A PROMISING NEW ANTI-WRINKLE INGREDIENT :

Pericarpium castaneae extracts

Beom-Jun Kim, Byoung-Kee Jo and Jeong-Ha Kim
Skin Biology Laboratory, R&D Center,
Coreana Cosmetics Co. Ltd., Cheonan, 333-830, KOREA

Key word ; pericarpium castaneae, anti-wrinkle, anti-oxidative activity, free radical scavenging activity, moisturizing, elasticity, cell adhesive protein

Abstract

Pericarpium castaneae extracts have variously potent activities, such as anti-oxidative activity and free radical scavenging activity. *in vivo* and *in vitro* studies both indicate that pericarpium castaneae extracts acts as a free radical scavenger (IC_{50}: 7.6μg/ml) stronger than gallic acid(IC_{50}:12.5μg/ml) and ellagic acid(IC_{50}:15μg/ml) which could prevent cutaneous UV damages and skin aging. The extracts showed a good effect as a anti-oxidant (IC_{50}:50μg/ml).

It was shown that the appearance of wrinkle in human skin was reduced by topical application of pericarpium castaneae extracts. And the treatment of human skin with the extracts increased the elasticity and moisture of the skin.

We investigated the effect of the pericarpium castaneae extracts on production of extracellular matrix using cultured A431 fibroblast cells. The results indicated that the extracts had no detectable effect on collagen synthesis. But synthesis of cell adhesion protein was increased by the extracts.

The results suggest that increase of cell adhesion protein synthesis by pericarpium castaneae extracts has closely related to reduction of wrinkle in skin.

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 metagenesis and carcinogenesis in addition with aging. To develop an active agent for

skin anti-aging, we screened biological activity of 285 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. The purpose of our investigation was to elucidate the effect on the reconstruction of extracellular matrix (ECM).

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. To isolate and identify the compound of the extracts, we purified the extract through column chromatography (Silica gel and Sephadex LH-20). The composition of the extracts is shown in Table 1.

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 (100 ㎕) and ethyl linolate (10 ㎕) was added to incubation medium (4.89 ㎖) containing 2% SDS, μ M ferrous chloride and 0.5mM hydrogen peroxide. After incubation at 55℃ for 16 hrs, the sample' 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(2 ㎖) was added to 2 ㎖ of 60μ M 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. *in vivo* assay on healthy volunteers

We studied the effect of the extracts on human skin twenty healthy volunteers. 3% pericarpium castaneae extracts solution (50% 1,3-butylene glycol) was topically applied on the left forearm twice a day for the indicated time period, while right forearm received only vehicle for the same period of time.

In order to evaluate the skin moisturizing effect, the degree of stratum corneum hydration was measured by capacitance method using a Corneometer CM 820 (C+K electronic GmbH, Germany) according to the previously published procedure (8-11).

For measuring skin elasticity, suction method was used with Cutometer SEM 575 (C+K electronic GmbH, Germany)(3).

The degree of wrinkle improvement was evaluated by measuring skin roughness using Skin-Visiometer SV 400 (C+K electronic GmbH, Germany)(4,5).

4. Determination of collagen synthesis

Fibroblast was placed in 96-well microplates ($2x10^4$cells/well) for 24hrs. The medium was changed for serum free medium containing pericarpium castaneae extracts for 48hrs of incubation. During the final 24hrs, ascorbic acid (50μg/ml) was added to wells to promote collagen synthesis. At the end of incubation, the wells were washed, and fresh serum free medium was added. After an additional 24hrs of incubation, supernatants were collected to determine the amounts of procollagen type I C-peptide with an enzyme-linked immunosorbent assay kit.

5. Determination of adhesion protein

Fibroblasts were placed in 6-well culture plates ($5x10^5$cells/well) for 48hrs. The medium was replaced with new medium containing pericarpium castaneae extracts for 48hrs of incubation. After incubation, cultivated cells was washed with PBS and collected by scraping and centrifugation. The protein increased by pericarpium castaneae extracts was purified and identified by SDS-PAGE and electroelution

Results and Discussion

In order to elucidate the biological activities of the ethanolic extract of pericarpium castaneae, *in vitro* assay such as anti-oxidative activity and free radical scavenging activity. As shown in Table 2, pericarpium castaneae extracts showed mild anti-oxidative activity (IC_{50} = 48 μg/mℓ), while BHT was the most potent anti-oxidative activity (IC_{50}=0.2 μg/mℓ). The free radical scavenging activity of pericarpium castaneae extracts is shown in Figure 1. Pericarpium castaneae extracts possessed potent free radical scavenging activity *in vitro* (IC_{50}=7.6 μg/mℓ) compared to garlic acid (IC_{50}= 12.5 μg/mℓ). 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. Citropten (coumarin) and ellagic acid were successfully isolated from pericarpium castaneae extracts. Ellagic acid is a well-known free radical scavenger, while citropten did not show the anti-oxidative and radical scavenging activity.

