PROCEEDINGS OF THE 20TH IFSCC CONGRESS

EFFICACY AND BIOLOGICAL ACTIVITIES OF A NEW ANTI-AGING AGENT OBTAINED FROM *ARECA CATECHU*

KUN-KOOK, LEE., KWANG-SIK, LEE., JEONG-HA, KIM., BYUNG-KEE, JO., AND JUNG-DO, CHOI.*

R & D Center, Coreana Cosmetics Co Ltd., 204-1, Cheonan-si, Chungnam, 330-830, Korea
**Derpartment of Biochemistry, College of Natural Science. Chungbuk National University, Cheongju, Chungbuk, 361-763, South Korea*

Key Words

Areca catechu, Elastase, Hyaluronidase, Anti-oxidative activity, Free radical scavenging, Anti-aging, Inhibition.

Abstract

Inhibitory effects of the new material obtained from *Areca catechu* seed (CC-516) according to a special process, and its applicability to the skin as a cosmetic raw material in terms of its efficacy were presented. *Areca catechu* extract out of 150 medicinal plants, exhibited high inhibitory effect on the porcine pancreatic elastase (IC_{50} : 40.8μg/ml). It also had an inhibitory effect on the human leukocyte elastase (IC_{50} : 48.1μg/ml), hyaluronidase (IC_{50} : 416μg/ml), antioxidative activity (IC_{50} : 45.4μg/ml), and free radical scavenging activity (SC_{50} : 10.2μg/ml). The cream contained 3% of CC-516 improved skin hydration above 16.5%. Especially, the skin elasticity increases more than 35% and skin wrinkles decreased more than 23%. The CC-516 was designed to be utilized in cosmetology. The cream containing 3% of this product has not only protecting effect on the skin mechanical properties provided by the collagen and the elastin in the derm but also restructuring effect of scarring or aging tissue.

Introduction

In UV-A-irradiated skin, mild inflammation occurs repeatedly in the dermis, and it is assumed that connective tissue proteins may be attacked by elastase released from polymorphonuclear leukocytes (PMNs), resulting in damage to elastin and collagen fibers and finally causing sagging (1). Recently, a number of studies have been interested in interaction between elastase and its inhibitors (2-4)

including unsaturated fatty acids, peptides, flavonoids and terpenoids (5-8). Plant sources have been evaluated for developing natural active agents that may be involved in anti-aging and anti-wrinkle care (9-10). To develop active agents for skin anti-aging, we have previously screened the inhibitory effects of 150 medicinal plants on elastase activity. The *Areca catechu* metanolic extract shows high inhibitory effects on porcine pancreatic elastase, compare to reference compounds (11), and we selected the *Areca catechu* extract (the seed of *Areca catechu* L) as a new anti-aging agent in cosmetic. *Areca catechu* L is widely cultivated, especially in the Southern Asia and its seed is as chewing material, anthelmintic and also in kompo-traditional medicine. Preparation containing *Areca* are used also as a digestive as *Areca* promotes the secretion of Saliva (12-13)

To clarify the mechanism of *Areca catechu* L extract against anti-aging, we studies the various biological activities *in vitro* and also assessed the efficacy *in vivo*.

Anti-aging effect of *Areca catechu* L extract studies the anti-elastase activity, anti-hyaluronidase activity, anti-oxidative activity and free radical scavenging effect as *in vitro*. Furthermore, we examined the skin mechanical properties for anti-wrinkle effect, skin moisturizing , and skin elasticity as *in vivo* study.

Materials and Methods

Preparation of CC-516

Areca catechu seed was purchased from the oriental medicinal market located in Seoul, South Korea. 100g of *Areca catechu* was soaked in 500ml of 90% ethanolic aqueous solution at room temperature for 7 days. After filtration, the ethanolic filtrate was evaporated to dryness under vacuum, and then completely dried by lyophilization. The dried extract was called the CC-516 . The dried CC-516 was used as the sample in this study.

Assay for elastase activity

Porcine pancreatic elastase (PPE : Sigma) and human leukocyte elastase (HLE, Sigma) was assayed spectrophotometrically by the modified method of James et al. (14), using N-Succ-$(Ala)_3$-nitoranilide (S.A.N.A) as the substrate, and monitoring the release of p-nitroaniline for 20 min at 25°C by measuring the absorbance at 410nm. The percentage of inhibition was calculated as :

Inhibition (%) = (1-B/A) x 100

Where, A is the enzyme activity without CC-516, and B is the activity in the presence of CC-516.

