

Original Research Article

Splash mask formulation of tangerine (*Citrus reticulata Blanco.*) peel extract and turmeric (*Curcuma longa L*) extract as a whitening agent

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ABSTRACT

Background: Indonesia is a tropical country that is exposed to the sun for 12 hours, one of the harm effects of sunlight which is damage to the skin. The purpose of this study was to determine tyrosinase inhibition of tangerin peel extract (*Citrus reticulata Blanco.*) from Garut, West Java, Indonesia then develop the splash mask from the extract.

Methods: The tangerine peel will be extracted by maceration method. The splash mask formulation containing tangerine peel extract which has a whitening agent. Tyrosinase inhibition determine by ELISA method with tyrosinase enzyme and L-DOPA substrat.

Results: The results showed that tangerine peel extract had tyrosinase inhibition with the IC50 of 30.000 ppm, which has less effective than kojic acid with IC50 81,10 ppm. Splash mask containing of 1000x IC50, evaluation carried out for 28 days showed that preparations made pharmaceutically stable.

Conclusions: In addition, the whitening agent of tangerine peel extract is in the weak category. It is hoped that this research can increase the value of tangerine peel waste.

Keywords: Splash mask, Tangerines peel extract, Tyrosinase, Whitening agent

INTRODUCTION

Hyperpigmentation is a skin problem caused by an increase in skin pigment. Increased melanin synthesis locally or uneven distribution of melanin which can cause local pigmentation or dark spots.¹⁻³ Melanin in the skin serves to absorb exposure to ultraviolet light, bind to free radicals produced in the cytoplasm, and protect the body from radiation exposure. Melanin as one of the dominant pigment components of skin color, plays a role in determining skin color variations between individuals. Melanin synthesis process occurs in melanosomes that are inside melanocytes. Melanosomes then move towards keratinocytes through dendrites. In keratinocytes, melanosomes release melanin, which is then distributed in epidermal keratinocytes.¹

In addition to turmeric, tangerines are widely used by people as a whitener because they contain large amounts of vitamin C, where vitamin C is a whitening agent with a mechanism of action that is exfoliation.⁴⁻⁸ It is hoped that the combination of these two products can increase the effectiveness in inhibiting the tyrosinase enzyme which plays a role in melanin synthesis. In addition, the Splash Mask formulation is expected to be able to maximize this effect, because the Splash Mask formulation is made with a concentrate system, where both ingredients are added to the solvent until the condition is saturated. With the high concentration of the splash mask, how to use it by diluting it in water.⁹

Basically, splash mask is a form of mask in the form of liquid with high concentration and is intended for people

who do not have much time to do facial treatments with a mask. Because of its high concentration and liquid form, the use of splash mask is only applied for 20-30 seconds until the preparation is washed on a dry face or completely absorbed.⁹ The purpose of this research is to utilize tangerine peels to be something useful and has an effect as a whitening agent.

METHODS

This research was conducted at the pharmaceutical technology laboratory, faculty of mathematics and natural sciences, Universitas Garut, Indonesia. This research took place from April to August 2019.

The equipment used in this study are Spektrofotometer UV-Vis- 1601 (Shimadzu, Japan), pH meter type 510 (eutech instrument, Singapur), optical microscope (Nikon Eclipse E 200, Japan), sentrifugator (Kubota 5100, Japan), oven (Mettler, German), waterbath (Mettler, Hongkong), analytic scales type 210-LC (Adam, USA), mikropipet Eppendorf (Socorex, Switzerland) dan alat-alat gelas.

Ingredients used in this study are tyrosinase from straw mushroom (Sigma, USA), levodopa (Sigma, USA), potassium dihydrogen phosphate (Merck, Indonesia), sodium hydroxide (Mallinckrodt, Swedia), etanol (Merck, Indonesia), glycerin (Brataco, Indonesia), DMDM hydantoin (Brataco, Indonesia), aquades, and DMSO (Merck, Indonesia).

Material processing and extraction

The first thing to do when processing a plant into an extract is to ascertain the correctness of the plant's identity, one of the ways that can be done is by plant determination. Determination was carried out at the Bandungense herbarium, school of life sciences, Bandung institute of technology. After the results of the material identity are obtained, then made a simplicia of tangerine peel by wet sorting, dry sorting, and chopping until a tangerine peel is obtained. The resulting powder is then extracted. After the simplicia is obtained, extraction is carried out. The extraction chosen was cold or maceration extraction with 96% ethanol for 3×24 hours. The liquid extract obtained is then concentrated until thick. The extract obtained was then calculated the percent yield and analyzed the extract characterization and phytochemical.⁹⁻¹¹

Tyrosinase inhibition

In vitro tyrosinase inhibition test. First a 2.5 mm L-DOPA solution is made. Then a 50 mm phosphate buffer is made with a pH of 6.8 and then a tyrosinase solution is made, this solution is stored at a low temperature (2-8° C).¹²

