The Effects of SCH-T2 Seaweed Extract on Melanogenesis

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Background : Melanocytes synthesize melanin pigment by the action of specific enzyme tyrosinase. Melanogenesis inhibitors such as ascorbic acid, kojic acid, arbutin, hydroquinone have been developed for use in cosmetic preparations for the skin hyperpigmentation, but they are still unsatisfactory to efficacy and tolerance.

Objective : In this study, the inhibitory effects of seaweed extract(SCH-T2) on tyrosinase activity and melanogenesis were investigated with B-16 melanoma cell line.

Methods : The seaweed was added with 21 organic solvents and extracted during 12 hours at 20-40℃. To evaluate the inhibitory effects of SCH-T2 on tyrosinase activity following to extraction time and temperature, this study was done at 4, 8, 12, 24, 72 hours and 4, 10, 20, 30, 40°℃. Also, the effects of whitening agents(kojic acid, arbutin, licorice extracts, SCH-T2) on tyrosinase activity were compared by measuring the IC50, the concentration of the compound at which half of the original tyrosinase activity is inhibited.

Results : 1. The inhibitory effects of SCH-T2 on tyrosinase activity were high at 60-80% ethanol as an extraction organic solvent and showed increase in proportion to concentration following to extract concentration, but showed little differences following to extraction time and temperature. 2. SCH-T2 has relatively stronger inhibitory effects on tyrosinase activity than arbutin and licorice extracts. 3. The inhibitory effects of SCH-T2 on melanogenesis following to extract concentration were greatly increased at more than 20 ㎍/ml.

Conclusion : This study showed that SCH-T2 seaweed extract has strong inhibitory effect on tyrosinase activity and melanogonesis. So, the results of this study suggest that SCH-T2 seaweed extract can be used as a new whitening agent. (Ann Dermatol 14(1) 1-5, 2002).

Key Words : SCH-T2, Tyrosinase, Melanogenesis

The skin color is classified into two groups; one is constitutive skin color which is determined on genetic & biochemical feature of melanocytes, and the other is facultative skin color which is determined on external stimuli such as UV irradiation. Generally sun protection factor(SPF) of epidermal melanin is about 3 and only this effect doesn't prevent skin damage or skin cancer production from UV light. Recently, industrial development has destroyed the ozone layer and economic development leads people to have outdoor vacations. As a result people are exposed to strong UV and consequently hyperpigmentation of the skin caused a very serious aesthetic problem to oriental people1. Even though the kojic acid2 and arbutin3 were the most famous and strong whitening materials, they are still unsatisfactory to efficacy and tolerance. Therefore the attempt to use plant materials was started.

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These plant materials, especially seaweed extracts were used for a long time and were thought to be safe for humans, and also their process of manufacture was safe and good enough, so recently they were highlighted as whitening materials and a lot of studies were done about them.

In this study, we investigated seaweed, especially Phyeophyta extracts living in the clean sea of southern Korea and Jaeju island and selected SCH-T2 among them. We studied the effects of SCH-T2 on tyrosinase activity and melanogenesis with B-16 melanoma cell line.

MATERIALS AND METHODS

1. Materials

1) Cell line and culture

Mouse-derived B-16 melanoma(ATCC CRL 6323) cell line was used for quantitative analysis of melanin. B-16 melanoma cell line was cultured in DMEM (Sigma Chem. Co. St. Louis, Mo., U.S.A.) supplemented with glucose 4.5 g/L, 10% fetal calf serum (FCS: Gibco, Grand Island, NY, U.S.A.), and 1% gentamicin (Sigma Chem. Co. St. Louis, Mo., U.S.A.). T-25 and T-75 tissue culture flask(Falcon Inc., Franklin Lakes, NJ, U.S.A.) were used as culture flask.

2) SCH-T2 Extraction

The Seaweed(Laminariaceae and Alariaceae) was collected from the clean sea of southern Korea and Jaeju island. The seaweed was washed with distilled water and dried in the shade. The dried seaweed was homogenized and added in 5 to 10 times with various concentrations of alcohol, acetone, ethylacetate, butylacetate, and chloroform etc. and extracted at 20°℃ for 12 hours(Table 1). The extracts were filtered with whatman No 5 and dried in the rotary vacuum evaporator at 65℃ and powdered. The powdered extracts(SCH-T2) were solubilized with 1,3 butylene glycol (50%; v/v), and were used as 1% of extract solution in this experiment.

2. Methods

The homogenized seaweed was added with 80% methanol(v/v) and extracted with the same condition described above for 4, 8, 12, 24 and 72 hours respectively. Also, the effects of extraction temperature of SCH-T2 were also investigated at 4, 10, 20, 30, and 40℃ respectively.

