The Depigmentation Effect of A New Material Extracted from Paper Mulberry and its Comparison by Three Colorimetric Instruments.

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Introduction

Skin color varies depending on age, racial background, seasonal change and pigmentation disorder. Whiter skin color is a desire of oriental women. Various whitening beauty cosmetic products for inhibiting pigmentation process prevails in the market. Measuring skin color is a popular clinical tool for evaluating depigmentation effect of these products. Therefore, the cosmetic scientists need to develop new effective depigmenting ingredients as well as powerful measuring tools for skin color.

Traditionally, measuring skin color depends on visual assessment. Since, however, subjective opinions of observers are likely to be incorporated, it is hard to communicate with other laboratory in this method. For this reason, commercial colorimeter is widely used in the cosmetic field. Still, objective evaluation of instrumental accuracy and sensitivity between the colorimeters is needed to choose proper instrumentation for their experiments.

Chromameter CR200(Minolta, Japan) is one of the most widely used colorimeter and its properties are extensively studied by many groups(1, 2). Chromameter emits white light and detects reflectance from the skin surface by silicon photocells. Results are shown as L*, a* and b* values according to CIE color space definition, where L* value represents darkness degree of skin color. Dermaspectrometer(Cortex Technology, Denmark) has two light emitting diodes; red(635 or 655nm) and green(565 or 568nm) and detects reflectance of this narrow band of spectrum to calculate erythema index and melanin index. Mexameter MX16(Courage + Khazhaka electronics, Germany) has the same measuring principle and generates the same type of indices as Dermaspectrometer. Unlike Chromameter and Dermaspectrometer, Mexameter, which was introduced in cosmetic field very recently, has not been compared to the other two yet.

Today, most of depigmenting materials in cosmetics are tyrosinase inhibitors. By mushroom tyrosinase inhibition assay, Jang *et al*(4) has developed a new powerful tyrosinase inhibitor. This new tyrosinase inhibitor extracted from paper mulberry(family Moracea, *Broussonetia kazinoki x B. papyrifera*) is named Kazinol F (5-[3-(2,4-dihydroxyphenyl)propyl]-3,4-bis(3-methyl-2-butenyl)-1,2-benzenediol). They showed in *in vitro* assay that Kazinol F has the same level of tyrosinase inhibition activity at a lower concentration compared with kojic acid, ascorbic acid and hydroquinone(4,5).

In this study, we performed clinical test to confirm depigmentation effect of Kazinol F *in vivo* and to compare the results obtained from three colorimetric instruments in the course of depigmentation process of tanned human skin.

Materials and Method

Test Subjects

24 volunteers(male, age 26-33) were equally divided into 2 groups(Group A, Group B) with 12 subjects each. Minimal erythema dose(MED) of each subject was measured according to US FDA test method. MEDs of 24 subjects were in the range of 105mJ/cm^2-270mJ/cm^2(mean 150mJ/cm^2).

Instruments

Pigmentation degree was measured with 3 commercial colorimeters : Chromameter CR200(Minolta, Japan), Dermaspectrometer(Cortex Technology, Denmark) and Mexameter(Courage+Khazhaka electronics, Germany).

Test samples

Four depigmenting materials were used for this clinical experiment. Test samples were as follows; lotion A(2% kojic acid derivatives), lotion B(2% Kazinol F), lotion C(2% arbutin), lotion D(2% hydroquinone), lotion E(lotion base as a vehicle). Kojic acid derivative is kojic acid monobenzoate(2-benzoyl-5-hydroyl-4H-pyran-4-one), arbutin and hydroquinone purchased from Sigma, and Kazinol F extracted from the bark of paper mulberry root(4).

Pigmentation

With artificial 2MED UVB radiation from xenon arc lamp, tanning was made. Erythema was induced within 24 hours and pigmentation process started within 3 days after radiation. After 24 hours of UVB radiation erythema degree was measured by Dermaspectrometer.

Measurement

Overall evaluation of the treated areas was performed at baseline, after 24 hours of radiation, and then twice per week(Monday and Thursday) for two months. The areas were measured 3 times with each of three colorimeters to obtain the mean values. Skin colors were visually assessed by two technicians and graded according to the following scale; 3, much darker; 2, darker; 1 slightly darker; 0, undetectable.

