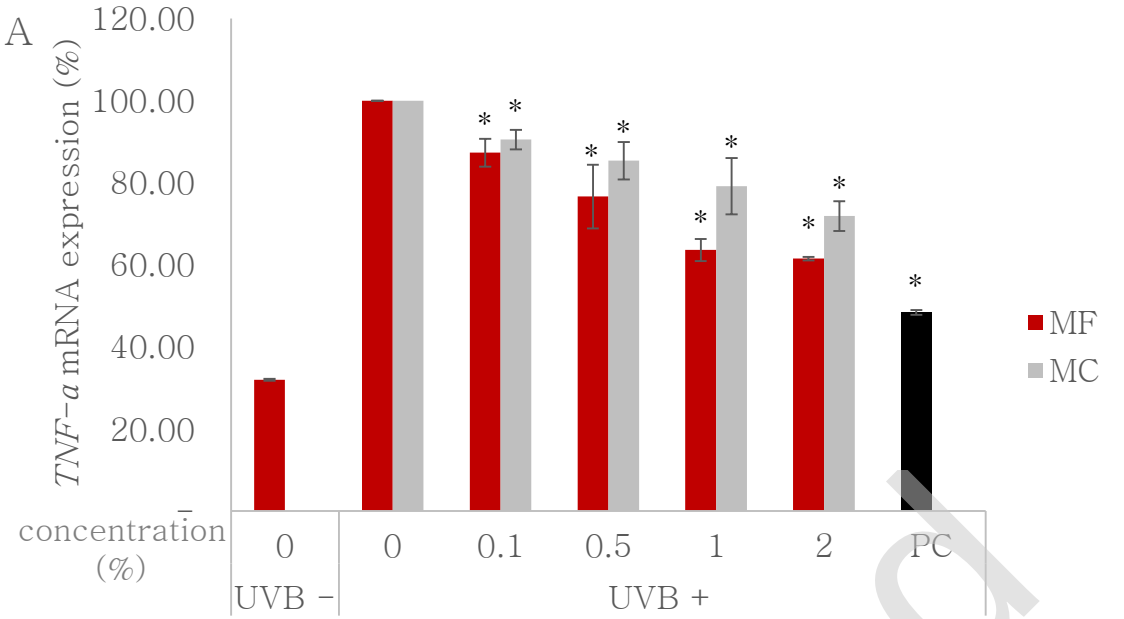
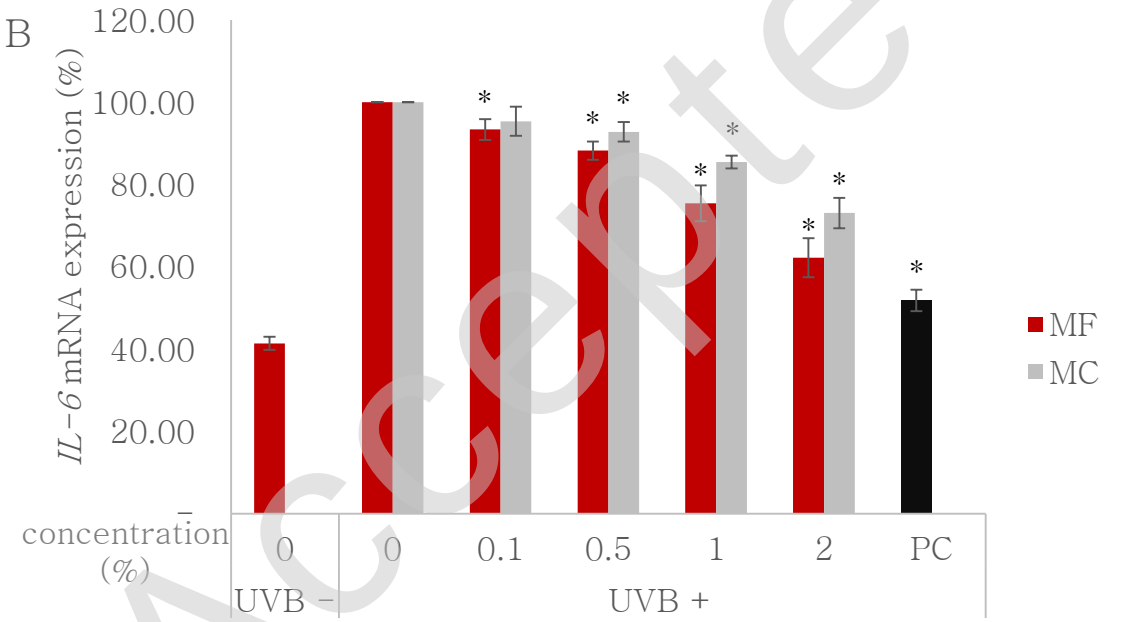


Figure 5 Effect of MC and MF on COL1A1 and HAS-2 mRNA expression in HDF cells.