Assay for hyaluronidase activity

Hyaluronidase activity was determined spectrophotometrically by measuring the amount of N-acetylglucosamine formed from sodium hyaluronate (15). Fifty µl of bovine hyaluronidase (7,900 units/ml, Sigma) dissolved in 0.1M acetate buffer (pH 3.5) was mixed with 100ul of a designated concentration of CC-516 dissolved in 5% DMSO, and then incubated in a water both at 37°C for 20

min. The control group was treated with 100ul of 5% DMSO instead of the CC-516. Optical density at 585nm of the reaction mixture was measured by using a spectrophotometer.

Antioxidative activity

A lipid peroxidation systerm was induced by Fenton s reagent. The known synthetic antioxidant, butylated hydroxytoluene (BHT) was used as a reference compound. The incubation medium was kept at 55 °C for 16 hrs. Each reaction mixture (0.2ml) was transferred into a test tube, followed by addition of 4% BHT (50µl) to prevent further oxidation. Antioxidative activity of the sample was measured using thiobarbituric acid(TBA) assay according to the method of Ohkawa et al. (16).

Free radical scavenging activity

Scavenging effect against free radical generation was measured following the procedure of Fugita et al. (17). The sample solution (2ml) was added to 2ml of 50µM 1,1-diphenyl-2-picryhydrazy (DPPH) ethanolic solution and kept room temperature for 30 min. The absorbance was measured at 520nm.

Assay of improvement of skin condition

The improvements of skin condition were evaluated for 3 aspects: moisture contents of the skin, skin elastic properties and wrinkles. Used instruments are Corneometer CM 820 (Courage + Khazaka c+k, Germany) for moisture contents of skin, Cutometer SEM 474 (c+k, Germany) for skin elasticity and Skin Visiometer SV 400 (c+k, Germany) for wrinkles. All the percentage values was calculated as: (%) = (value at measuring point value at initial point x 100) and the statistical significance of the two test groups was verified by paired t-test as the significant level $p<0.05$. SEM examination was performed on dyed silicon replica of the tested areas that had been air dried, coated with a thin layer of gold-palladium and viewed in a Scanning Electron Microscope (JSM-840A, JEOL Co.,) at 25 kV. Application of test sample was applied twice a day on the eye and cheek regions. The measurements were taken before first treatment and 1 week, 2 weeks, 4 weeks and 6 weeks after test sample treatment.

Results and Discussion

We have previously screened the inhibitory effect on porcine pancreatic elastase from methanolic extracts of 150 medicinal plants. The *Areca catechu* extract shows high inhibitory effect comparable to reference compounds (11). For inhibitory effect of elastase on several solvent extracts showing high ethanolic extract, the CC-516 was used as the sample in this study. The composition analysis of the CC-516 examined other assay methods, and investigated the contents of free amino acids by Amino Acid Analyzer. Figure 1 shows the concentration-dependent inhibition of PPE and HLE by CC-516. The CC-516 showed a similar pattern of inhibition on the hydrolytic activity of PPE and HLE. The CC-516 at 10 to 500 µg/ml as the final concentration exhibited more than 37% to 98% inhibition, IC_{50} values is 40.8 µg/ml (PPE) and 48.1 µg/ml (HLE), respectively. In anti-hyaluronidase activity assay, CC-516 exhibited more than 65% and 78% of inhibition at the concentration of 0.5 mg/ml and 1mg/ml, respectively. As shown in Fig. 2, the CC-516 at 0.1 1 µ g/ml as the final concentration exhibited 10% to 78% inhibition. Anti-hyaluronidase activity of *Glycyrrhiza uralensis* as control

exhibited 10-84% of inhibition at the concentration of 0.1-1.0μg/ml. IC_{50} values of Glycyrrhiza uralensis and CC-516 were found to be 330μg/ml and 416μg/ml, respectively. Major constituents of CC-516 are phenolic compounds.

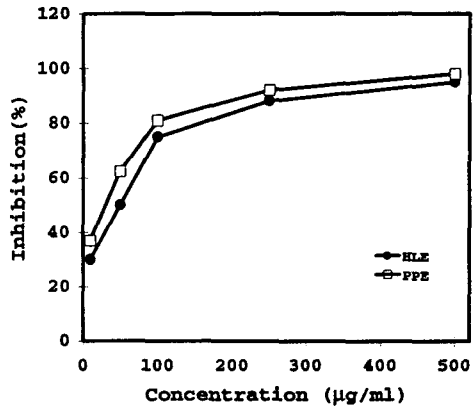

Fig 1. Anti-elastase activity of CC-516. Dose-response curves for the inhibitory effect on PPE(IC_{50} : 408 μg/ml) and HLE(IC_{50} : 48.1 μg/ml).