To elucidate the effect of pericarpium castaneae extracts on skin, we studied *in vivo* biological activities of the extracts throughout topical application of the cream containing 3% pericarpium castaneae extracts. As shown in figure 2, pericarpium castaneae extracts increased skin moisturizing of about 20% compared to control.

And pericarpium castaneae extracts also increased skin elasticity about 10% compared to control (Fig. 3) and greatly roughness of skin wrinkle about 26% (Fig. 4).

Also, in collagen synthesis through fibroblast culture, pericarpium castaneae extracts increased protein synthesis, but had no detectable effect on collagen synthesis. The production of a protein (MW 60,000) with pericarpium castaneae extracts was increased significantly (Fig.5). Increased of the protein synthesis correlated in vivo biological activities of pericarpium castaneae extracts. We suggest that the protein induced by pericarpium castaneae extracts may be a one of a number of cell adhesion protein.

5. References

1. Ohakawa, T., Ohishi, N., and Yagi, K., *Anal. Biochem.*, 1997, *95*, 351
2. Fugita, Y., Uera, I., Morimoto, Y., Nakajima, M., Hatano, C., and Okuda, T.,: *Yakugaku Zasshi*, 1998, *108*, 129
3. Elsner, P.: Skin elasticity. In: Berardesca, E., Elsner, P., Wilhelem, K.P., and Maribach, H.I., Eds., : *Bioengineering of the skin* : Methods and instrumentation : CRC, Boca Raton, FL, 1995, Chapter 6, 53-64
4. Nissen, H.P., Comparative studies of skin roughness measurements by profilometry and a new image analysis system: *Cosmetics and toiletries manufacture world wide*, 1995, 247-248
5. Gassmueller, J., Kecskes A., Jahn, P., : Stylus method for skin surface contour measurement., *Hand-book of non-invasive methods and the skin*, 1995, 83-87
6. Davies, K.T.A. : Oxidative damage and repair, Pergamon press, New York, 1991
7. Simic, M.G., and Bergtold, D.S. : *Mutation Res.*, 1991, *250*, 17
8. Hartman, P.E., and Shankel, D.M. : *Environ. Mol. Mutagen.*, 1990, *15*, 145
9. Wattenberg, L.W. : *Cancer Res.*, 1992, *52*, 2015s
10. Masaki, H. : Active oxygen scavenging activity in plant extracts, *Fragrance J.*, 1995, *8*, 64
11. Fukuda, T., and Kitada, Y. : Reactive oxygen species scavenging effect of crude drug, *Fragrance J.*, 1995, *18*, 75
12. Yoshikawa, M., and Yamahara, J. : *Chem. Pharm. Bull.*, 1992, *40(8)*, 2248

Table 1. Composition of Pericarpium castaneae extracts

Components(%)	Content(mean± SD)(%)
Lipid	0.3 ± 0.1
Protein	22.0 ± 1.5
Carbohydrate	35.5 ± 4.8
Ash	1.4 ± 0.1
Citropten (Compound I)	0.0028 ± 0.0006
Unknown(Compound II)	0.0056 ± 0.0001
Ellagic acid	0.09 ± 0.02
Tannic acid	2.5 ± 0.5

Table 2. Anti-oxidative activity of Pericarpium castaneae extracts

Group	*Absorbance (OD_{535nm})*	*% inhibition*
Control		
(vehicleonly)	0.351± 0.002	-
Pericarpium castaneae extracts		
5g/mℓ	0.291± 0.010	16.9
50g/mℓ	0.178± 0.010**	50.9
100g/mℓ	0.130± 0.005**	63.0
d*l*-*a*-tocopherol		
5g/mℓ	0.275± 0.025	22.7
30g/mℓ	0.184± 0.035	47.6
50g/mℓ	0.121± 0.028	65.6
l-ascorbic acid		
100g/mℓ	0.287± 0.007**	18.3
200g/mℓ	0.188± 0.006*	46.5
300g/mℓ	0.139± 0.028	60.4
BHT		
1g/mℓ	0.114± 0.010	67.5
10g/mℓ	0.054± 0.022	85.6
100g/mℓ	0.009± 0.001**	97.5

* $P < 0.05$, **: $P < 0.01$, significantly different from control(n=3)

