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Anti-tyrosinase activity of orange peel extract and cosmetic formulation

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Abstract

The aims of this study were to determine anti-tyrosinase activity of ethanolic extract of orange peel, then develop the whitening cream from the extract and finally evaluate the satisfaction by users. The results showed that ethanol crude extracted had anti-tyrosinase activity with the IC_{50} of 255.10 µg/ml, which was less effective than kojic acid (23 times). Whitening cream containing orange peel extract 2% w/w was then formulated and tested for the satisfaction among 20 volunteers for 1 month compared with cream base. The results revealed that volunteers satisfy the properties of orange peel cream, i.e., texture, spread ability, absorption, and moisturization at high level, while odor properties was moderate satisfaction level. The average point of overall preference was high. After using orange peel cream for 1 month, the whitening improvement of volunteers' skin color had been observed by skin color bar. Skin melanin content was measured before application of creams and then 1 month after using by Mexameter®. It was found that orange peel cream could reduce melanin pigment 17.33%. In addition, none of the volunteers developed irritation during the test period. It can be concluded that California Navel orange peel extract can be applied in cosmetic industry to increase the value of orange peel waste.

Introduction

Skin whitening products are commercially available for cosmetic purposes in order to obtain a lighter skin appearance. They are also utilized for clinical treatment of skin pigmentation disorders such as melasma and post-inflammatory hyperpigmentation. Hyperpigmentation (dark skin areas) is characterized by an increased production and accumulation of melanins or an increased number of melanocytes. Melanins are dark colored pigments produced by melanocytes. Whitening agents act at various levels of melanogenesis (melanin production) in the skin. Many of them are known as competitive inhibitors of tyrosinase. Tyrosinase is the enzyme involved in melanogenesis and catalyzes the oxidation process of tyrosine to dihydroxyphenylalanine (DOPA) and from DOPA to DOPA quinone. Some whitening agents inhibit the maturation of this enzyme or the transport of pigment granules (melanosomes) from melanocytes to surrounding keratinocytes. Whitening agent such as hydroquinone, arbutin, kojic acid and azelaic acid are widely used in cosmetic products as active substances (Prota 1996; Mora and Baradi 2000; Nakayama *et al*., 2000; Petit and Pierard 2003; Ozer *et al*., 2007). In addition, many plant extracts have a good inhibitory effect on melanin formation such as *Morus alba* L. (Moraceae) or *Glycyrrhiza glabra* Linneva. (Leguminosae) have been used as whitening agents (Lee *et al*., 1997; Bernard and Berthon 2000; Baurin *et al*., 2002) with relatively fewer side effects (Kim and Lee 1998). Orange juice is one of the most widely-consumed beverages today and the cultivation of oranges has become a major industry in Thailand. Seventy percent of orange is used for manufacturing orange products and produced 50-60% of orange peel waste (peel, seeds and membrane residue) (Wilkins *et al*., 2007). The previous studies found that citrus peel *(Citrus unshiu)* have antioxidant and anti-tyrosinase activity (Sang-Suk *et al*., 2008). Moreover, it was found that the active compound of citrus peel was nobiletin which act as a tyrosinase inhibitor (Sasaki and Yoshizaki, 2002). It may suggest that the peel of Citrus fruit could be a new source in the field of hyperpigmentation, with potential as a skin whitening cosmetic material (Kenroh and Fumihiko, 2001). This study, the California Navel orange peel (*Citrus sinesis* L.) was collected from orange juice shop in CentralPlaza, Chiang Rai (Chiang Rai, Thailand). The orange peel was the waste from the process of orange juice. The aims of this study were to determine anti-tyrosinase activity of ethanolic extract of orange peel, then develop the whitening cream from the extract and evaluate the satisfaction by users.

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Table 1. The composition of cream base and cream containing orange peel extract

Ingredients	Cream base (g)	Orange peel Cream (g)
Emulbase® (Polyacylamide and Laureth-7)	5	5
Creamaflow® (Hydrogenate polydecene)	5	5
Orange peel extracts (Active)	-	2
Propylene glycol	2.5	2.5
Glycerol	2.5	2.5
Preservative (Conc paraben)	0.5	0.5
Water	84.5	82.5

Materials and Methods

Plant material

California Navel orange peel (*Citus sinesi*s L.) was the waste derived from orange juice process. It was collected from fruit juice shop at CentralPlaza, Chiang Rai during August-September 2012.

