Effect of *Asparagus Racemosus* Extract on Production of Matrix metalloproteinase-1 (MMP-1) and Type-I Procollagen in Ultraviolet-A (UVA)-Irradiated Fibroblasts


Abstract—The aim of this study was to determine MMP-1 activity and type I procollagen level in UVA-irradiated primary human dermal fibroblasts treated with *A. racemosus* extract. The root of *A. racemosus* grown in Thailand was extracted by acetonitrile. ELISA analysis revealed that *A. racemosus* contained total saponins (related to shatavarin IV) was 40.19 ± 5.79 w/w. The primary human dermal fibroblasts were used for determination of the cytotoxicity, MMP-1 activity and level of type I procollagen of the extract. The *A. racemosus* extract at concentration more than 0.5 mg/mL showed cytotoxicity to primary human dermal fibroblasts but, at a lower concentration, *A. racemosus* didn’t show cytotoxicity. *A. racemosus* extract at 0.1 and 0.2 mg/mL was found to increase type I procollagen level and decrease MMP-1 production in UVA-irradiated primary human dermal fibroblasts. The results of this study suggest that *A. racemosus* extract has potential as anti-aging cosmetic ingredients.

Keywords— *Asparagus racemosus*, type 1 procollagen, matrix metalloproteinase-1, Ultraviolet A-irradiated fibroblasts

I. INTRODUCTION

 Nowadays, skin aging problem is interesting in cosmetic field. It is commonly associated with increased wrinkles and deep lines. Skin aging is a complex biological phenomenon consisting of two components; the passage of time (intrinsic aging) and from cumulative exposure to external factors (extrinsic aging) such as ultraviolet exposure which promotes aging formation and loss of skin elasticity [1]. Intrinsic skin aging is largely genetically investigated. An important event in the process of intrinsic skin aging is the increased matrix metalloproteinase-1 (MMP-1) activity (interstitial collagenase), decreased type 1 procollagen [2] and estrogen [3].

MMPs are a family of calcium-dependent zinc-containing proteinase enzymes, which are responsible for the tissue degradation of the extracellular matrix (ECM), including collagens, elastins, and glycosaminoglycans [4]. In particular, MMP-1 (interstitial collagenase) is thought to be important in extracellular matrix degradation during the aging process of human skin [5]. It can be up-regulated by UV irradiation both in vivo and in vitro cultured cells [6]. Moreover, reduction of type 1 procollagen closely related to skin aging [7]. Therefore, the agents with ability to increase the type I procollagen and decrease the MMP-1 can prove to use for the development of anti-aging ingredients.

A number of pharmaceutical and cosmetic ingredients have been used to improve skin aging disorder such as tretinoin (retinoic acid) [8] and alpha hydroxyl acid (AHA) [9]. However, they irritate skin and also lead to photosensitivity of the skin [8, 9]. For this reason, we are particularly interested in finding anti-aging agent from natural sources. An effectiveness of MMP-1 inhibitors extracted from natural sources has been reported for their potential applications in improving skin aging disorders. For example, extracts from *Panax ginseng* C.A. Meyer (Ginseng) [10] and *Vitis vinifera* L. (Grape seed extract) [11] have been used as skin anti-aging agent.

One of the most recognized estrogenic activity obtained from Thai herbs is *Asparagus racemosus* (Satavar, Shatavari, or Shatamull). It is an important medicinal plant of tropical India and Thailand. The major active constituents of *A. racemosus* are steroidal saponins (Shatavarins) presented in the roots. A number of studies in the recent years have proved phytoestrogen, antioxidant and anti-microbial activity of these compounds [12]. Moreover, the chemical structure of...
shatavarins resembles with ginsenoside in ginseng which increase type I procollagen level and decrease MMP-1 activity in UVA-irradiated primary human dermal fibroblasts [10]. Therefore, in the present study, we investigated the effect of *A. racemosus* extract on MMP-1 activity and type I procollagen level in UVA-irradiated primary human dermal fibroblasts.

II. MATERIALS AND METHODS

A. Preparation of *A. racemosus* extract

The root part of *A. racemosus* was collected from Rayong Province, Thailand. It was chipped, exposed to the sun and dried at 50°C by a hot-air oven. Then, the dried root was ground into powder. After that, 100 grams of the *A. racemosus* powder were extracted with 500 mL of acetonitrile. The solution of *A. racemosus* acetonitrile extract was filtered through a piece of cloth in order to remove unwanted residue and evaporated by Buchi Rotavapor at 45°C. Finally, the extract was dried in a desiccator and stored in airtight dark bottles at 4°C.