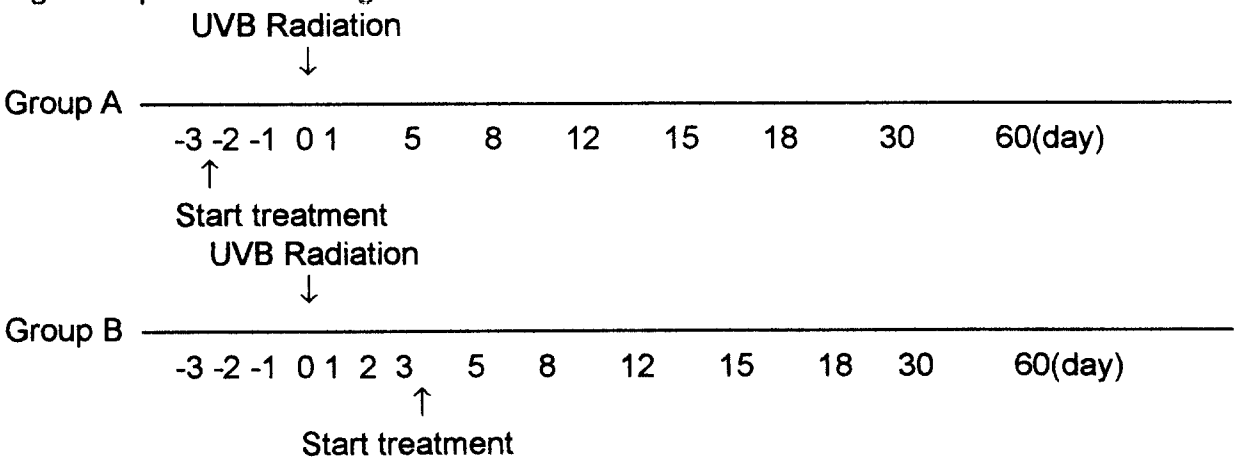
Experimental Design

The study was conducted in 2 groups of volunteers, each with 12 subjects. Inside of lower arm of the each subject was divided into 6 circular clinical areas(5.34 cm^2 each). Miniml erythema dose(MED) of each subject was measured and 2MED of artificial UVB from xenon arc lamp radiated for tanning. During two months, each circular area was treated with one of 5 test lotions. One area was for non-treated control site. Each test lotion was applied twice per day : 10am and 3pm. Baseline measurements were taken with three colorimeters before first application. Darkness degree of each area after tanning was measured twice per week for two months. Also, visual assessment was made by two technicians at the time of colorimetric measurement.

Group A was treated with each lotion for 3 days before radiation. Group B started the treatment 3 days after radiation. To minimize the site variation in the lower arm within the group, right arms were used as treat site for 6 subjects and left arms for the other 6 subjects. Within a person, 5 lotion treat sites were randomized as well.

Treat period was in the spring and all measurements were made in a temperature and humidity controlled room(24±2°C, 40±3%). All subjects took at least 15 minutes before measurement.

Fig. 1. Experimental design



Statistics

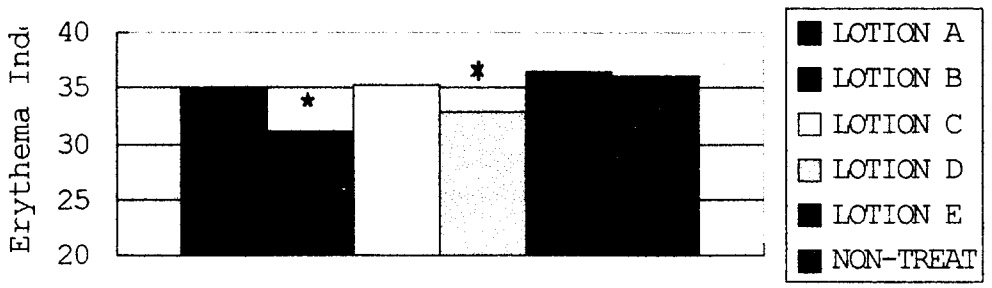
To confirm the significance of depigmentation effects of each lotion, we used one-way ANOVA test method. Correlation coefficient was calculated for instrumental correlation, and coefficient of variation(Standard deviation / Mean) for the instrumental sensitivity. All statistical computation was done with SPSS ver 6.0.1.

