New anti-wrinkle cosmetics

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Abstracts

In the aged skin especially in the face and eyelid, deep and slight wrinkles are one of the remarkable phenomena of aging and the cause of wrinkle is various. Among the cause of wrinkles an oxidative stress plays an important roles in wrinkle formation process. It caused the lipid peroxidation of cell membrane, the increase of the MMPs(MatrixMetalloProteinase) gene expression and cellular DNA damage. These ROS induced materials may cause the degradation of collagen matrix system in the dermis and cause the formation of skin wrinkle. So, it is very important for protecting skin wrinkle formation to regulate ROS activity.

In this study, we developed one active ingredient having multi functional activities such as activation of collagen synthesis, inhibition of MMPs activity, lipid peroxidation and free radical scavenging activity and inhibition of free radical induced DNA damage *in vitro*.

Pericarpium castaneae extracts showed collagen synthesis increase in Normal Human Fibroblast and the inhibition of elastase activity (IC_{50} of Elastase: 43.9 ㎍/㎖). It showed also anti-oxidative activity (IC_{50} : 48 ㎍/㎖) and free radical scavenging activity(IC_{50}: 7.6 ㎍/㎖).

Conclusively, Pericarpium castaneae extracts may be used as an ingredient for new anti –wrinkle cosmetics.

Introduction

The biological activity of various plant extracts had been screened for cosmetic use. Furthermore, plant sources have been evaluated for developing natural anti-aging agents. Many endogeneous plant

compounds possess anti-oxidative activity and free radical scavenging activity. Recent studies indicate that the compounds are able to inhibit mutagenesis and carcinogenesis in addition with aging. To deveolp an active agent for skin anti-aging, we screened biological activity of plant extracts. From the results, we selected pericarpium castaneae as a new anti-aging. pericarpium castaneae has been used as a popular remedy for anti-wrinkle from the ancient time in Korea and in an ancient literature such as Dong-Eu-Bo-Gam. Many anti-wrinkle cosmetics induce the production of extracellular matrix such as collagen. Retinoic acid (RA) or Retinol(vitamin A) like materials can increase the production of collagen and make wrinkles less visible. Also, Matrix degradation is the most important factor of wrinkle formation. ROS caused the expression of MMP(MatrixMetalloProteinase) and these MMPs degraded collagen matrix system in the dermis. So, it is ideal for protecting wrinkle formation to increase collagen synthesis and regulate the MMPs expression induced by ROS and collagen synthesis

In this study, we developed one active ingredient possessing potential activities such as activation of collagen synthesis, inhibition of MMPs activity and free radical scavenging activity *in vitro.* Pericarpium castaneae extracts showed collagen synthesis increase in Normal Human Fibroblast and the inhibition of collagenase and elastase activity *in vitro.*

Materials and Methods

1. Preparation of pericarpium castaneae extracts

We used only was chestnut (*Castaneae crenata*) that had been grown in Korea. We extracted dried pericarpium castaneae with 70% ethanolic aqueous solution and evaporated to dryness using a vacuum evaporator.

2. Anti-oxidative activity and free radical scavenging activity

For measuring anti-oxidative activity, we used lipid peroxidation system induced by Fenton reagent. Briefly, each sample (100μl) and ethyl linolate (10μl) was added to incubation medium (4.89ml) containing 2% SDS, μM ferrous chloride and 0.5mM hydrogen peroxide. After incubation at 55℃ for 16 hrs, the sample's anti-oxidative activity was measured using thiobarbituric acid(TBA) assay according to the method of Ohkawa et al(1). For measuring scavenging activity, each sample(2ml) was added to 2ml of 60μM 1,1-diphenyl-2-picryl hydrazyl(DPPH) ethanolic solution and kept at room temperature for 30min according to the procedure of Fugita et al(2)

3. Collagen synthesis assay

Human normal fibroblast was inoculated to 48-well microplate ($5x10^4$ cell/well) and cultivated for 24 hours. After the culture, culture medium was changed to serum-free DMEM medium containing sample extracts in the following and cultivated for 24 hours. Control group was cultivated without sample extracts. After the culture, supernatants of each well was collected and amount of procollagen type IC-peptide (PICP) was measured by using PICP assay kit (Takara, Kyoto, Japan). TGF-β was used as positive control(10ng/ml).

4. Assay for elastase activity in vitro

Porcine pancreatic elastase(PPE: Sigma Type IV) was assayed spectrphotometically by the method of James et al(3), using N-Succ-(Ala)3-nitroanilide(S.A.N.A) as the substrate and monitoring the release of p-nitroaniline for 20min at 25℃. The amount of p-nitroaniline was determined by measuring the absorbency at 410nm. The reaction mixture contained 0.2M Tris-HCl buffer(pH 8.0), 1μg/ml elastase, 0.8mM Succinyl-Ala-Ala-Pro-p-nitroanilide(ESIV; elastase substrate IV, Calbiochem) as substrate and CC-516 dissolved. The reaction was started by the substrate. Blanks contained all the components except the enzyme.