B. Quantification of total saponins related to shatavarin IV in the extract

To control the extract quality of each batch, the content of the total saponins, a major component of the *A. racemosus* root extract, was determined by an Enzyme-linked immunosorbent assay (ELISA). The 96 wells plate (Maxisorb Nunc, Roskilde, Denmark) was coated with 100 µL of 1 µg mL⁻¹ shatavarin IV–HSA conjugate in 50 mM carbonate buffer (pH 9.6) and incubated at 37°C for 1 hour. The plate was washed three times with 0.05% tween 20 phosphate buffer saline (TPBS). After that, the plate was treated with 300 µL of 0.2% gelatin in phosphate buffer saline (GPBS) for 1 hour to reduce non-specific binding. 50 µL of various concentrations of shatavarin IV or the extract in 20% MeOH were incubated with 50 µL of the polyclonal antibodies (PAbs) solution for 1 hour. The plate was washed three times with TPBS and then the antibody was mixed with 100 µL of a solution of a 1:1000 diluted solution of peroxidase conjugated goat IgG fraction to mouse IgG Fc and kept for 1 hour. After washing the plate three times with TPBS, 100 µL of substrate solution consisted of 100 mM citrate buffer (pH 4.0) 0.3 mg mL⁻¹ of ABTS was added and incubated for 15 minutes. The plate was determined by using a microplate reader (Model CeresUV900, Bio-Tek Instrument, Winooski, Vermont, USA) at 405 nm [13].

C. Primary human dermal fibroblasts preparation

The primary human dermal fibroblasts were obtained from Thai healthy female with 51 years old (Source of abdominal skin). Dermis tissue was isolated form full skin. The dermis was cut into three-millimeter by a biopsy punch. Two-three pieces of tissue were placed in tissue culture flask (25 cm²) and incubated in CO₂ incubator (37°C with 5% CO₂) for 1 hour. After incubation, the culture medium (DMEM + 10% FBS + 1% penicillin/streptomycin solution) in an amount of 3 mL was added and incubated in CO₂ incubator. After incubation for 3 weeks in the CO₂ incubator, the fibroblast cells were removed by trypsin-EDTA solution and seeded at 1x10⁵ cells/cm² in 25-flasks using the same medium [14]. The medium was changed every 2 days. The passage numbers 4th-8th was used in this study.

D. Cell viability assay

Cell proliferation kit II (XTT) was used in this study. This assay is based on the cleavage of the yellow tetrazolium salt XTT to form an orange formazan dye by metabolic active cells. Therefore, this conversion only occurs in viable cells. Firstly, the cell suspension was transferred from the 25-cm² flask into a 96-well plate (1x10⁴ cells/well) and kept in CO₂ incubator (37°C with 5% CO₂) for overnight for complete adhering of the cells on the culture plate. After incubation, the cell-free supernatant was removed and replaced with 200 µL serum-free DMEM. The *A. racemosus* extract at various concentrations was added into the primary human fibroblast cells. After incubation for 24, 48, 72 h, the volume of 50 µL of the activated XTT solution was added to each well and incubated for 4 h. The optical density (OD) was measured with microplate reader (Model CeresUV900, Bio-Tek Instrument, Winooski, Vermont, USA) at 490 nm. Cell viability was compared with control (0.1%DMSO+DMEM, non-treated cell) that the absorbance of control was adjusted to 100%. The measurements were performed in triplicate.

E. Effect of *A. racemosus* extract on production of matrix metalloproteinase-1 (MMP-1) and type I procollagen in UVA-irradiated fibroblasts

To study the effect of *A. racemosus* extract on MMP-1 production and type I procollagen level before UVA irradiation on fibroblasts. This method was described individually below.

Firstly, the cell suspension was transferred from the 25-cm² flask into a 96-well plate (1x10⁴ cells/well) for MMP-1 assay and into 24-well plate (1x10⁵ cells/well) for type I procollagen assay and kept in CO₂ incubator (37°C with 5% CO₂) for overnight for complete adhering of the cells on the culture plate. After incubation, the cell-free supernatant was removed and replaced with serum-free DMEM. The *A. racemosus* extract at various concentrations, all-trans retinol (50µg/mL) and vitamin C (Ascorbic acid 100 µg/mL) were added into the primary human fibroblast cells. After incubation for 24 hours, the culture medium was replaced with phosphate buffer saline (PBS) and the culture was irradiated with 15 J/cm² UVA light. After irradiation, PBS was changed with fresh 200 µL (for MMP-1 ELISA kit assay) and 500 µL (type I procollagen assay) serum-free DMEM. The MMP-1 and type I procollagen was determined for each culture after incubation for 6 and 24 hours.
F. Determination of the amount of MMP-1

MMP-1 known as interstitial collagenase is an enzyme that correlates with skin aging [4]. After incubation, the cell-free supernatant was collected. The amount of MMP-1 was determined by a commercial human MMP-1 ELISA kit (RayBiotech, Inc., Georgia, USA) at the wavelength of 450 nm using microplate reader (Model CeresUV900, Bio-Tek Instrument, Winooski, Vermont, USA. The determinations were performed in triplicate.

G. Determination of the amount of type I procollagen

In this study, type I procollagen assay was used polyclonal antibodies against type I procollagen carboxy-terminal peptide. Thus, the amount of the free propeptiyes reflects with the amount of collagen molecules synthesized [15]. After incubation, the cell-free supernatant was collected. The amount of type I procollagen was determined by a commercial human procollagen type-I C-peptide EIA kit (Takara Bio Inc., Shiga, Japan) at the wavelength of 450 nm using microplate reader (Model CeresUV900, Bio-Tek Instrument, Winooski, Vermont, USA). The determinations were performed in triplicate.