Result

1. Depigmentation Effect

In Group A, lotion B(Kazinol F) and lotion D(hydroquinone) treated areas showed signficantly low erythema level at 24 hours after radiation(Fig 2).

Fig. 2. The erythema indices of each lotion treated areas(Dermaspectrometer)



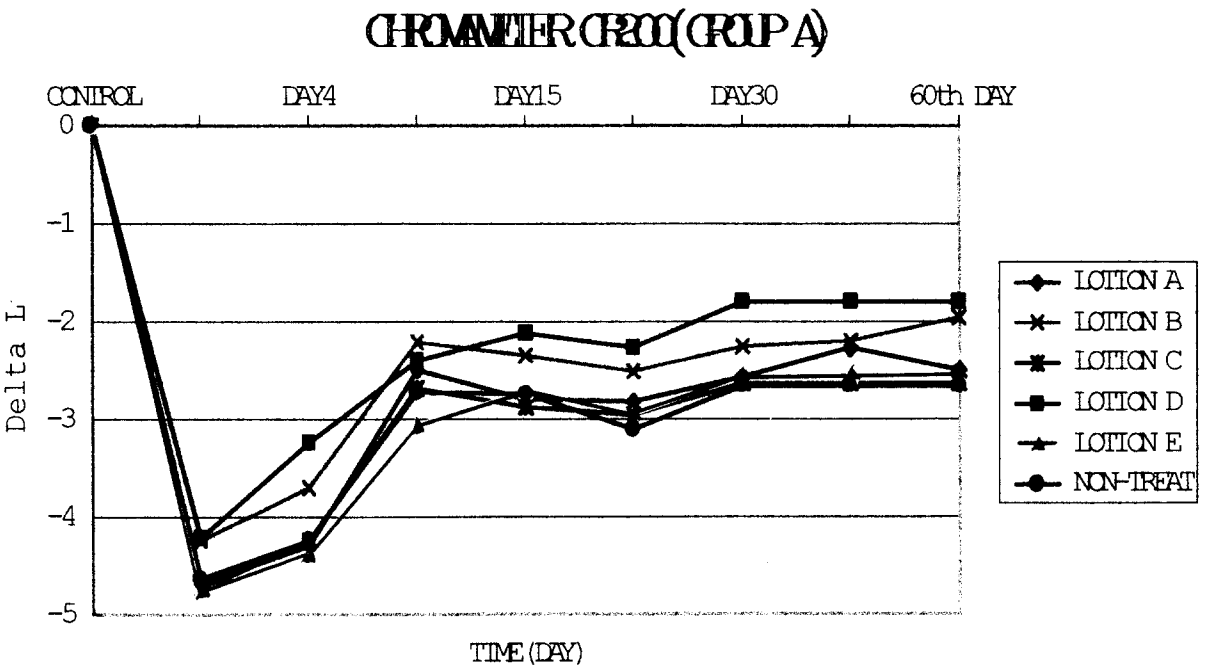
* : represents significance at 0.05 level

The result, that Kazinol F and hydroquinone brought down the erythema level, resulted in lower the pigmentation degree. Lotion B(Kazinol F) and lotion D(hydroquinone) treated areas showed low pigmentation level than other lotion treated areas in the test period(Fig. 3, 4).