Measurement of tyrosinase inhibitory activity was carried out by means of four test tubes prepared (A, B, C, D), in each tube piped 1.0 ml of 2.5 mm LDOPA solution and 1.8 ml of 50 mm phosphate buffer (pH 6.8) then incubated for 10 minutes. After incubation, added to each tube, tube A: 0.1 ml phosphate buffer and 0.1 ml tyrosinase enzyme solution, tube B: 0.2 ml phosphate buffer, tube C: 0.1 ml sample solution and 0.1 ml tyrosinase enzyme solution, and tube D: 0.1 ml phosphate buffer and 0.1 ml sample solution. The tubes were incubated for 25 minutes, then their absorbance was measured at a wavelength of 475 nm with a UV-Vis spectrophotometer. The tyrosinase enzyme inhibitory activity value was obtained by calculating the doparomine inhibition formed using the following formula.^{13,14}

$$(\%) \text{ Inhibisi} = \frac{(A-B)-(C-D)}{(A-B)} \times 100\%$$

A is absorption of negative blank solution with enzymes, B is absorption of negative blank solution without enzyme, C is absorption of sample solution with enzyme, and D is absorption of sample solution without enzyme. The inhibitory activity of the test sample is determined by the IC50 value, i.e., the concentration at which the test sample can inhibit the tyrosinase enzyme activity by 50%.¹³

Splash mask preparations

Splash mask first conducts a base orientation to see and know a stable base. Base orientation is carried out by making three formulas without orange peel extract by varying the concentration of glycerin.¹⁵ The splash mask base was then evaluated for 28 days and selected the most stable base from the evaluation results obtained (Table 1).⁹

The process of making a splash mask base is done using glycerin, DMDM hydantoin and aquadest.¹⁶ Preparation of the base formula is started by dissolving DMDM hydantoin with a little distilled water and then adding glycerin and distilled water up to 30 ml.¹⁵ Then stir until homogeneous.

Tangerine and turmeric splash mask preparation

The most stable splash mask base is used as the basis for the next splash mask formulation. The splash mask formulation was made with a concentration of tangerine peel added 1000×IC50 from the tyrosinase inhibition of tangerine peel extract (Table 2).

The preparation of the splash mask formula begins with dissolving the extract of tangerine with distilled water, then filtered, add DMDM Hydantoin which has been dissolved with a little distilled water, then add glycerine and aquadest to 30 ml. Stir until homogeneous.

RESULTS

The tangerines are taken and the skin is made into simplicia (Figure 1). Material is processed into simplicia by means of material collection, wet sorting to separate impurities from tangerine peel and ensure that no fruit is damaged or rotten, then washing with running water to clean from dirt or dust attached to plants, stripping tangerine peels from the fruit, coarse chopping to reduce the size so it is easy to dry, the drying process is carried out with a drying cabinet, after drying dry sorting is done to separate from the material that is not good, then crushed with a grinding machine (Figure 2).



Figure 1: Tangerine fruit.



Figure 2: Tangerine peel.

Simplicia produced is as much as 800 grams. Then extraction by maceration method using 96% ethanol solvent. Maceration is done for 3x24 hours. Extraction results obtained from the tangerine peel from 700 grams simplicial to 129.48 grams of extract with yield extract is 18.49% (Table 1). The results of tyrosinase inhibition test can be seen in the figure 3. After the extract concentration was obtained with IC50 values, it was followed by optimization of the splash mask preparation base, with the formulation (Table 2).

Table 1: Splash mask base formula.

Ingredients	Base (%)		
	B1	B2	B3
Glycerine	20	25	30
DMDM hydantoin	0.53	0.53	0.53
Aquadest	Add 30 ml	Add 30 ml	Add 30 ml

Table 2: Formula for splash mask tangerin peel extract.

Ingredients	Concentration
Tangerine peel extract	1000xIC50
Turmeric extract	q.s.
Glycerine	25%
DMDM hydantoin	0.53%
Aquadest	Add 30 ml

Table 3: Extract rendement.

Symplicia (gram)	Etanolic extract (gram)	Rendement (%)
700	129.48	18.49

$$\begin{aligned}
 (\%) \text{ Rendement} &= \frac{\text{weight Ekstrak}}{\text{weight dry sample}} \times 100\% \\
 &= \frac{129.48}{700} \times 100\% \\
 &= 18.49\%
 \end{aligned}$$

The splash mask base does not have a color that is clear or clear transparent, because all the materials used are colorless, and the odor of the three splash mask bases has a distinctive smell of glycerine, because it has not been added to the extract of tangerine and its texture resembles water. Of the three bases there were no significant changes.

