Brazilin as a new sunless tanning agent

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Abstract

To develop an active material for skin darkening (tanning), we examined the effect of 300 plants on tyrosinase activity and found only *Caesalpinia sappan* has an ability to increase tyrosinase activity highly and melanin contents in B-16 melanoma cells. A compound increasing tyrosinase activity and melanin production was isolated from *Caesalpinia sappan* Lignum. Brazilin was identified as a new active agent. Brazilin increases the tyrosinase activity and melanin production of B-16 melanoma cells. In conclusion, it seems that brazilin can be used as a new sunless tanning agent.

Introduction

In Europe, unlike East Asia, well-tanned skin is considered as a sign of activity, fitness, youth and health. So many women want to tan their skin in spite of solar's danger. Natural tanning of the skin means the increase of melanin, the skin's natural pigment. Melanin is a protective dark pigment which gives the skin its color and can be increased by UV exposure in nature. Melanin synthesis is a chemical reaction of the skin which protects itself and thus gets adapted naturally to the sun. But it is very harmful to expose one's skin to UV radiation because UV can penetrate one's skin, reach the dermis and cause skin damage like skin cancer or skin aging. In these days, natural tanning materials that do not damage skin and increase melanin contents of the skin have been researched and developed for cosmetic purposes. Generally, dihydroxyacetone (DHA) has been used for producing a simulated suntan on the skin (3). It can react with the amino acids in the skin and with the keratin itself, to change the skin color. But skin color from tanning products containing DHA cannot last for a long time (1). Meanwhile, Gilchrest et al have reported that topical application of diacylglycerol (DAG) to animal skin produces long-lasting pigmentation that is clinically and histologically indistinguishable from UV-induced tanning (2). Also, they reported that DNA can enhance tanning (4).

Using in vitro tests, we have examined whether plant extracts can have tanning effects on the skin and found that *Caesalpinia sappan* extracts increase tyrosinase activity and melanin contents in B-16 melanoma cells.

In this paper, we report that *C. sappan* increases the tyrosinase activity and melanin production of B-16 melanoma cells and brazilin isolated from *C. sappan* extracts show the same effects.

Materials and Methods

1. Preparation of Plant Extracts

Extraction by various solvents: *Caesalpinia sappan* is found in Korea. We prepared *C. sappan* lignum by cutting and pulverization. It was immersed in various solvents (ethanol, methanol, propanol, butanol, acetone, water, butyl acetate, isopropanol) for 5-7 days. After removing the lignum, filtrate was concentrated under reduced pressure and the remaining extracts were dissolved in propylene glycol.

Extraction by ethanol: Dried lignums were extracted with 70% ethanol and concentrated to dryness in a vacuum evaporator. The ethanol extracts were dissolved in propylene glycol.

2. Tyrosinase Activity Tests

To test tyrosinase activity, spectrophotometric analysis is used. Total volume of reaction mixtures was made to 1.5. It consists of 0.5 of 0.1 mg/mL L-tyrosine(Sigma Chemical Co. Ltd.), 0.5 of 200 Units mushroom tyrosinase (Sigma), and sample solutions up to 0.5 or 0.5 of 0.05 M sodium phosphate buffer (pH 6.8).

Then, the reactants are incubated for 10 min at 37°C. After incubation, the enzyme activity was measured by spectrophotometer at 475 nm. Increasing enzyme activity of the sample was calculated as follows

% increasing activity (%)= {(B-A)/A}100
A: Absorbance at 475 nm without test sample after incubation.
B: Absorbance at 475 nm with test sample after incubation.

3. Cell Culture and Melanin assay

B-16 melanoma cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Sigma Chemical Co. Ltd.) supplemented with 10% fetal bovine serum (FBS). After 24 hrs cultivation, the medium was replaced with fresh medium and *C. sappan* extracts at various concentration were added to cultured cells and the cells were incubated for 2 days.

The cells were harvested with trypsinization and pelleted by centrifugation. At the same time, the MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed to estimate cytotoxicity of the tested plant extracts.

The melanin content in B-16 melanoma cells was quantified with the following procedure. The cells were washed with phosphate buffer saline (PBS), then collected by trypsinization and centrifugation. The pellets were treated with 5% trichloroacetic acid and solubilized in 1N NaOH. The melanin contents were quantified by measuring absorbance at 475 nm. A standard curve for melanin determination was prepared using synthetic melanin (Sigma Chemical Co Ltd.)

4. Brazilin isolated from *C. sappan* extracts.

Brazilin was purified from the *C. sappan* extracts (5) and tyrosinase activity test and melanin assay are performed. Kojic acid is used to compare with brazilin.

Results and Discussion.