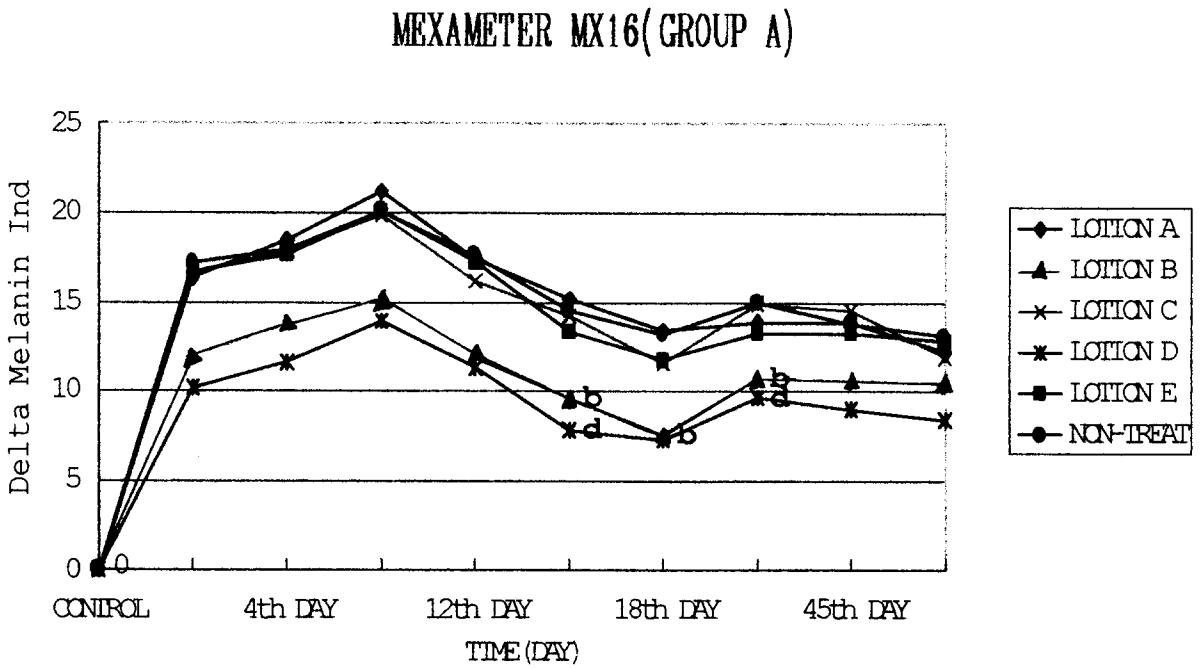
While, in Group B, statistically significant difference was not detected in the same period.

Fig. 3. Comparison of depigmentation effect.

