Chestnut extracts as new Anti-aging agent

Beom-Jun Kim*, Jeong-Ha Kim[1], Hyun-Pyo Kim[2], and Moon-Young Heo[2]

김범준*, 김정하[1], 김현표[2], 허문영[2]

R&D Center, Coreana Cosmetics Co. LTD., Cheonan, Korea[1]

코리아나화장품연구소

College of pharmacy, Kwangwon University, Chuncheon, 200-701, Korea[2]

ABSTRACT

Inner nutshell of *Castanea crenata*(chestnut) has been used as an anti-aging and anti-wrinkle agent from the ancient time in east Asia. In order to develop new anti-aging and anti-wrinkle, ethanolic extract of inner nutshell of *Castanea crenata*(Cor-285) was prepared and various biological activities were evaluated. Cor-285 showed potent anti-oxidant activity. Especially, Cor-285 possessed potent free radical scavenging activity *in vitro*(IC_{50}:7.6μg/ml) compared to gallic acid(IC_{50}:12.5μg/ml). Cor-285 showed the preventive effect against UV-induced cytotoxicity of fibroblast at concentration of 25-250μg/ml. When Cor-285 was evaluated for its anti-allergic activity, it effectively inhibited histamine release from mast cells induced by compound 48/80(86% inhibition at 10mg/ml). The inhibitory activity was stronger than that of glycyrrhizinate . Cor-285 also showed *in vivo* inhibition against delayed hypersensitivity as well as croton-oil induced ear edema in mice when topically applied. These results strongly suggest that Cor-285 may reduce immunoregulatory/inflammatory skin trouble. From the attempts to isolate the

scavenger, were isolated. In a clinical trial of twenty healthy volunteers with aged skin, 6 weeks application of Cor-285(3% cream) decreased wrinkle about 26% and increased moisturizing 20% on the skin. All of these results indicate that Cor-285 may be an effective anti-aging and anti-wrinkle agent.

Introduction

The biological activity of various plant extracts have been screened for cosmetic use. Furthermore, plant sources have been evaluated for developing natural anti-ageing agents. Many endogeneous plant compounds possess anti-oxidative activity and free radical scavenging activity. Recent studies indicate the compounds is able to inhibit metagenesis and carcinogenesis in addition with ageing. We have screened biological activities of 300 plants for cosmetic use.

We selected *castanea crenata*(chestnut) as a anti-ageing agent. Chest nut has been used as a popular remedy for anti-wrinkle from the ancient time in Korea. In an ancient literature such as Dong-Eu-Bo-Gam, Chestnut has been used for anti-wrinkle. Recently, retinol or AHA has been using as a anti-wrinkle agent in the skin care products, but retinol or AHA has problems of skin irritation and stability. To clarify the mechanism of anti-ageing, we studied the anti-oxidative activity, free radical scavenging effect, inhibition of histamine release as *in vitro* study and anti-inflammatory/inhibition of delayed hypersensitivity, skin roughness for anti-wrinkle effect, skin moisturizing, and skin elasticity as *in vivo* study.

Experimental Methods

Preparation of chestnut extracts

Castanea crenata was obtained from the oriental medicinal market located in seoul, South Korea. The 1Kg of *castanea crenata* was soaked in 5L of 70% ethanolic aqueous solution at room temperature for 3 days. After filtration, the ethanolic filtrate was evaporated to dryness under reduced pressure. The extracts was dissolved in 50% 1,3-butylene glycol and then used for each biological study.

Anti-oxidative activity

A lipid peroxidation system was induced by Fentons reagent. Each test sample(0.1ml) and ethyl linoleate(10μl) were added to incubation medium(4.89ml) containing 2% sodium dodecyl sulfate, 1μM ferrous chloride and 0.5mM hydrogen peroxide. The known synthetic anti-oxidants, butylated hydroxyltoluene(BHT) was used as a reference compound. The incubation medium was kept at 55℃ for 16hrs. Each reaction mixture(0.2ml) was transferred into a test tube, followed by addition of 4% BHT(50μl) to prevent further oxidation. Anti-oxidative activity of the sample was measured using the thiobarbituric acid(TBA) assay according to the method of Ohkawa *et al.*[(1)]. The absorbance was measured at 535nm.

Free radical scavenging effect

Scavenging effect against free radical generation was measured by the procedure of Fugita *et al.*[(2)]. The sample solution(2ml) was added to 2ml of 60μM 1,1-diphenyl-2-picryl hydrazyl(DPPH) ethanolic solution and kept at room temperature for 30min. The absorbance was measured at 520nm.

