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Attenuating properties of *Agastache rugosa* leaf extract against ultraviolet-B-induced photoaging via up-regulating glutathione and superoxide dismutase in a human keratinocyte cell line

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ABSTRACT

Agastache rugosa Kuntze, known as a Korean mint, is an herbal medicine that has been used for the treatment of diverse kinds of symptoms in traditional medicine. This work was undertaken to assess the protective properties of *A. rugosa* leaves against UV-B-induced photoaging in HaCaT keratinocytes. They were evaluated via analyzing reactive oxygen species (ROS), promatrix metalloproteinase-2 (proMMP-2) and -9 (proMMP-9), total glutathione (GSH), total superoxide dismutase (SOD), cellular viability, flavonoid content and in vitro radical scavenging activity. Total flavonoid content of ARE, a hot water extract of *A. rugosa* leaves, was 22.8 ± 7.6 mg of naringin equivalent/g ARE. ARE exhibited $ABTS^{+}$ radical scavenging activity with an SC_{50} of 836.9 μg/mL. ARE attenuated the UV-B-induced ROS generation. It diminished the UV-B-induced elevation of proMMP-2 and -9 at both activity and protein levels. On the contrary, ARE was able to enhance the UV-B-reduced total GSH and total SOD activity levels. ARE, at the used concentrations, was unable to interfere with the cellular viabilities of HaCaT keratinocytes under UV-B irradiation. Taken together, ARE possesses a protective potential against UV-B-induced photoaging in HaCaT keratinocytes, possibly based upon up-regulating antioxidant components, including total GSH and SOD. These findings reasonably suggest the use of *A. rugosa* leaves as a photoprotective resource in manufacturing functional cosmetics.

1. Introduction

The skin acts as a barrier that protects the human bodies against microbial pathogens, oxidant stress, chemical compounds, and mechanical resistance, and is repeatedly exposed to various environmental factors, including sunlight and air pollutants. It is also subject to aging, described as the changes in the appearance and characteristics of the skin, particularly pronounced on the face and hands. Skin aging is categorized into two basic types, intrinsic or chronological aging, which is determined by genetic factors, and extrinsic aging, frequently referred to as photoaging or premature aging, which is influenced by external environmental factors [1]. Intrinsic aging is more or less inevitable, whereas photoaging can be prevented in many ways. Photoaging is characterized by deep coarse wrinkles, sagging, laxity, thickness, roughness, mottled pigmentation and histologic alterations, which is led by the degradation of extracellular matrix (ECM) proteins, such as collagens, elastin, proteoglycans and fibronectin [1].

UV radiation in sunlight, via impairing the cellular redox status, disrupts the capability of the skin to protect itself from excessive generation of reactive oxygen species (ROS), which leads to consequent damage to the cutaneous tissues through a process commonly known as photoaging [2]. It is responsible for approximately 80% of the visible signs of skin photoaging [3]. UV radiation, especially UV-B radiation, causes acute and chronic photodamages, such as sunburn, photoaging and skin cancer, to the human skin, which subsequently leads to the degradation of the dermal ECM and chronic alteration in skin. UV-B radiation is the most damaging component of the solar UV radiation reaching the earth and acts chiefly on the epidermal basal layer of the skin, and one of the most crucial external stimuli that affects the skin by inducing immunosuppression, cancer, photoaging, inflammation, and cell death [4].

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Matrix metalloproteinases (MMPs), a family of structurally related zinc-containing endopeptidases with an extensive range of substrate specificities, degrade various components of ECM proteins and are categorized into five main subgroups, such as collagenases (MMP-1, -8, -13 and -18), gelatinases (MMP-2 and -9), stromelysins (MMP-3, -10 and -11), matrilysins (MMP-7 and -26) and transmembrane MMPs (MMP-14, -15, -16, -17, -24 and -25) [5]. They are known as major participants in both extrinsic aging and photoaging of skin. Both MMP-2 and -9 digest a number of ECM components such as collagen type I and IV, and additionally degrade other substrates such as collagen type V, VII and X, fibronectin, and elastin, and are essential in degrading fibrillar fragments after their initial degradation by collagenases [5]. Exposure of skin cells to UV-B irradiation up-regulates the synthesis of diverse MMPs, such as MMP-1, -2, -3, -7, -8, -9 and -12, which are implicated in photoaging [6]. MMP-2 and -9, overexpressed by UV-B irradiation in skin cells, contributes to the acceleration of photoaging and the development of skin cancer [7]. Hydrogen peroxide also causes the increase in the activities of MMP-2 and -9 in human skin fibroblasts and an increase in MMP-2 activity can partly contribute to the decrease in the amount of collagen secreted into the medium [8]. MMP-2 and -9 also participate in angiogenesis by disrupting the vessel basement membrane and other ECM barriers and enabling endothelial cell migration through the surrounding tissues ([9]. Since diverse MMPs, including MMP-2 and -9, are induced by UV radiation-generated ROS, the blockade of ROS generation with antioxidants, for example, certain types of phytochemicals, is considered as a valuable strategy to protect against UV-B-induced skin photoaging.