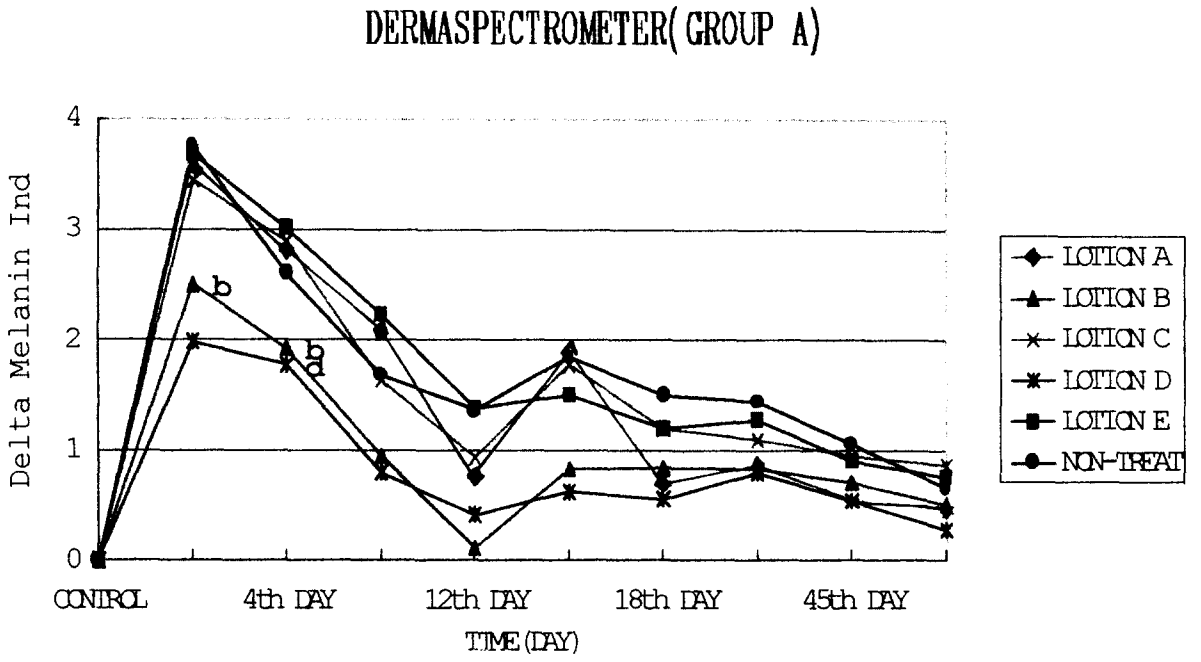
a) Chromameter CR200



b) Mexameter MX16



c) Dermaspectrometer



2. Colorimetry

In this study, Mexameter showed significant difference in skin darkness between lotion B treated areas and lotion E treated areas at 15th,18th and 30th day.
Dermaspectrometer showed significant difference between lotion B treated areas and lotion E treated areas at 1st day and 4th day but Chromameter and visual assessment didn't show any difference ($p<0.05$).(Table 1)

Table 1. Significance of difference between lotion B or D treated areas and lotion E treated areas

	DAY 1	DAY4	DAY8	DAY15	DAY18	DAY30	DAY60
Chromameter	-	-	-	-	-	-	-
Mexameter	-	-	-	b,d	b	b,d	-
Dermaspectrometer	b	b,d	-	-	-	-	-
Visual assessment	-	-	-	-	-	-	-

b; represents significant difference between lotion B treated areas and lotion E treated areas.
d; represents significant difference between lotion D treated areas and lotion E treated areas.
-; represents non-significant difference

Correlation of three instruments with visual assessment was shown in Table 2.

Table 2. Correlation of instruments and visual assessment.

	Chromameter	Mexameter	Derma-spectrometer	Visual assessment
Chromameter				
Mexameter	r=-0.2796 p=0.806			
Derma spectrometer	r=0.0856 p=0.000	r=0.0206 p=0.987		
Visual assessment	r=0.1364 p=0.288	r=0.0178 p=0.552	r=0.005 p=0.355	

* Spearman correlation (n=103)

Three colorimetries showed low correlation with visual assessment in this study. Chromameter showed higher correlation(r=0.1364, p=0.288) with the visual assessment than the other two colorimeters did. Mexameter and Chromameter showed higher correlation(r=-0.2796 p=0.806) compared with Dermaspectrometer vs Chromameter or Dermaspectrometer vs Mexameter.

Coefficient of variations of three colorimeters were summerized in Table 3.

Table 3. Coefficient of variation(SD/M) score of three colorimeters

	Chromameter	Mexameter	Derma-spectrometer	Visual assessment
SD/M	1.073	0.674	7.299	1.558

Mexameter gave less variation between the measurements and Dermaspectrometer gave greater variation.

Discussion

Recently, most of depigmenting materials incorporated in cosmetics were tyrosinase inhibitors. In this study, 24 participants were equally divided into two groups. Group A , 12 volunteers, was treated with 5 lotions for 3 days before UVB radiation. It takes at least 48 hours for the topically applied material to give cellular tyrosinase inhibition. So, pre-treatment of Group A for 3 days before UVB radiation would be sufficient for inhibiting tyrosinase activation. Group B to get treatment 3 days after UVB radiation. Inflammation induced by UV radiation starts within 24 hours and tanning starts within 3 days. So tyrosinase inhibitor treated 3 days after radiation would work against tanning process only. As a result, Kazinol F and hydroquinone in Group A not only brought down the erythema level at 24 hours after radiation but showed statistically significant depigmentation effect different from other materials. On the other hand, Group B has no significant difference among treated materials. From these results, we conclude that pre-treated Kazinol F and hydroquinone have dual functions; anti-erythema and tyrosinase inhibition. Kazinol F, if treated before UV radiation, inhibits inflammation and pigmentation, comparable to hydroquinone. According to the previous results, free radical scavenging activities of Kazinol F was relatively good compared with -tocopherol(5, 6). The result of safety test showed that Kazinol F has no primary irritation and sensitization potential(5, 6). In this study we confirmed the clinically significant depigmentation effect of Kazinol F. Kazinol F that has good safety, stability and powerful depigmentation effect is a desirable ingredient in whitening cosmetics. Depigmentation effect of Kazinol F depending on its anti-inflammatory fucntion remains to be investigated.