The expression levels of *COL1A1* and *HAS-2* were measured by RT-PCR in HDF treated with indicated increasing concentrations of MC or MF. Each value represents the mean±SD of three individual experiments. Retinyl palmitate (50 μM) was used as a positive control (PC1) for *COL1A1*. Hyaluronic acid (50 μg/mL) was used as a positive control (PC2) for *HAS-2*. *Statistically significant (p<0.05).


A
120.00
100.00
80.00
60.00
40.00
20.00
TNF-α mRNA expression (%)
MF
MC
concentration (%)
0
0
0.1
0.5
1
2
PC
UVB −
UVB +


B
120.00
100.00
80.00
60.00
40.00
20.00
IL-6 mRNA expression (%)
MF
MC
concentration (%)
0
0
0.1
0.5
1
2
PC
UVB −
UVB +

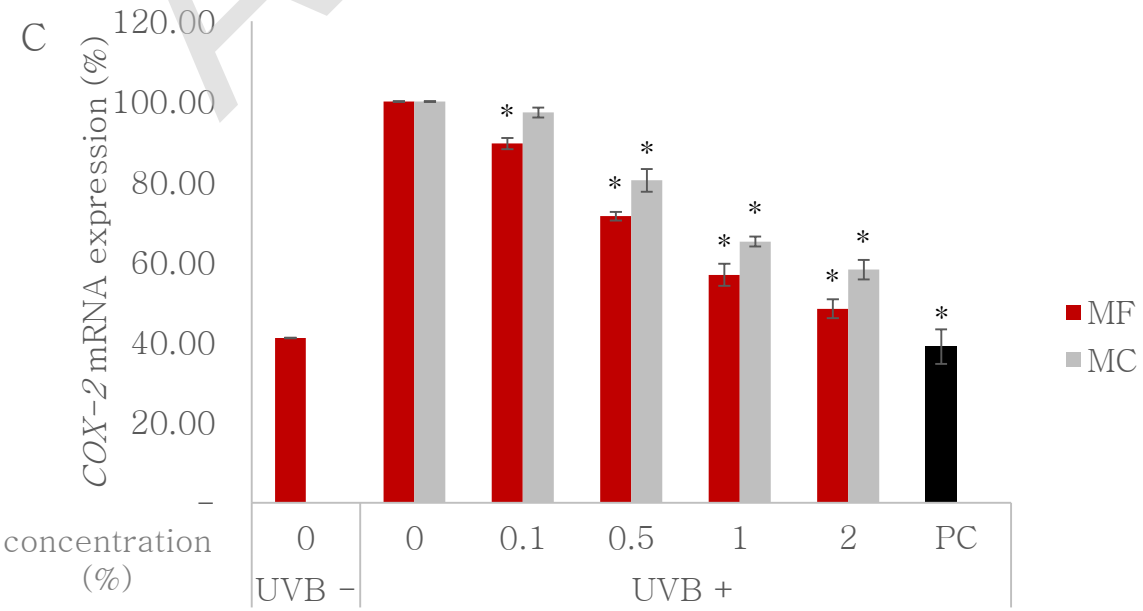

C
120.00
100.00
80.00
60.00
40.00
20.00
COX-2 mRNA expression (%)
MF
MC
concentration (%)
0
0
0.1
0.5
1
2
PC
UVB −
UVB +

Figure 6 Effect of MC and MF on inflammatory-related mRNA expression in HEKa cells.

The expression levels of *TNF-a* (A) *IL6* (B) *COX-2* (C) were measured by RT-PCR in HEKa treated with indicated increasing concentrations of MC or MF. Each value represents the mean±SD of three individual experiments. Allantoin (0.01 μg/mL) was used as a positive control (PC3).