1. Effect of *C. sappan* on Tyrosinase Activity

C. sappan extracts by various solvents were prepared and tyrosinase activity test was perfomed. *C. sappan* extracts by ethanol increase tyrosinase activity highly. It means ethanol is the most suitable solvent for extraction of *C. sappan* (Figure 1).

To determine the extraction system, we carried out extraction by various ethanol concentration from 10% to 100% (v/v), extraction time, extraction temperature and concentration system. So 70% ethanol is determined as extraction solvent (Data not shown).

Tyrosinase activities were increased as increasing the concentration of *C. sappan* (Figure 2). *C. sappan* increases highly tyrosinase activity above a concentration of 0.4 mg/mL. These results suggest that *C. sappan* directly activate tyrosinase and show the possibility of its tanning agent.

2. Kinetic Studies of Tyrosinase Using *C. sappan*

To analyze the effect of *C. sappan* concentration on the tyrosinase-catalyzed reaction, we examined the kinetic studies of tyrosinase. The result is shown in Figure 3. In kinetic diagram, tyrosinase reactions activated by *C. sappan* show hyperbolic pattern. In the diagram of tyrosinase activity versus *C. sappan* concentration (Data not shown), the relationship is linear. The rate of the tyrosinase-catalyzed reaction increases gradually with increasing *C. sappan* concentration. It means *C. sappan* is an active agent that directly activates tyrosinase (Figure 3).

3. Effect of *C. sappan* on Melanogenesis

We examined the effect of *C. sappan* on melanogenesis in B-16 melanoma cells. In this experiment, *C. sappan* showed a potent enhancing effect. The effect of *C. sappan* on melanogenesis in B-16 melanoma cells is shown in Figure 4.

C. sappan above a concentration of 0.1 mg/mL had an enhancing effect on melanogenesis. *C. sappan* showed cytotoxicitic effect above a concentration of 0.2 mg/mL.

We quantitatively examined the effect of *C. sappan* on melanogenesis. *C. sappan* increased the melanin production in B-16 melanoma cells. At a concentration of 0.6 mg/mL, *C. sappan* increased the melanin content to 191.2 ± 10.6% compared those of control cells (Fig. 4)

4. Effect of Brazilin on Tyrosinase Activity and Melanogenesis.

We isolated brazilin from *C. sappan* according to the method of Perkin and Robonson (5) and examined the effect of brazilin on tyrosinase activity (Figure 5). Brazilin increased tyrosinase activity at all testing concentration. Brazilin is more potent enhancing effect than *C. sappan* extracts. To confirm the effect of brazilin, kojic acid was used. Figure 5 shows that brazilin increases tyrosinase activity but kojic acid inhibits.

The examination of the effect of brazilin on melanogenesis in B-16 melnoma cells was performed. The result is shown in Figure 6. Brazilin had an enhancing effect on melanogenesis at all testing concentration. At a concentration of 10.0 mg/mL, brazilin increased melanin content to 271.4 ± 15.3% compared to those of control cells.

In these experimental conditons, brazilin had low cytotoxicity.

Conclusion

C. sappan and brazilin increase tyrosinase activity and have potent enhancing effect on melanogenesis in B-16 melanoma cells. From these results, *C. sappan* and brazilin can be used as a new type of pigmentation (or tanning) agent from natural origin.

References

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3. K Laden et al, *J Soc Cosmet Chem* **16**, 777-782 (1982)
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5. WH Perkin and R Robonson, *J Chem Soc* **91**, 1073 (1907)

Figure 1. The influence of various solvents system on tyrosinase activity of Caesalpinia sappan.

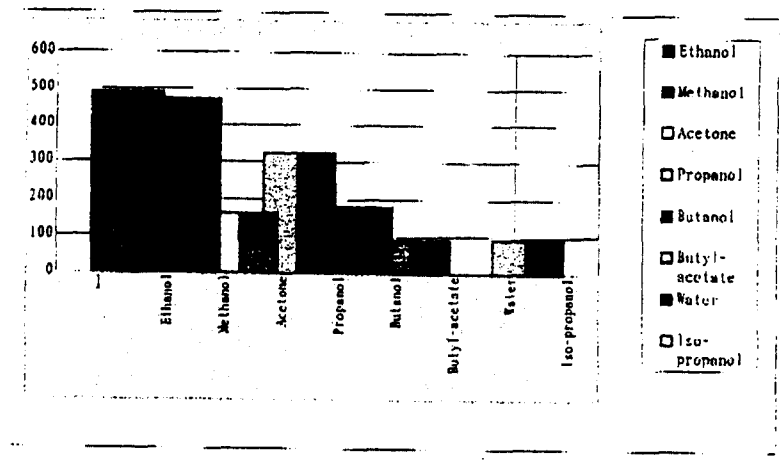

Figure 2. The effects of *C. sappan* extracts on tyrosinase activity.