According to Vanny method, the test tubes were filled with 0.5 ㎖ of L-tyrosine solution(Sigma Chem. Co. St. Louis, Mo., U.S.A.) and 0.5 ㎖ of various concentrations(1, 2, 5, 10, 20, 100 ㎍/㎖) SCH-T2. And 0.5 ㎖ of tyrosinase(200 ㎍/㎖, Sigma Chem. Co. St. Louis, Mo., U.S.A.) was added to above solution and reacted at 37℃

$$\text{Tyrosinase activity inhibition (\%)} = 100 - \left(\frac{\text{absorbance of extract}}{\text{absorbance of control}} \times 100\right)$$

for 10 minutes. The test tubes of control were filled with buffer solution instead of SCH-T2. After 10 minutes, test tubes were moved into ice for stopping reaction. The inhibitory effects of SCH-T2 extract on tyrosinase activity were determined by measuring absorbance at 475 nm with spectrophotometer.

The effects of SCH-T2 on tyrosinase activity were compared with kojic acid, arbutin (Sigma Chem. Co. St. Louis, Mo., U.S.A.), and licorice extracts by measuring the IC50, concentrations of agents at which half of the standard tyrosinase activity is reduced.

To evaluate the inhibitory effects of SCH-T2 on melanogenesis, cultured melanoma cells were seeded in 50 ㎖ T-flask at density of 4.51×106 and cultured in 5% CO2 at 37℃. When cells were proliferated abundantly they were treated with B-16 melanoma cell line which was cultured in DMEM medium supplemented with optimum concentration of SCH-T2, and were cultured following 5 days. After 5 days, the culture medium was removed and cells were isolated with 0.05% trypsin-EDTA solution.

Table 1. The inhibitory effect of SCH-T2 on tyrosinase activity following to organic solvents which is uesd to extraction. Values are mean± SD.

Organic solvents		Tyrosinase inhibition(%)
1	H_2O	56.7 ± 1.2
2	10% Ethanol (V/V)	20.6 ± 0.9
3	20% Ethanol	24.3 ± 0.5
4	30% Ethanol	49.1 ± 0.9
5	40% Ethanol	64.9 ± 1.0
6	50% Ethanol	84.8 ± 0.7
7	60% Ethanol	92.1 ± 0.9
8	70% Ethanol	94.4 ± 0.9
9	80% Ethanol	91.5 ± 0.7
10	90% Ethanol	85.3 ± 1.6
11	100% Ethanol	84.2 ± 0.8
12	80% Methanol	90.3 ± 0.8
13	100% Methanol	85.3 ± 1.2
14	n-Propanol	78.4 ± 0.9
15	iso-Propanol	90.4 ± 0.8
16	2-Butanol	87.9 ± 0.5
17	Acetone	87.6 ± 0.6
18	Chloroform	48.3 ± 1.5
19	Ethylacetate	91.4 ± 1.8
20	Butylacetate	88.4 ± 0.8
21	1,3-Butylene glycol	66.4 ± 1.2

The isolated cells were buffered with 30% calf serum and centrifuged (1,000 rpm, 5 minutes) for separation of melanocytes. This process was duplicated at 0℃ and the obtained melanocytes were washed with buffer solution. Melanocytes were centrifuged 2 times in ether: ethanol solution(1:3, v/v) and washed with 1 ㎖ ether and then dried. The dried melanocytes were added to 1 ㎖ of 10% DMSO containing 1 N NaOH and then heated in the water bath(90℃) for 10 minutes. The inhibitory effects of melanogenesis following each extract concentration were determined by measuring absorbance at 475 nm with spectrophotometer.

$$\text{Melaninogenesis inhibition}(\%) = \frac{\text{absorbance of control} - \text{absorbance of treatment}}{\text{absorbance of control}} \times 100$$

Table 2. The inhibitory effect of SCH-T2 on tyrosinase activity following extraction time. Values are mean±SD.

Extraction time	Yield(%) SCH-T2	inhibition(%)-50 ㎍/ml Tyrosinase
4hrs	7.8 ± 0.5%	85.4 ± 0.8%
8hrs	10.2 ±1.1%	87.7 ± 1.3%
12hrs	11.7 ± 1.0%	90.1 ± 0.7%
24hrs	11.9 ± 0.8%	92.2 ± 1.5%
72hrs	12.8 ± 1.3%	91.7 ± 1.0%

Also melanin concentration was determined from the standard concentration curve of synthetic melanin(Sigma Chem.. Co. St. Louis, MO., U.S.A.).