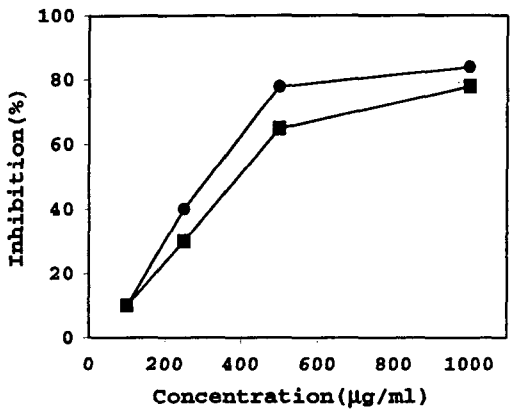

Fig 2. Dose-dependent inhibition of hyaluronidase activity. The effect of CC-516 (■) and Glycyrrhiza uralensis (●) on enzyme activity are indicated as % inhibition compared to the control. Significances of the data are P > 0.01. IC50 values is 416 μg/ml (■) and 330 μg/ml (●), respectively.

Table I.1 showed the antioxidative activity of the CC-516 and reference compounds such as dl-α-tocopherol, L-ascorbic acid and BHT, which gave good dose response relationships. IC_{50} value of BHT was 1.5μg/ml, while other reference compounds, dl-α-tocopherol and L-ascorbic acid showed 33.6μg/ml and 219μg/ml, respectively. IC_{50} value of CC-516 was exhibited 45.4μg/ml, which showed the similar to dl-α-tocopherol or more potent activity than L-ascorbic acid. Some of plant extracts may act at the initiation stage of peroxidation interfering with Fenton s reaction, thus breaking the chain reaction.

Table I.2 showed the free radical scavenging activity of CC-516 and several reference compounds. L-ascorbic acid was the most potent scavenger. SC_{50} values of ascorbic acid, dl-α-tocopherol and BHT were found to be 29.7μg/ml, 33.5μg/ml and 37.2μg/ml, respectively. On the other hand, SC_{50} values of CC-516 (SC_{50}:10.2μg/ml) showed much lower activity than the well-known reference compounds. Free radical damage to bio-system is one of the major processes that contributes to degenerative disease like cancer and aging (19).

Components	IC_{50}(μg/ml) [1]	SC_{50}(μg/ml) [2]
BHT	1.5	37.2
dl-α-tocopherol	33.6	33.5
Ascorbic acid	219.0	29.7
CC-516	45.4	10.2

Table I. 1. Inhibition of lipid peroxidation of the CC-516 by Fenton s reagent linoleate system.
2. Free radical scavenging activity of CC-516 by DPPH free radical generating system.

Fig. 3 showed the skin moisturizing effect of cream contained 3% CC-516. The elasticity of the skin depends on its water content and on the swelling capacity of the fibers of its connective tissue. Evaluation of the stratum corneum hydration by capacitance method showed that skin moisturizing increased about 16.5% against untreated cream. In the skin hydration, all measurements were done in triplicate and the average values were used for statistical evaluation. From the results, which are represented in Fig. 4 as means of 5 test samples, a smaller depth of penetration of the skin into the tube and thus an elasticity increased by 35% are obtained. In the case of treated skin (control) on the contrary, no modification could be observed.

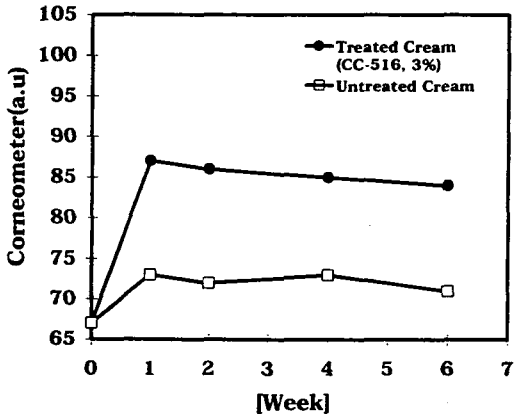

Fig.3. The effect of skin hydration on eye region.