Anti-wrinkle Effect

The anti-wrinkle activity of chestnut extracts on intact skin was evaluated *in*

vivo. We applied a cream containing 3% of chestnut extracts twice a day for 6 weeks[3,4].

The degree of wrinkle improvement was evaluated by measuring skin roughness using a SKIN-VISIOMETER SV 400(C+K electronic GmbH, Germany).

Skin Moisturizing effect

Evaluation of skin moisturizing effect was measured the degree of stratum corneum hydration by capacitance method using a CORNEOMETE CM 820(C+K electronic GmbH, Germany)[5-7].

We applied 2mg/cm^2 of a control cream or a cream containing 3% of chestnut extracts twice a day for 6weeks, then measured changes in degree of hydration, comparing the skin treated with the extracts vs. areas treated with only vehicle[8].

Skin elasticity

Experimental data on skin biomechanics have led to the conclusion that the skin can be described as a complex material with elastic, viscous and plastic characteristics[9]. Various instruments have been developed to measure skin elasticity. We was evaluated the change of skin elasticity by suction method using a CUTOMETER SEM 575(C+K electronic GmbH, Germany).

We applied 2mg/cm^2 of a control cream or a cream containing 3% of chestnut extracts twice a day for 6weeks on twenty volunteers.

Inhibition of Histamine release

The inhibitory effect of the chestnut extracts on type I allergic reaction was evaluated *in vitro*. Rhinitis and dermatitis are induced by histamine released from mast cells residing in the mucosa and skin as a result of allergic

reaction and reaction to exogenic stimulation. Therefore, anti-allergic effect and sedative effect is throught to be obtained by inhibiting histamine release. We isolated the intraperitoneal mast cells from rat(Wistar) according to the method of Uvans[10]. After preincubation of 1.8mL of rat intraperitoneal mast cells solution at 37℃for 10min. the chestnut extracts was added and incubated for 5min. 0.1mL of compound 48/80(10μg/mL) was added to the solution and incubated for 10min. The reaction was stopped by cooling and then centrifuged for 5min. at 1200rpm. The histamine of supernatant was quantified by the procedure of Shore *et al.*[11]. 0.7mL of the supernatant was added to the mixture of 1.4mL of H_2O, 0.4mL of 1N NaOH and 0.1mL of 1% *O*-phthal-dialdehyde methanol, and incubated for 4min. After stopping of the reaction by addition of 0.2mL of 3N HCl solution, the reaction solutions was centrifuged for 5min. at 3000rpm. The extract is purified and stained with *O*-phthal-dialdehyde and then the fluorescence intensity is measured with 350nm excitation wave length light and 450nm fluorescence wave length light[12] in order to distinguish the histamine release control rate from the histamine release rate.

We measured the histamine of supernatants by fluorescence at 450nm and excitation wavelength at 360nm using Spectrophotometer(Shimazu). And 2mL of PBS was added to the pellets and homogenized by ultrasonic instrument. We calculated the percent inhibition of histamine release as follows

$$\text{Histamine release \%} = \frac{\text{histamine released with compound 48/80} - \text{spontaneously released histamine}}{\text{total histamine}}$$

$$\text{Inhibition \%} = \frac{\text{\% histamine release without test sample} - \text{\% histamine release with test sample}}{\text{\% histamine release without test sample}} \times 100$$

Anti-inflammatory activity

For measuring the topically anti-inflammatory activity, mouse ear edema assay was employed. According to the modified method of Kim *et al.*[13], based on the original procedure of Tonneli *et al.*[14], cream containing 3% of chestnut was topically applied to right ears of mice(18-22g) three times at 3hrs interval. Thirty minutes after the final treatment of the test compounds, 2.5% croton-oil or 2% arachidonic acid dissolved in acetone(25μl/ear) was applied topically to ears of mice. And the ear thickness was measured 5hrs after croton-oil treatment or 1hr after arachidonic acid treatment. Percent inhibition of ear edema was calculated compared to the control group having vehicle and inflammgen only.

Inhibition of delayed Hypersensitivity

Inhibitory activity against delayed hypersensitivity was measured according to the method of Tarayre *et al.*[15]. Briefly, 3% picryl chloride(acetone) was applied to abdomen of mice(18-22g). One week later, 3% picryl chloride was applied to ears of mice and ear thickness was measured 24hrs after the treatment of picryl chloride solution. We applied the cream comtaining 3% chestnut extracts to ears of mice daily for 7days starting from 0 day. The differences between ear thickness of the extract treated group and the control group treated with picryl chloride and vehicle only were regarded as an inhibitory activity.