Orange peel extraction

Orange peel was cut into small pieces and dried in hot air oven at 50°C for 48 hours. Dried orange peel was soaked in 95% ethanol in the ratio of 1:3 and incubated in incubator shaker at 25°C, 150 rpm for 72 hours then filtered through Whatman® No.1. The filtrate was evaporated under pressure by rotary evaporator to yield crude extract and kept in -4°C refrigerator until used.

Anti-tyrosinase activity assay

Anti-tyrosinase activity of orange peel extract was determined using L-Dopa as substrate and kojic acid as standard according to the modified method of Chang *et al*., 2008. One KU (1000 unit/ml) mushroom tyrosinase was pre-incubated with kojic acid (or orange peel extract) at various concentrations in 50 mM phosphate buffer pH 6.8 (total volume of each reaction mixture tube was 1.80 ml) at 37°C for 5 min. L-Dopa (25 mM, 0.20 ml) was added to the reaction mixture and incubated at 37°C for 10 min. The enzyme reaction was measured for formation of DOPA chrome by measuring absorbance at 475 nm. The percent inhibition of tyrosinase activity was calculated by using the equation of % inhibition = $(A_{475}$ control - A_{475} sample)/ A_{475} control x 100. Where, A_{475}control is the absorbance of the control solution without kojic acid (or orange peel extract) and A_{475} sample is the absorbance of solution with kojic acid (or orange peel extract). The result was expressed as the half maximal inhibitory concentration (IC_{50}) of orange peel extract compared with kojic acid. IC_{50} represents the concentration of extract (or kojic acid) that is required for 50% inhibition of tyrosinase activity.

Formulation of whitening cream containing orange peel extract

The composition of cream base and cream containing orange peel extract were shown in Table 1. For cream base preparation, Emulbase® and Cremaflow® were mixed together with stirring. Propylene glycol and glycerol were added into the mixture and mixed well. Water was slowly added with continuous stirring then preservative and color (FD&C yellow no.5 and red no.3) were added. The preparation of cream containing orange peel extract was the same as cream base preparation but 2% w/w orange peel extract was additionally added in the formula. Prepared creams were stored at the room temperature during tested.

Subjects

Twenty female volunteers were used in this study. They were 21-23 years old and studied in major Beauty Technology, School of Cosmetic Science, Mae Fah Luang University, Chiang Rai.

Skin irritation test

To assess the skin irritation, the closed patch test was performed. The test materials (cream base, orange peel cream, orange peel extract, and DI water) were put with a sufficient amount on each aluminum disk and occluded on the back of volunteers. After occluded for 24 hours, the patches were opened and observed for any signs of skin irritation.

User satisfaction test

Cream containing orange peel extract was tested by 20 volunteers for their satisfaction compared

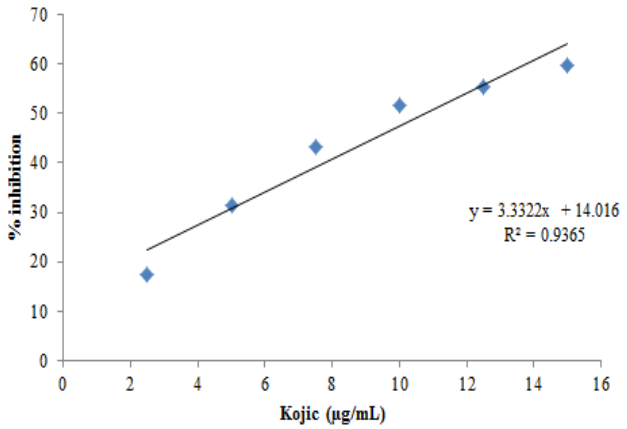

Figure 1. Tyrosinase inhibitory activity of kojic acid at different concentration

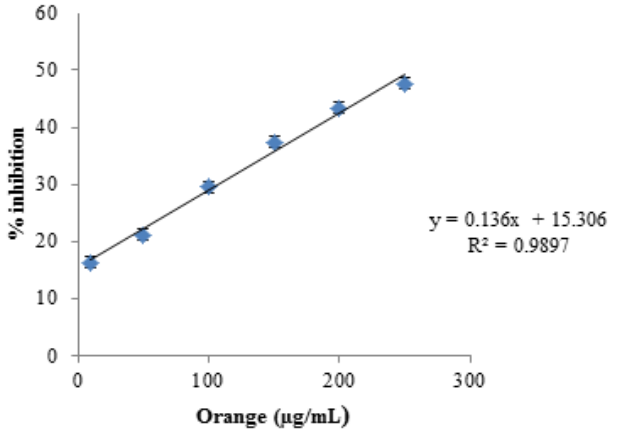

Figure 2. Tyrosinase inhibitory activity of orange peel extract at different concentration

with cream base using randomized controlled trial. Volunteers were asked to apply cream base on left leg and orange peel cream on right leg twice daily for 1 mouth. The satisfactions of volunteers were determined by using 5-point Likert scale questionnaire. The satisfaction scores were calculated as class intervals to classify 5 levels of satisfaction: very high (score 4.21-5.00), high (score 3.41-4.20), medium (score 2.61-3.40), low (score 1.81-2.60) and very low (score 1.00-1.80).