III. RESULTS AND DISCUSSION

A. The characteristics of A. racemosus root extract

The appearance and percentages of yield of the acetonitrile extract were shown in Table 1 and Fig. 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>A. racemosus root extract</th>
</tr>
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<tbody>
<tr>
<td>% yield</td>
<td>4.12</td>
</tr>
<tr>
<td>Appearance</td>
<td>Brown paste</td>
</tr>
</tbody>
</table>

Fig. 1 The appearance of A. racemosus acetonitrile extract

B. The content of total saponins related to shatavarin IV in A. racemosus extract

To increase sensitivity of the analysis of the total saponins in A. racemosus extract. Therefore, ELISA was used as a analysis method. In this study, the amount of total saponins related to shatavarin IV in the acetonitrile extract was 40.19 ± 5.79 w/w.

C. Cell viability assay

Cell viability was determined to investigate the effect of A. racemosus extract on fibroblasts. The results were shown in Fig. 2, at 0.5 and 1 mg/mL of A. racemosus extract for 1, 2, and 3 days, the percent cell viability of fibroblasts was lower than the control (DMEM, non-treated cell). Thus, at 0.5 and 1 mg/mL of A. racemosus extract have cytotoxicity on fibroblasts. But, at 0.02, 0.05, 0.1 and 0.2 mg/mL of A. racemosus extract, the percent cell viability of fibroblasts was not different from cells of the control.

D. Effect of A. racemosus extract on production of MMP-1 in UVA-irradiated fibroblasts

MMP-1 known as interstitial collagenase is an enzyme that correlates with skin aging [4]. Previous studies have been reported that UVA induces the level of MMP-1 expression in human skin, which causes inhibition of de novo synthesis of collagen that results in much connective tissue damage [6]. Fig. 3 shows that, at 6 and 24 hr of incubation, the concentration of 0.1 and 0.2 mg/mL of the extract was found to significantly decrease MMP-1 production in primary human dermal fibroblasts as well as all-trans retinol and vitamin C at 50 and 100 µg/ml, respectively. Therefore, the extract at these concentrations might be improving collagen metabolism in dermal fibroblasts.

E. Effect of A. racemosus extract on production of type I procollagen in UVA-irradiated fibroblasts

UVA irradiation is mainly responsible for aging formation in human skin through decrease the amount of collagen in dermis layer [6]. Type I collagen is a predominant constituent of dermis extracellular matrix. Thus, reduction of type I collagen is regarded as sign of skin aging [4]. Previous studies have been reported that the amount of pro-collagen type I protein is decreased after UVA irradiation [6]. Fig. 4 shows that, at 6 and 24 hr of incubation, the concentration of 0.1 and 0.2 mg/mL of the extract was found to significantly increase type I pro-collagen production in primary human dermal fibroblasts when compared with UVA untreated control. Moreover, the effect of UVA irradiation on morphology of
primary human dermal fibroblasts is shown in Fig. 5. The results under the microscopic observation showed that UVA irradiation at intensity of 15 J/cm² changed morphology of the primary human dermal fibroblasts whereas 0.1 and 0.2 mg/mL of the *A. racemosus* extract, all-trans retinol (50 µg/mL) and vitamin C (Ascorbic acid 100 µg/mL) didn’t change morphology of the primary human dermal fibroblasts. It’s possible that the extract could protect cell structure from UVA irradiation.

**Fig. 3** Inhibitory effect of *A. racemosus* extract in acetonitrile on MMP-1 production by UVA-irradiated fibroblasts (Mean (ng/mL) ± SD.)

**Fig. 4** Effect of *A. racemosus* extract in acetonitrile on type I pro-collagen synthesis in UVA-irradiated fibroblasts (Mean (ng/mL) ± SD.)

![Image](image1)

**Fig. 5** Morphology of primary human dermal fibroblasts. a) No UVA irradiation, b) UVA irradiation at intensity of 15 J/cm², c) UVA irradiation at intensity of 15 J/cm² and treated with 50 µg/mL all-trans retinol, d) UVA irradiation at intensity of 15 J/cm² and treated with 100 µg/mL ascorbic acid, E) UVA irradiation at intensity of 15 J/cm² and treated with 0.1 mg/mL of *A. racemosus* extract and F) UVA irradiation at intensity of 15 J/cm² and treated with 0.2 mg/mL of *A. racemosus* extract for 24 hours at magnification of 10X

### IV. CONCLUSION

Chronic UVA exposure could also lead to skin aging. Therefore, determination of the mechanisms of anti-aging could be used to develop the active agents to retard the aging process. UVA-irradiation increased MMP-1 which causes collagen degradation, leading to the formation of skin aging. In this study, *A. racemosus* extract could increase type I procollagen level, decrease MMP-1 production and didn’t show cytotoxicity in primary human dermal fibroblasts.
dermal fibroblasts. Therefore, *A. racemosus* extract has performance in preventing skin aging induced by UVA and can be used as anti-aging for cosmetic application.

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REFERENCES