Results and Discussion

We find that the chestnut extracts have various biological activities for cosmetic use. Table 1 demonstrated analysis results of the composition of the chestnut ethanolic extracts by HPLC and other various quantitative

analysis.

Table 1. Composition of the ethanol extracts of *Castanea crenata*

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 represented the anti-oxidative activity of the chestnut extracts and reference compounds such as d*l*-α-tocopherol, *l*-ascorbic acid, and BHT, which gave good dose-response relationships. BHT was the most potent inhibitor of TBA-reactive material formation. IC_{50} value of BHT was 5μg/ml, while other reference compounds, d*l*-α-tocopherol and *l*-ascorbic acid, showed the 33.6μg/ml and 219μg/ml, respectively. IC_{50} value of chestnut extracts was 48.7μg/ml, which showed similar potency with d*l*-α-tocopherol and more potent activity than *l*-ascorbic acid.

Fig. 1 showed the free radical scavenging activity of chestnut extracts and several reference compounds. IC_{50} values of reference compounds such as *l*-ascorbic acid, d*l*-α-tocopherol, BHT, and gallic acid were found to be 29.7μg/ml, 33.5μg/ml, 37.2μg/ml, and 12.5μg/ml, respectively. Gallic acid was the most potent scavenger. IC_{50} value of chestnut extracts showed 7.6μg/ml. From the results, the extracts of *castanea crenata* showed much higher

Table 2. Anti-oxidative effects of the EtOH extracts of *Castanea crenata*

Group	*Treatment*	*Absorbance, OD535nm*	*% inhibition*
Control (vehicle only)		0.351±0.002	-
Cor-285	5 μg/mℓ	0.291±0.010	16.9
	50 μg/mℓ	0.178±0.010**	50.9
	100 μg/mℓ	0.130±0.005**	63.0
d*l*-*α*-tocopherol	5 μg/mℓ	0.275±0.025	22.7
	30 μg/mℓ	0.184±0.035	47.6
	50 μg/mℓ	0.121±0.028	65.6
l-ascorbic acid	100 μg/mℓ	0.287±0.007**	18.3
	200 μg/mℓ	0.188±0.006*	46.5
	300 μg/mℓ	0.139±0.028	60.4
BHT	1 μg/mℓ	0.114±0.010	67.5
	10 μg/mℓ	0.054±0.022	85.6
	100 μg/mℓ	0.009±0.001**	97.5

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

activity than the well-known reference compound. Free radical damage to biosystem is one of the major process that contributes to the degenerative disease like cancer and aging[16]. Detailed free radical mechanisms and their quantitative contributions are still not clear. Despite these uncertainties, it is clear that free radical scavengers may inhibit endogeneous, metabolically driven, oxidative DNA damage, as well as mutation and aging by exogeneous agents[17-19]. There are many plant which were reported to show their free radical scavenging effect[20, 21]. Most of these plants have phenolic or

polyphenolic compounds such as tannins and flavonoids that may contribute to their free radical scavenging activity. Yoshikawa[22] reported that *paeonia suffruticosa* have a strong scavenging activity against DPPH radicals, and isolated galloyl glucose as active compound. We isolated ellagic acid and tannic acid as polyphenolic active compound from ethanolic extracts of chestnut.

In a clinical study, we found that chest nut extracts showed the improvement of skin wrinkle and increease of skin moisturizing and elasticity.

Fig. 2 showed the skin moisturizing effect of chest nut extracts. Evaluation of the stratum corneum hydration by capacitance method showed that skin moisturizing increased about 20% against control.

Fig. 3 showed the skin elasticity increased by application of cream containing 3% of chestnut extracts. Skin elasticity increased about 10% against control.

Fig. 4 showed improvement effect of skin wrinkle with application of cream containing 3% of chestnut extracts twice a day on designated skin. After 6 weeks, roughness of skin wrinkle improved about 26%. The chestnut extracts inhibited histamine released from rat intraperitoneal mast cells induced by compound 48/80. The chestnut extracts showed 86% inhibition of histamine release at 10μg/ml. Its inhibitory activity was stronger than that of dipotassium glycyrrhiznate(Data not shown).