5. Single cell gel electrophoresis

Cells were embedded in agarose on frosted microscopic slides by the method of Sing et al(4). First, 0.65% NMPA in PBS(100μl) at 65 ℃ was dropped onto slides and they were covered with glass coverslip(18×18mm, No1). After leaving on ice for 10min, the cover slip was removed. Cells were mixed with 200μl of 0.5% LPMA and 50ul of cell suspension was immediately pippeted onto the layer of agarose on the same slide. After covering with a coverslip, the slide was left on ice for 10min. A final layer of agarose(100ul of 0.5% LMPA) was applied in the same way. The slide without coverslip ws immersed in ice-cold lysis solution(10mM Tris, pH 10.0, containing 2.5M NaCl, 100mM EDTA, 10% DMSO and 1% Triton X-100) at 4 ℃ for 1hr. Electrophoresis was carried out in a tank containing 300mM NaOH, 1mM EDTA, pH 13.0 for 15min under 25V and 300mA. Slides were then transferred to 0.4M Tris buffer(pH 7.5), washed three times and gently dried. Ethidium bromide(2μg/ml) was dropped onto the gel to stain DNA. Slides were examined at X400 magnification using a BH2 fluorescence microscope(Olympus, Japan) equipped with a 20BG-W2 dichroic mirror(excitation filter: 515nm, barrier filter: 590nm). Image analysis was performed with the software Kormet(Version 3.0, Kinetic Imaging, Liverpool, UK) on 25 randomly selected cells. DNA damage was quantified by the increase of the tail moment, which was defined as a product of comet length and amount of DNA in the tail by Olive et al(5).

Results and Discussion

In order to elucidate the biological activities of the ethanol extracts of pericarpium castaneas *in vitro*, we did the various tests such as anti-oxidative activity and free radical scavenging activity. As shown in Table 1, pericarpium castaneae extracts showed mild anti-oxidative activity (IC_{50} = 48 ㎍/㎖), while BHT was the most potent anti-oxidative activity (IC_{50} = 0.2 ㎍/㎖). The free radical scavenging

activity of pericarpium castaneae extracts is shown in Table 2. Pericarpium castaneae extracts possessed potent free radical scavenging activity *in vitro* (IC_{50} = 7.6 ㎍/㎖) compared to Vit-E(IC_{50} = 8.5 ㎍/㎖). There have been numerous report (6-12) that certain plant extracts possessed anti-oxidative and free radical scavenging activities. Most of these active plant extracts contained phenolic or polyphenolic compound such as flavonoids and tannins that would contributes to their anti-oxidative and radical scavenging activities.

Also, in collagen synthesis through fibroblast culture, pericarpium castaneae extracts increased collagen synthesis about 110.9ng/ml at 50μg/ml(control 55.9ng/ml), but over the concentration 100μg/ml it did not show more increasing activity than 50μg/ml(Data not shown).

Pericarpium castaneae extracts showed inhibitory activity on Elastase activity (IC_{50}= 43.9ug/ml) and collagenase activity(Data not shown). It means that Pericarpium castaneae extracts can be used as anti-MMP agents for wrinkle treatment products(Table 4).

Table 5 showed that Pericarpium castaneae extracts demonstrated the inhibitory activity against free radical induced DNA damage measured by tail moment in single cell gel electrophoresis. It showed a significant reduction in the tail moment. DL-α-Tocopherol used as a reference compound also showed a reduction of the tail moment concentration dependently.

From these results we concluded that Pericarpium castaneae extracts may be used as anti aging agent having multi functions such as anti-oxidative activity, free radical scavenging activity, collagen synthesis activity, anti-MMP activity and anti-DNA damage effects induced by free radical.

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Table 1. Anti-oxidative activity of Pericarpium castaneae extracts

Group	% inhibition
Control(vehicle only)	
Pericarpium castaneae extracts	

5μg/mℓ	16.9
50μg/mℓ	50.9**
100 μg/mℓ	63.0**
BHT	
50μg/mℓ	27.5*
100μg/mℓ	42.4**
200 μg/mℓ	61.3**

N=3, Significantly different from the positive control group(Student's t-test)

*P<0.05, **: P<0.01

Table 2. Free radical scavenging activity of Pericarpium castaneae extracts

Group	% inhibition
Control(vehicle only)	-
Pericarpium castaneae extracts	
5μg/mℓ	38.9**
10μg/mℓ	59.9**
20 μg/mℓ	71.4**
Vit-E	
5μg/mℓ	33.0**
10μg/mℓ	64.9**
20 μg/mℓ	78.8**

n=3, Significantly different from the positive control group(Student's t-test)

*P<0.05, **: P<0.01

Inhibition = [OD positive – OD control] / QD positive×100

Table 3. Effects of the Pericarpium castaneae extracts on collagen synthesis

Pericarpium castaneae extracts(μg/ml)	Abs at 450nm	Collagen (ng/ml)
0	1.574	55.9
5	2.231	83.2
10	2.374	90.7
50	2.479	110.9

*Positive control : TGF-beta : Abs- 2.894, Amount of collagen : 112.5ng/ml

Table 4. Inhibition effects of the Pericarpium castaneae extracts on Elastase activity

Pericarpium castaneae extracts(μg/ml)	Abs at 410nm	Elastase inhibition(%)
0	0.902	-
5	0.758*	15.9
10	0.352**	60.9
50	0.306*	66.1
100	0.251**	72.1
200	0.194*	78.5

N=3, Significantly different from the positive control group(Student's t-test)

*$P<0.05$, **: $P<0.01$

Inhibition = [OD positive – OD control] / OD positive×100

Table 5. Inhibitory effects of the Pericarpium castaneae extracts on the DNA damage

Group	Olive Tail moment	% inhibition
Control(vehicle only)	19.08 ± 0.55	
Pericarpium castaneae extracts		
5μg/㎖	13.54±2.91 *	29.0
10μg/㎖	10.65±4.10 *	44.1
20 μg/㎖	7.21±1.07 **	62.2
Vit-E(M)		
10^{-6}	13.04±2.35 *	31.6
10^{-5}	7.45±1.98 **	60.9
10^{-4}	7.04±0.96 **	63.1

N=3, Significantly different from the positive control group(Student's t-test)

*P<0.05, **: P<0.01