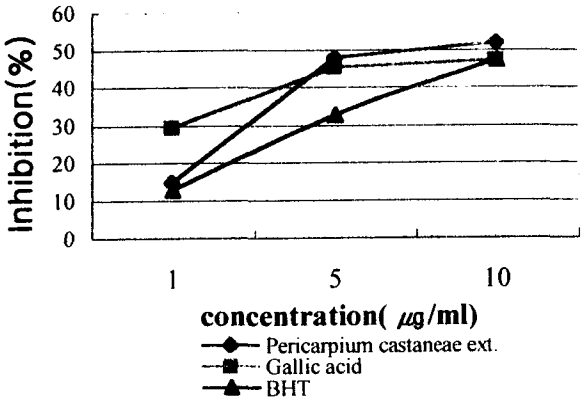


Fig. 1 Free radical Scavenging activity of Pericarpium castaneae extracts

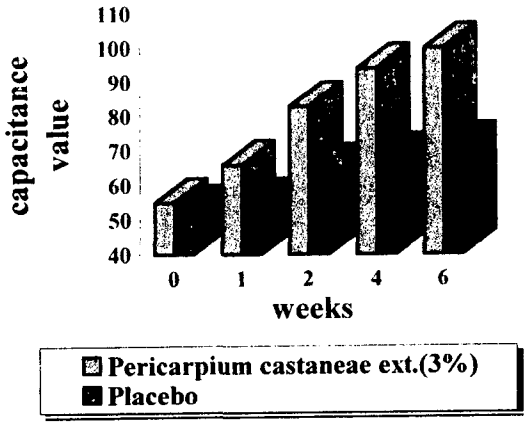


Fig. 2 Effect of Pericarpium castaneae extracts on skin moisturizing

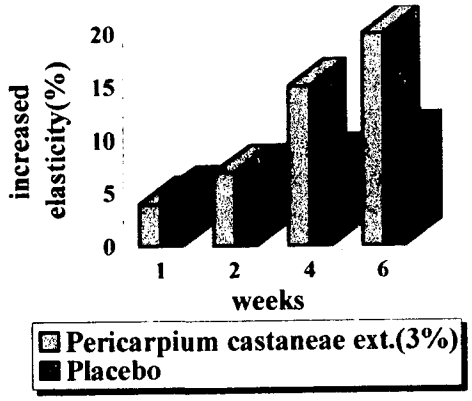
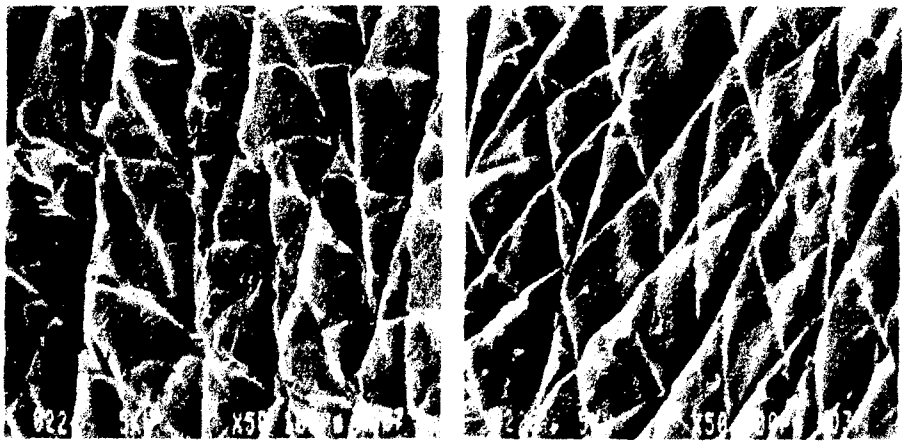


Fig. 3 Effect of Pericarpium castaneae extracts on skin elasticity



Before After

Fig. 4 Effect of Pericarpium castaneae extracts on skin roughness(anti-wrinkle)

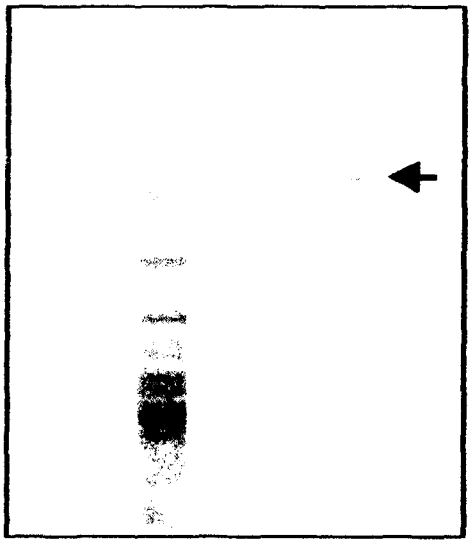


Fig.5 Increasing effect of cell adhesion protein of Pericarpium castaneae extracts by electrophoresis (SDS-PAGE)