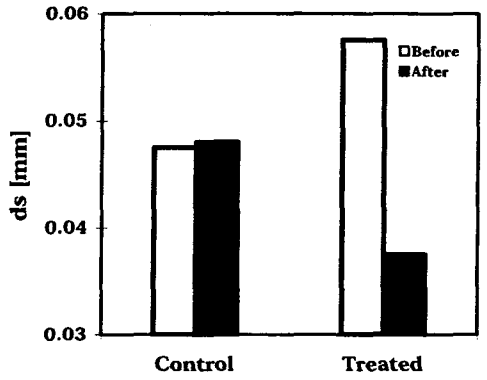

Fig.4. The skin elasticity of the cream treated 3% CC-516. Control is untreated cream before and after 6 weeks treatment. Treated is treated cream (3% CC-516) before and after 6 weeks treatment. The smaller ds, the more elastic the skin.

Fig.5 and 6 showed the anti-wrinkle effect by three-dimensional Image Analyzing. The average values were used for statistical evaluation. Applications of test sample (CC-516 3% cream) are topically treated by twelve volunteers twice a day for 6 weeks on designated eyes. The results obtained on the treated side show significant reductions of roughness (-16.7%) and of the average depth of the cutaneous relief 23%, but untreated skin (control) shows no significant difference.

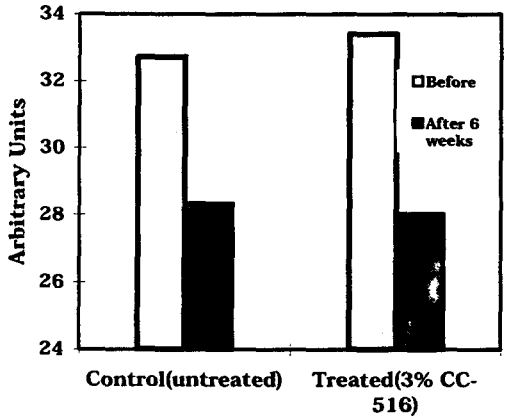

Fig.5.-The anti-wrinkle effect on roughness of the skin.-The results obtained treated side show Significant reduction-16.7% of roughness of the skin compared to before treatment.

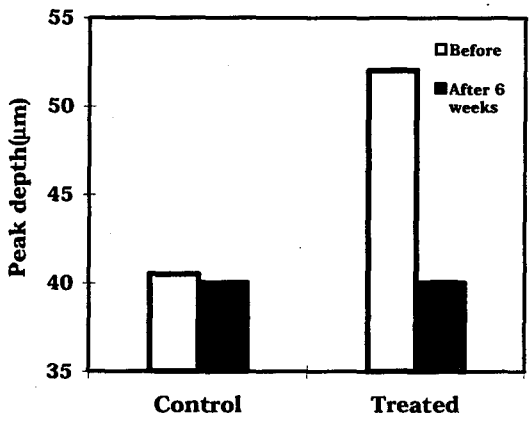

Fig.6.-The anti-wrinkle effect on mean depth of the skin.-The results obtained treated side show significant reduction, 23% of the average depth of the skin compared to before treatment.

The study on the wrinkles using SEM reveals apparent reduction in the depth of the wrinkles against untreated skin (photo 1, 2), but control cream show no significant difference before treatment and after treatment. In conclusion we suggests that the use of CC-516 as an active ingredient for anti-aging may be sufficient to satisfy the needs of users and cosmetic scientists.

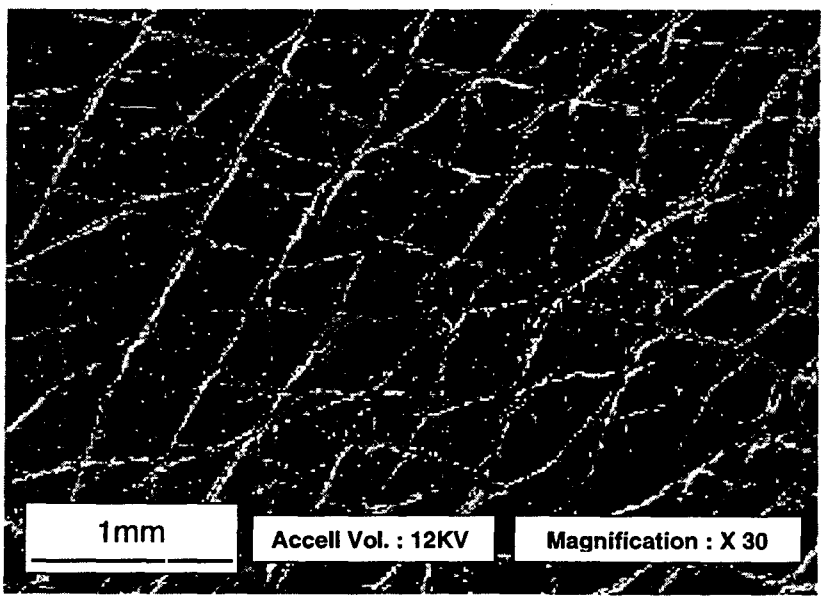

Photo 1.-The wrinkles of the skin surface before treatment.