Table 3 and 4 presented the topical anti-inflammatory activity of the chestnut extracts. Its activity was found to be weak compared to the potent activity of the reference compound, prednisolone, but the chestnut extracts has anti-edematic activity as well as anti-hypersensitivity and may be a useful agent to treat various skin troubles.

Conclusively, these results suggested that the chestnut extracts may be effective on anti-ageing.

Table 3. Mouse ear edema inhibition of the EtOH extracts of *Castanea crenata*

	Dose/ear	Thickness increase	% inhibition
Control	-	0.23±0.02mm	-
Prednisolone	0.1mg	0.18±0.03mm*	22
	1.0mg	0.10±0.02mm*	57
YP-70% EtOH	0.2mg	0.18±0.02mm*	22
	1.0mg	0.15±0.01mm*	35
Citropten	0.2mg	0.21±0.01mm	-
	1.0mg	0.21±0.02mm	-

croton-oil induced ear edema, *: $P < 0.05$, $n = 10$

Table 4. Inhibition of delayed hypersensitivity by the EtOH extracts of *Castanea crenata*

	Dose/ear	Thickness increase	% inhibition
Control	-	0.13±0.010mm	-
Prednisolone	0.01mg	0.10±0.005mm**	23
	0.10mg	0.07±0.005mm**	46
YP-70% EtOH	0.20mg	0.10±0.007mm*	23
	1.00mg	0.08±0.005mm*	38
Citropten	0.01mg	0.11±0.008mm	8
	0.05mg	0.11±0.007mm	8

** : $P < 0.01$, * : $P < 0.05$, $n = 10$

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. 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
4. 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
5. Courage, W. : Hardware and measuring principle : CORNEOMETER. In : *Bioengineering of the skin* : water and the stratum corneum : Elsner P., Berardesca E. and Maibach H.I., Eds., CRC, Boca Raton, 1994, 171-176
6. Barel, A., Clarys, P. : Measurement of epidermal capacitance. In : *Handbook of non-invasive methods and the skin* ; Serup, J. and Jemec, GBE, Eds., CRC, Boca Raton, 1995, 165-197
7. Distante, F., Berardesca, E. : Hydration. In : *Bioengineering of the skin* : Methods and instrumentation : Berardesca, E., Elsner, P., Wilhelm, K.P., and Maibach, H.I., Eds., CRC, Boca Raton, 1995
8. Stab, F., Sauermann, G., Hoppe, U. : Evaluation of moisturizers. In : *Bioengineering of the skin* : Skin surface imaging and analysis : Wilhelm, K.P., Elsmer, P., Berardesca, E. and Maibach, H.I, Eds., CRC, Boca Raton, 1997, 315-330
9. 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
10. Uvans, I.L, Thon, *Exp. Cell Res.*, 1959, *18*, 512

11. Shore, P.A., Burkhalter, A., Cohn Jr. V.H., *J. Pharmacol. Expel. Therap.*, 1959, *127*, 182-186
12. Hidehiko, H., Ryouichi, E. and Yukimasa, O., *Japaness J. Allergy*, 1970, *19(3)*, 193-198
13. Kim, H.K., Namgoong, S.Y. and Kim, H.P., *Arch Pharm. Res.*, 1993, *16*, 18
14. Tonneli, G., Thiabalt, L. and Ringgler, I., *Endocrinal.*, 1965, *77*, 625
15. Tarayre, J.P., Barbara, M., Aliaga, M. and Tisneversailles, *J. Arzneim. Forsch./Drug Res.*, 1990, *40(11)*, 10, 1125
16. Davies, K.T.A. : Oxidative damage and repair, Pergamon press, New York, 1991
17. Simic, M.G. and Bergtold, D.S. : *Mutation Res.*, 1991, *250*, 17
18. Hartman, P.E. and Shankel, D.M. : *Environ. Mol. Mutagen.*, 1990, *15*, 145
19. Wattenberg, L.W. : *Cancer Res.*, 1992, *52*, 2015s
20. Masaki, H. : Active oxygen scavenging activity in plant extracts, *Fragrance J.*, 1995, *8*, 64
21. Fukuda, T. and Kitada, Y. : Reactive oxygen species scavenging effect of crude drug, *Fragrance J.*, 1995, *18*, 75
22. Yoshikawa, M. and Yamahara, J. : *Chem. Pharm. Bull.*, 1992, *40(8)* 2248