All experiments were evaluated with 5 samples of SCH-T2, kojic acid, arbutin and licorice extracts.

RESULTS

Table 3. The inhibitory effect of SCH-T2 on tyrosinase activity following extraction temperature. Values are mean ± SD.

Extraction temperature	SCH-T2 Yield(%)	Tyrosinase inhibition(%)-50 ㎍/ml
4℃	8.5 ± 0.7%	94.1 ± 0.7%
10℃	9.5 ± 0.6%	92.5 ± 0.9%
20℃	11.7 ± 1.0%	90.1 ± 1.6%
30℃	12.5 ± 1.3%	91.4 ± 1.2%
40℃	15.1 ± 2.1%	82.7 ± 1.0%

1. The inhibitory effects of SCH-T2 extracted with organic solvents on tyrosinase activity were investigated(Table 1). The final concentration of SCH-T2 was 50 ㎍/㎖ and among them, 60-80% ethanol showed higher effects than others.

2. The inhibitory effects of SCH-T2 on tyrosinase activity following to extraction time were investigated with powder yields and inhibition of tyrosinase activity(Table 2). The yields of SCH-T2 were increased as

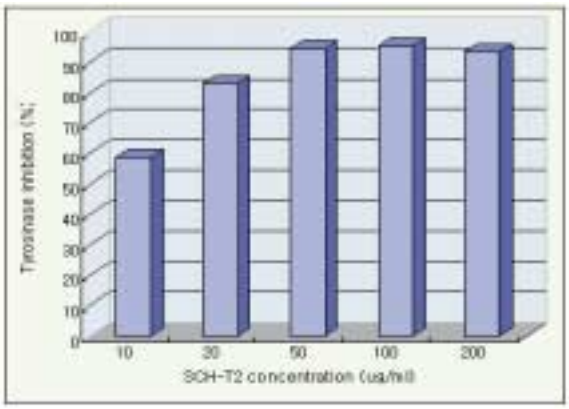

Fig. 1. The inhibitory effect of SCH-T2 on tyrosinase activity following extract concentration.

extraction time increased until 12 hours, but longer than 12 hours showed almost similar results. So, the inhibitory effect of SCH-T2 on tyrosinase activity showed no significant differences among extraction times.

3. The inhibitory effects of SCH-T2 on tyrosinase activity following to extraction temperature were investigated with powder yields and inhibition of tyrosinase activity were investigated(Table 3). The results fol-

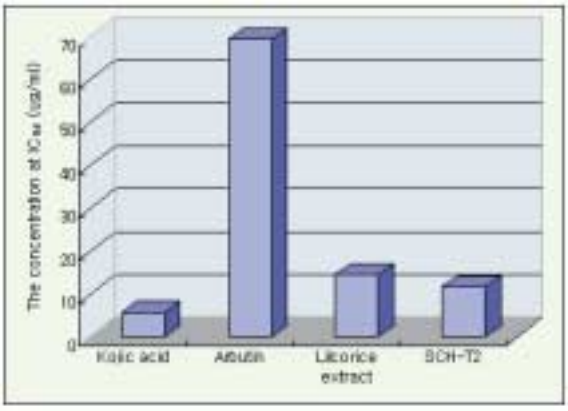

Fig. 2. The concentration required for selected tyrosinase inhibitors to reduce tyrosinase activity by 50%(IC $_{50}$).

lowing to extraction temperature showed no significant differences but showed much decrease at 40℃.

4. The inhibitory effects of SCH-T2 on tyrosinase activity following to 10 to 100 ㎍/㎖ of extract concentration were increased in

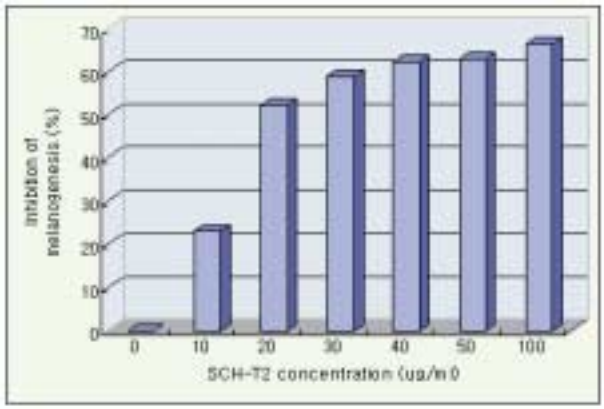

Fig. 3. The inhibitory effect of SCH-T2 on melanogenesis following extract concentration.

proportion to concentration from 58.7 ± 0.9% to 95.2 ± 1.2% and then decreased a little at 200 ㎍/㎖(Fig. 1). The SCH-T2 was solubilized with 70% ethanol(v/v) in this experiment.