The improvement of skin color level

The level of the skin color of each volunteer was measured by compared with skin color bar (modified from von Luschan's chromatic scale) before using cream and after 1 month of application.

Efficacy test using Mexameter®

The amount of melanin pigment in skin was measured before using cream and after 1 month of application by Mexameter® MX18, Courage-Khazaka, Germany. The device based on absorption and reflection of 3 specific wavelengths (green: λ = 568 nm, red: λ = 660 nm, infrared: λ = 870 nm). As the quantity of emitted light is defined, the quantity of light absorbed by the skin can be calculated as the amount of melanin pigment.

Results and Discussion

Anti-tyrosinase activity assay

The orange peel crude extract was obtained with the yield of 16.00±1.15%. The orange peel extract was examined for anti-tyrosinase activity compared with kojic acid. IC_{50} of kojic acid was 10.80 µg/ml (Figure 1) whereas IC_{50} of orange peel extract was 255.10 µg/ml (Figure 2) which was less effective than kojic acid (23.62 times). The previous study found that peel of Citrus fruit as a by-product of the citrus juice industry contains a large amount of flavonoids (Sasaki and Yoshizaki, 2002). Some of the flavonoids were identified as tyrosinase inhibitors, including nobiletin (3',4',5,6,7,8-hexamethoxyflavone), naringin (5,7,4'-tyihydroxyflava-none), and neohesperidin (5,7,3'-trihydroxy-4'-methoxyflavone) but the inhibitory strength of the three inhibitors was found to be poorly activity toward mushroom tyrosinase compared with kojic acid (Zhang *et al*., 2007; Itoh *et al*., 2009).

Irritation test

Before new skin care products and ingredients are introduced into the market, the testing for potential adverse skin effects (irritation and allergy) is essential. This dermatological test for irritation effect on human was performed to ensure consumer safety. The result was found that all test materials (cream base, orange peel cream, orange peel extract, and DI water) did not induce skin irritation under the test condition. In addition, none of the volunteers developed irritation during the test period. It can be concluded that both creams were not likely to induce skin irritation under normal condition of use.

User satisfaction test

Twenty volunteers were asked to answer the questionnaire for evaluate their satisfaction on both creams after used. The satisfactions of volunteers were determined by 5-point Likert scale and were showed in Table 2. For physical appearance, the volunteers were satisfied the texture of both cream at high level whereas the odor of both creams were at medium level satisfaction. However, their overall preference on both cream were at high level. For feeling during use, the volunteers were satisfied spread ability of both creams at very high level. In addition, they were satisfied the absorbable and moisturized feeling of both creams at high level. In

summary, the volunteers were satisfied both creams at high level. In addition, there were no changes in appearance of both creams during 1 month tested. For product improvement, fragrance might be added for better odor of cream.

The improvement of skin color level

The improvement of skin color was measured using skin color bar (modified from von Luschan's chromatic scale) before and after 1 month of cream application. The scores were calculated by the improvement of skin color level. The results were indicated that orange peel cream had effect on the improvement of skin color (different level = 1.15±0.93) more than cream base (different level = 0.70±0.64). In conclusion, orange peel cream had more whitening effect than cream base and most volunteers (80%) were satisfied on whitening property of orange peel cream more than cream base.

Efficacy test using Mexameter®

The effect of both creams on the production of skin melanin was examined. Skin melanin content was measured before and then 1 month after using creams by Mexameter®. The results were expressed in arbitrary units and shown that both creams were effect on melanin amount on different extent. The skin melanin content after 1 month of cream base using was reduced from 182±1.7 to 162.4±1.7 (10.77%), while orange peel cream using was reduced melanin content from 172±0.9 to 142.2±0.8 (17.33%). According to the results, it can be concluded that orange peel cream had whitening property more than control cream.

Conclusion

In the present study, the orange peel (California Navel orange peel) crude extract possessed anti-tyrosinase activity and skin whitening property. This research provided to increase the value of orange peel from the wastes after juice extraction. However, further research need to be elucidated for active compound in California Navel orange which may suggest the California Navel orange peel extract could be a new source as the active ingredient for whitening agent in cosmetic products.

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