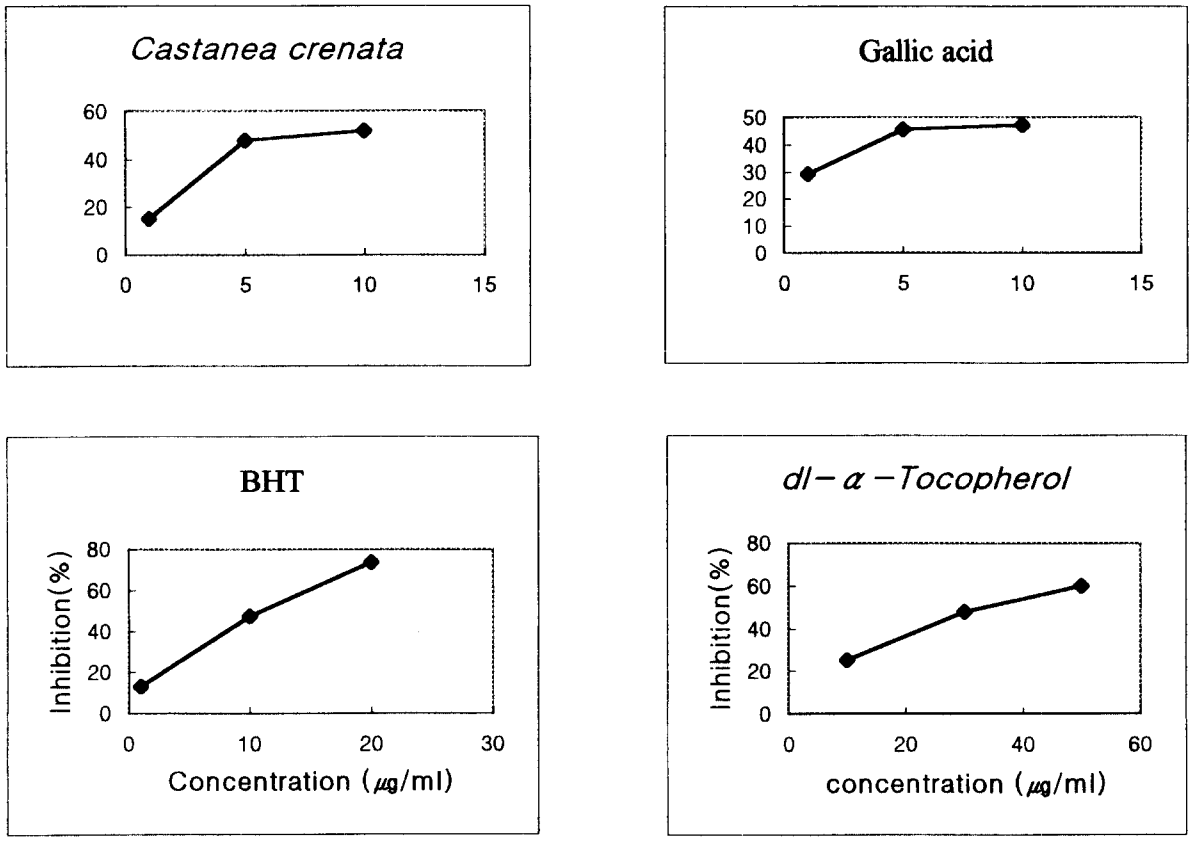


Fig 1. Free radical scavenging activity of chestnut extracts determined by DPPH free radical generating system.

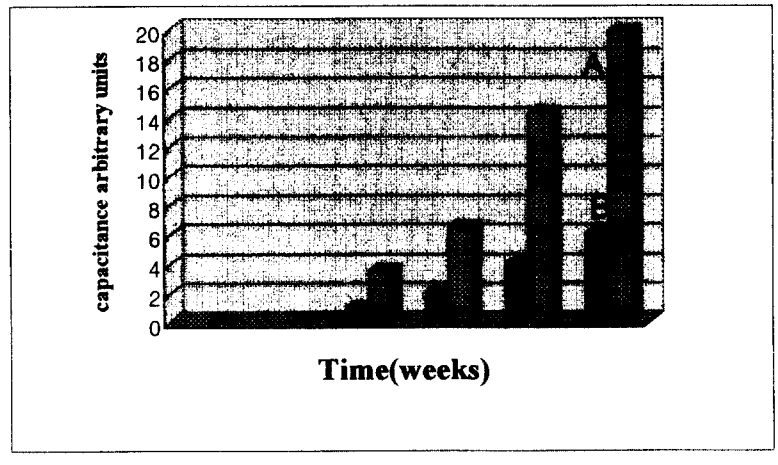


Fig2. Skin moisturizing effect after twice-daily application of cream at 3% chestnut extracts(A) against placebo(B) for 6 weeks