5. Comparing with the IC50, concentrations of agents at which half of standard tyrosinase activity is inhibited, the effects of whitening agents on tyrosinase activity showed 5.88 ± 0.8 ㎍/㎖(Kojic acid), 11.68 ± 0.6 ㎍/㎖(SCH-T2), 14.62 ± 1.0 ㎍/㎖ (licorice extracts), and 69.5 ± 0.9 ㎍/㎖ (arbutin). The SCH-T2 showed significant stronger effect than that of arbutin on tyrosinase activity but showed less inhibitory effect than kojic acid. Also, licorice extract showed no significant difference with SCH-T2(Fig. 2).

6. The inhibitory effects of SCH-T2 on melanogenesis were investigated with culture media containing 0, 10, 20, 30, 40, 50 and 100 ㎍/㎖ concentrations of SCH-T2. More than 50% inhibitory effect of SCH-T2 on melanogenesis appeared in all concentrations execept 10 ㎍/㎖(Fig. 3).

DISCUSSION

Normal human skin color is determined by exogenous factors like carotene which is deposited in epidermis, and endogenous factors like hemoglobin in dermal vessels, thickness of

horny layer and epidermal melanin. The major determinant of differences in skin color is melanin4. The melanin pigmentary system is composed by melanocytes which are distributed in various sites; skin, hair, eye, ear, and central nervous system. The melanin is inside a unit membrane and is deposited on an internal filamentouse and/or microvesicular matrix. The melanin pigments are made up of tyrosine which combines with cysteine to form cysteinyl dopa; this leads to yellow pigment. The tyrosine is converted to dopaquinone by an aerobic copper-containing oxidase, named tyrosinase5. Melanin is an important defense of human skin against the harmful effect of UV light6 due to its ability to absorb and reflect UV energy, and its ability to scavenge oxidative free radicals7,8. Pigmentation in humans is also an important cosmetic role which can be compromised in certain hyperpigmentary skin condition and/or in lesions such as melasma, age spots, and postinflammatory hyperpigmentation8,9. Ascorbic acid10, Kojic acid, arbutin, hydroquinone11, and licorice extracts11 were used to inhibit melanin production in skin for whitening agents. Kojic acid has strong inhibitory action to tyrosinase by chelating copper ion of tyrosinase, but it is not useful for whitening agent because it is unstable during getting mixed with base. Ascorbic acid is not suitable for whitening agent because of relatively low effect for inhibition of tyrosinase activity and less molecular safety. Also hydroquinone inhibits the tyrosinase activity powerfully but has a defect which irritates skin. To solve these problems including the excellent whitening effect, tolerance to human and material safety during manufacture process, various studies have been investigated.

Sea has plentiful natural resources, which supply the seaweed, fish, olein, and minerals to human beings. Korean peninsula has lots of seaweed such as laver, brown seaweed, and kelp. Seaweed has chlorophyll, and also has blue to red pigments. Blue-green algae(Cyanophyta), green algae(Chlorophyta), brown algae(Phaeophyta), and red algae(Rhodophyta) live worldwide according to climate and environmental conditions12. Brown algae is the biggest one among them and has fewer species than that of red algae, but can thrive in sea of most Temperate zone and Atlantic Ocean. Phaeophyta include Alariaceae and laminariaceae. Laminariaceae include Costaria costata, Agarum cribrosum, and Kjellmaniella crassifolia which are found in East sea. But they can be cultured in Southern sea of Korea by gametophyte culture even though they like to live in sub arctic sea. Alariaceae include Undaria which are only found in Korea and Japan sea. U. pinnatifida are found in all of Korea sea and U. peterseniana only in Jaeju sea12,13,14. The extracts of this seaweed are already used for materials of cosmetics and are also excellent for safety to human and manufacture process. Recently new materials for whitening agents are necessary for cosmetics, and so many extracts of seaweed are investigated for the whitening effect and cosmetic ingredient.

In this study, we investigated the inhibitory effects of seaweed, especially brown algae which live in clean sea of Southern Korea and Jaeju island, on tyrosinase activity and then selected the best one, named SCH-T2. And then we studied the inhibitory effects of tyrosinase activity and melanin synthesis following to organic solvents, extraction time and temperature, and concentration of SCH-T2.

In conclusion, SCH-T2 seaweed extract has excellent inhibitory effects on tyrosinase activity and melanin synthesis. So, we suggest that SCH-T2 seaweed extract can be used for treatment of hyperpigmentation and as a new whitening agent.

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