In the cosmetic field measuring skin color is an important tool for evaluating the efficacy of whitening products. Various types of colorimeters are introduced in the cosmetic field, but objective comparison of their accuracy and sensitivity was necessary to choose a proper instrument in each experimental design. In this study, we compared three commercial colorimeters (Chromameter CR200, Demaspectrometer, Mexameter MX16). Mexameter made the most significant difference between the test samples. The coefficient of variation(SD/M) score showed that Mexameter gave less variation between measurements. Gray scale measured by Chromameter was somewhat different from melanin index measured by Mexameter or Dermaspectrometer. With this result, we can conclude that Chromameter is good in its accuracy in general, but Mexameter better for the skin color measurement. Recently introduced in cosmetic field for measuring skin color, Mexameter has better resolution power and is easy to use. In this study, visual assessment doesn't make any significant difference. However, visual assessment is better for rough evaluation and instrumental measurement is appropriate for making quantifiable data.

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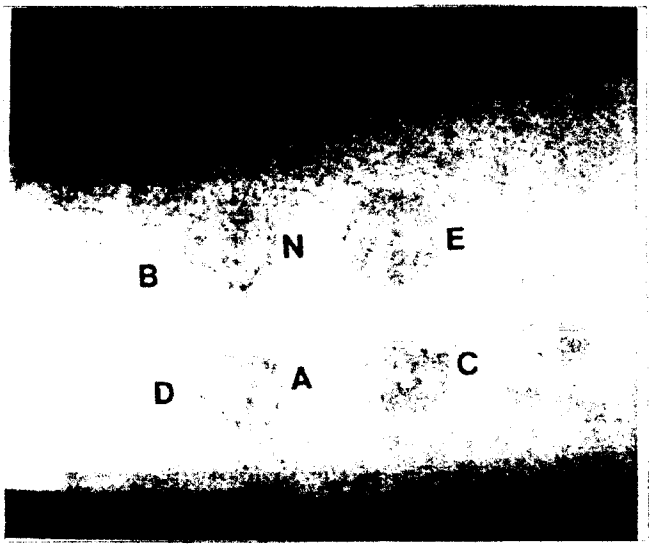
a)



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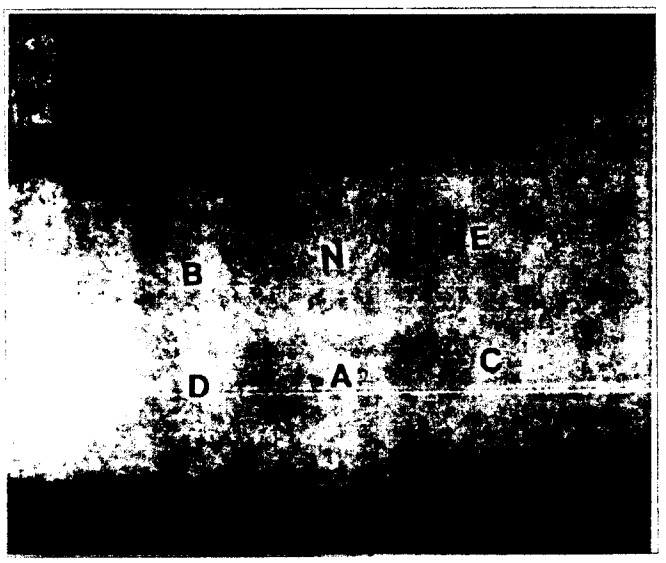

Fig 4. Clinical changes of treated areas.
a) 1 day after radiation b) 30 days after radiation
* A ; Lotion A (Kojic acid derivatives, B ; Lotion B(Kazinol F),
C ; Lotion C (Arbutin), D ; Lotion D(Hydroquinone),
E ; Lotion E(Cream Base) , N ; non-treat