Agastache rugosa Kuntze, a perennial herb, belongs to the mint family (Lamiaceae), which is widely distributed throughout East Asian Countries, including Korea, China and Japan. It is being used to treat anorexia, cholera, vomiting, miasma and other disorders in traditional folk medicine. *A. rugosa* has been shown to contain diverse essential oils, including methyleugenol, estragole and eugenol, and several kinds of flavonoids, including acacetin-7-*O*-β-D-glucopyranoside (tilianin), acacetin, linarin, agastachoside and rosmarinic acid [10]. Two diterpenoid compounds, agastanol and agastaquinone, and two lignin compounds, agastinol and agastenol, are also contained in *A. rugosa* [11,12]. *A. rugosa* extracts and their several constituents possess a range of biological and pharmacological activities, including antimicrobial, *anti*-fungal, insecticidal, antiviral, antihypertensive, anti-inflammatory, anticancer, antioxidant, antiatherogenic and vasorelaxant activities [13–17]. However, the beneficiary properties of *A. rugosa* on the skin have yet to be evaluated.

In the present work, the dermatological benefits of *A. rugosa* leaves, using their hot water extract (ARE), could be in part understood in human epidermal keratinocytes. ARE exhibited suppressive effects on the UV-B-induced ROS and proMMP-2 and -9 production, while it could up-regulate the UV-B-reduced total GSH content and SOD activity in HaCaT keratinocytes. These findings reasonably suggest the attenuating potential of ARE against the UV-B-induced photoaging of the human skin.

2. Materials and Methods

2.1. Chemicals

Bradford reagent, diethylene glycol, naringin, 2,2-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), ammonium persulfate, ascorbic acid (AA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), dihydrorhodamine 123 (DHR-123), dihydroethidium (DHE), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), glutathione reductase (GR), catalase, xanthine, xanthine oxidase and NADPH were from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Cell lysis buffer [25 mM Tris-phosphate (pH 7.8), 2 mM 1,2-diaminocyclohexane-*N*,*N*,*Nv*,*Nv*-tetraacetic acid, 2 mM dithiothreitol, 10% glycerol, 1% Triton X-100] was obtained from Promega Korea (Seoul, Korea).

2.2. Plant Material

Dried *A. rugosa* leaves were obtained at a local market, Chuncheon, Korea, in September 2015, and authenticated by Prof. Ki-Oug Yoo, Department of Biological Sciences, Kangwon National University, Chuncheon, Korea. The voucher specimen of the plant material was deposited in the herbarium of Department of Biological Sciences, College of Natural Sciences, Kangwon National University under the acquisition number KWNU90446.

2.3. Preparation of Hot Water Extract (ARE)

Dried *A. rugosa* leaves were ground under liquid nitrogen, mixed with 10-fold distilled water in 2-liter conical flask, and extracted under reflux by placing in a water bath at 90 °C for 4 h. After being chilled and filtered through a filter paper, the hot water extract was evaporated to dryness in a freeze dryer, and the extract powder was named ARE. The yield was determined to be 10.4%. For the experiments, ARE was dissolved in dimethyl sulfoxide, and control cells were treated with vehicle only (0.1% dimethyl sulfoxide). The vehicle used had no effect on cell viability.

2.4. Determination of Total Flavonoid Content

Total flavonoid content in ARE was determined according to a previously described procedure [18] with a slight modification. Half a mL of diethylene glycol and 0.05 mL ARE at varying concentrations were mixed with 0.05 mL of 1 N NaOH and incubated for 1 h at 37 °C. Change in absorbance at 420 nm was measured using a microplate reader. Naringin was used as a reference marker for total flavonoids at a concentration range of 0 to 1 mg/mL. Total flavonoid content was expressed as mg of naringin equivalent/g ARE (mg/g NE).

2.5. Quantitation of Total Antioxidant Activity

Total antioxidant activity of ARE was determined using $ABTS^+$ radical scavenging assay [19] with a slight modification. ABTS radical cations ($ABTS^+$) were generated by reacting ABTS stock solution (0.07 mM) with 0.12 mM ammonium persulfate. The mixture was allowed to stand in the dark at room temperature for 16 h before use. The varying concentrations of ARE (each 0.01 mL) were mixed with 0.29 mL of $ABTS^+$ solution and the final volume was made up to 1 mL with ethanol. The reaction mixture was incubated for 15 min in the dark at room temperature. AA was used as a positive control. The absorbance was measured at 745 nm and the percent inhibition by ARE was calculated using the formula, Inhibition (%) = [(Control − Test)/Control] × 100. Results were expressed as the ARE or AA concentration eliciting 50% scavenging of $ABTS^+$ radicals (SC_{50}).

2.6. Cell Culture

A human immortalized HaCaT keratinocyte cell line (ATCC, Manassas, VA, USA) was grown in DMEM containing 10% heat-inactivated FBS, 100 units/mL penicillin and 100 μg/mL streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

2.7. UV-B Irradiation

As an UV-B source, an ultraviolet lamp (peak, 312 nm; model VL-6 M, Vilber Lourmat, Marine, France) was used with a radiometer (model VLX-3 W, Vilber Lourmat, Marine, France) equipped with a sensor (bandwidth, 280 to 320 nm; model CX-312, Vilber Lourmat, Marine, France).