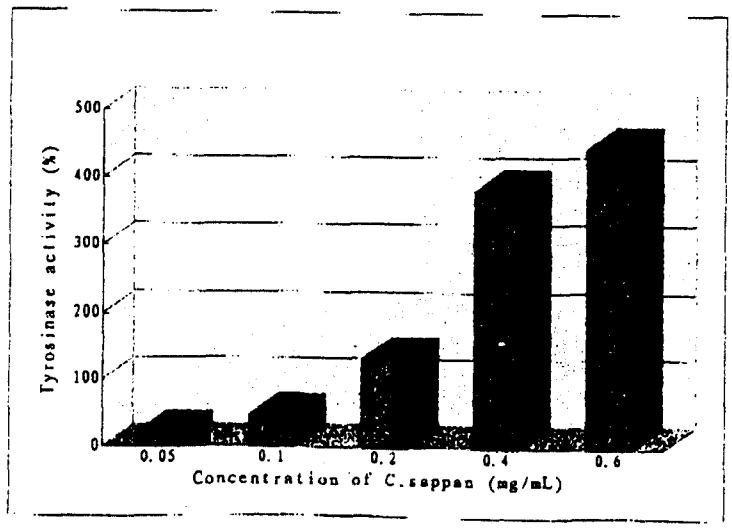

Figure 3. Kinetic study of tyrosinase activity by *C. sappan*.

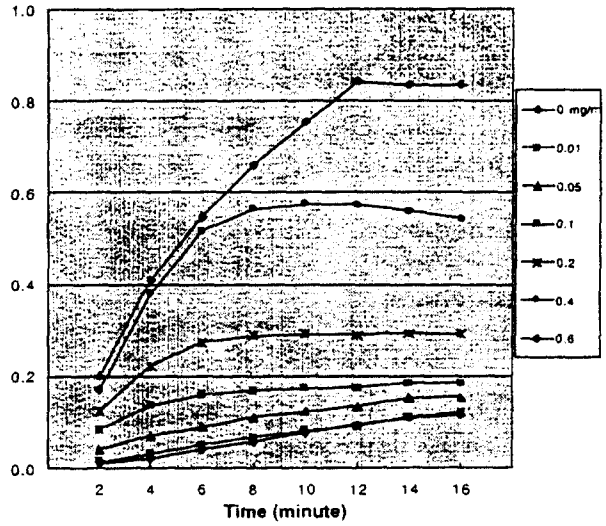

Figure 4. Effect of *C. sappan* on melanin contents in B-16 melanoma cells

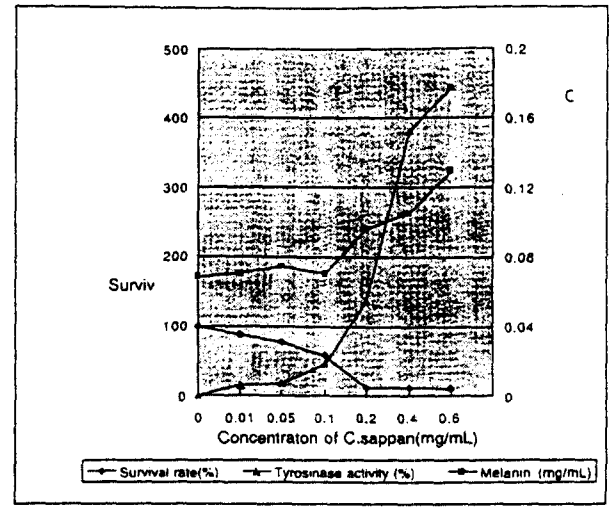

Figure 5. Effect of brazilin on tyrosinase activity.

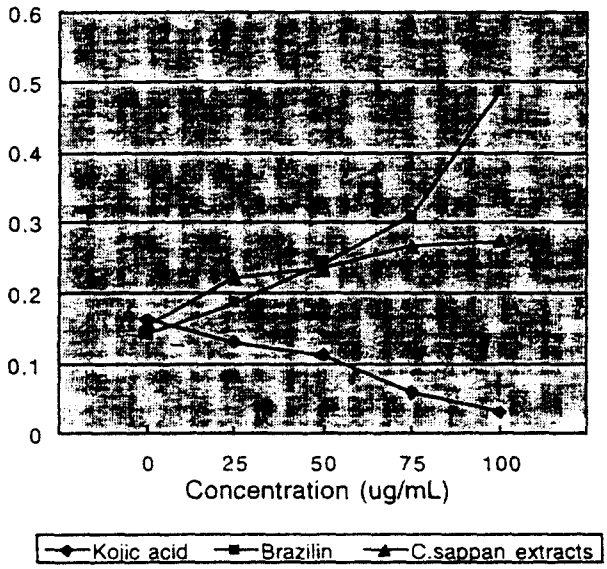

Figure 6. Effect of brazilin on Melanin contents in B-16 melanoma cells