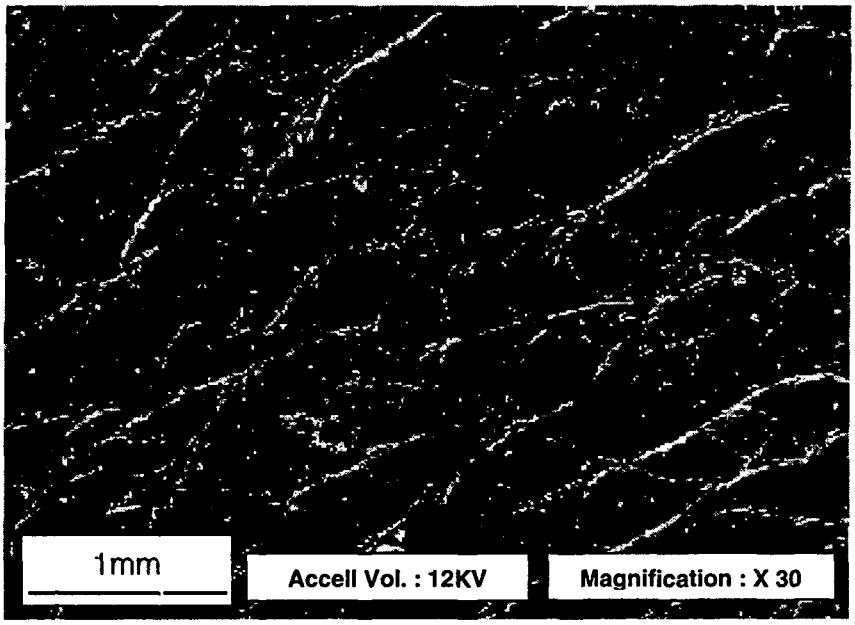

Photo 2. The wrinkles of the skin surface after treatment. The wrinkles decrease in depth after application of the treated cream (3% CC-516).

References

1. Motoyosh K. and Takenouch M., Preceedings of the 19 th IFSCC Congress, Sydney, R053(1996).
2. Philip D.E. and Peter R.B., *Med. Res. Rew.*, 14(2), 127-197 (1994).
3. Suresh C.T. and Sanford R.S., *Biochemistry.*, 29(42), 9970-9977 (1990).
4. Bizot-Foulon V., Codeat G. and Horneveck W., *Int. J. Cosm. Sci.*, 17, 255-264(1995).
5. Yamahara j., *Chem Pharm, Bull.*, 27, 1464-1468 (1979).
6. Centro Tecnico Res Pharma. *Cosmetic News N.*, 71, 88-90 (1990).
7. Mahato S.B., Ganguly A.N. and Sahu N.P., *Phyto chemistry.*, 21, 959-978 (1982).
8. Annoni F., Mauri A. and Resele L. F., *Arzneim. Forsh/Drug Res*, 29, 672-675 (1979).
9. Wattenberg L. W. and Lark L. K. T., Plenun Press., New York, pp1 (1981).
10. Kim Y.S., Noh Y.K. and Min K.R., *Kor. J. Pharma.*, 26(3), 265-272(1995).
11. Lee K.K., Kim J.H., Cho J.J. and Choi J.D., *Int. J. Cosm. Sci.*, 20, 1-12(1998).
12. Shiau Y.Y. and Kwan H.W., *Oral Surg.*, 47, 453(1979).
13. Dockrat J. and Shear M., 4 th Proc. Int. Acad. Oral Path., P57, Gordon and Breach Science Publishers. Inc., New York (1970).
14. James A.E.K., Timothy D.W. and Gordon L., *Biochemistry.*, 35, 9090-9096(1996).
15. Reissig G.L. and Leloir L.F., *J. Biol. Chem.*, 217, 959-963 (1955).
16. Ohkawa H., Ohishi N. and Yagi K., *Anal. Biochem.*, 95, 351(1979).
17. Fugita Y., Uehara J., Morimoto Y. and Okuda T., *Yakugak Zasshi*, 108, 129-135 (1988).
18. Wang C.K. and Lee W.H., *J. Agri. Food. Chem.*, 44(8), 2014 (1996).
19. Davies K.T.A.: Oxidative damage and repair, Perganon Press, N.Y. (1991).