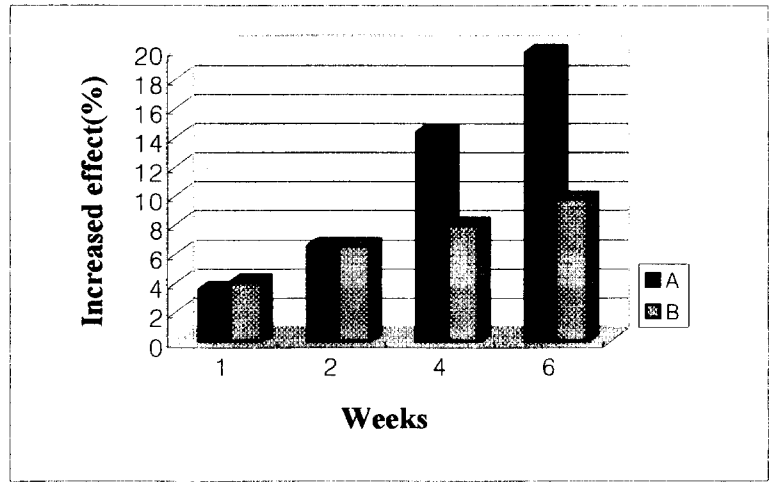
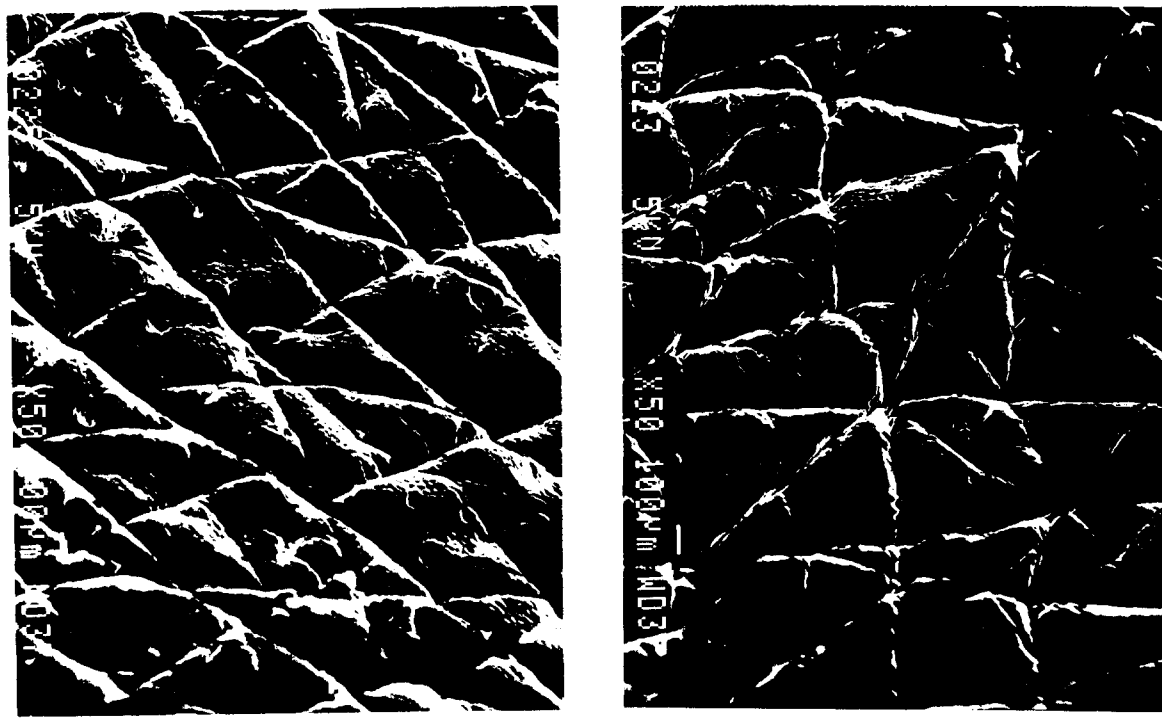


Fig 3. Skin elasticity effect after twice-daily application of cream at 3% Chestnut extracts(A) against placebo(B) for 6 weeks



A B

Fig 4. Skin anti-wrinkle effect after twice-daily application of cream at 3% chestnut extracts(A) against placebo(B) for 6 weeks