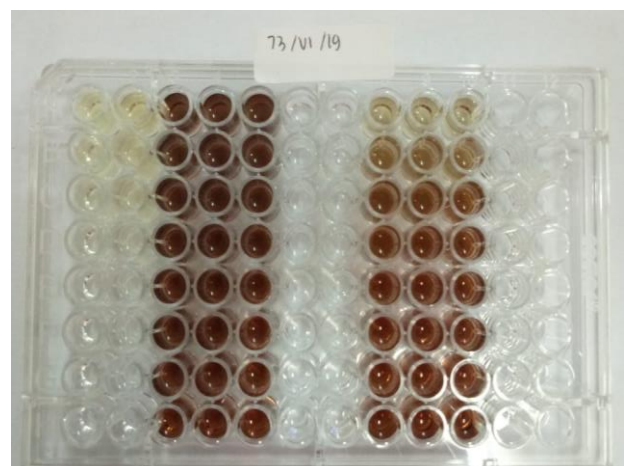


Figure 3: Microwell plate reader for tyrosinase inhibition.

Table 4: Inhibition tangerine peel extract.

Concentration (ppm)	Inhibition L-DOPA			
	Rep 1 (%)	Rep 2 (%)	Rep 3 (%)	Inhibition (%)
30000	51.054	50.865	50.009	50.643
10000	34.326	34.512	33.023	33.953
7500	32.558	32.744	33.488	32.930
5000	29.395	30.977	29.860	30.078
2500	33.302	26.977	27.256	29.178
1000	28.000	29.023	28.093	28.372
500	26.326	27.628	29.488	27.814
250	22.977	23.163	22.698	22.946
0	-	-	-	-

Table 5: Inhibition kojic acid.

Concentration (ppm)	Inhibition L-DOPA			
	Rep 1 (%)	Rep 2 (%)	Rep 3 (%)	Inhibition (%)
500	85.512	83.256	83.256	83.008
250	73.395	74.512	75.349	74.419
125	64.186	65.302	66.140	65.209
62.5	42.605	41.953	36.930	40.496
31.25	28.000	26.698	25.395	26.698
15.63	12.186	13.488	12.186	12.620
7.81	12.372	12.279	12.651	12.434
0	-	-	-	-



Figure 4: Splash mask with tangerine peel extract.

After obtaining the most stable base, then an inhibition test of the tyrosinase enzyme was carried out by the ELISA method (Figure 3). The test results of the tyrosinase enzyme inhibition activity were continued with a linear regression equation analysis test then determined the Inhibition concentration 50% tyrosinase (IC50). The IC value of inhibition of tyrosinase the result IC50 tangerin peel extract is 30.000 ppm with weak category and kojic acid 81,10 ppm with strong category (Table 4 and Table 5). The IC50 tangerin peel extract is higher than kojic acid, which is 30.000 ppm (tangerine) and 81,10 ppm (kojic acid). So, the lightening activity

possessed by tangerine peel extract is lower than kojic acid.

After obtaining a stable splash mask base, which is base 2, a splash mask preparation formulation was made with the addition of extracts of tangerine fruit to the formula, as many as 1000x IC50 (Figure 4).

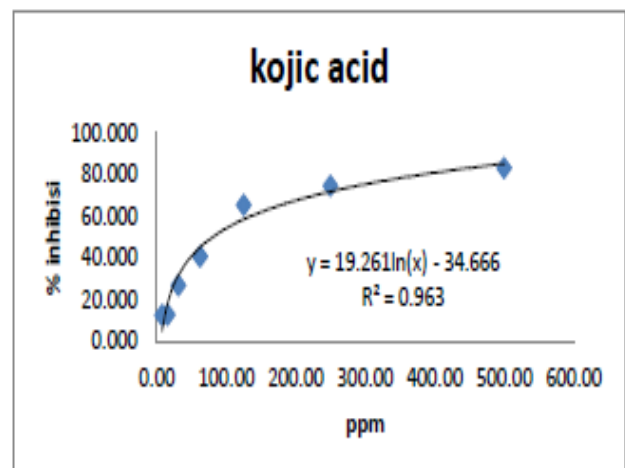


Figure 5: Inhibition of kojic acid.

DISCUSSION

This study uses tangerine obtained from the village of Cibolerang, Karang Sari village, Karangpawitan district,

Garut, West Java, Indonesia. To determine the identity of the arrowroot tangerines plant determination was carried out at Bandungense herbarium at Bandung institute of technology (ITB).

Phytochemical screening to determine the presence of secondary metabolites contained in simplicia and extracts from the tangerine peel. The metabolite compounds contained in the skin of tangerines are flavonoids, alkaloids, saponins, and steroids/triterpenoids.⁹ This supports the testing of antioxidants because flavonoids are known to have an antioxidant effect by interrupting free radical reactions that are very reactive, and tend to form new radicals.^{10,17} Phytochemical deterrence results showed that tangerine peel extract contained: flavonoids, alkaloids, saponins, and triterpenes. Where flavonoids are secondary metabolites that play a major role as antioxidants. Besides triterpenoids also act as antioxidants because it is able to give the hydrogen atom to free radicals.

Before making preparations, optimization of splash mask bases with various concentrations is carried out in order to find out which base is more stable in storage and then made in splash mask preparations containing tangerine peel extract. On a basis of optimization done for 28 days, made in 3 formulas B1, B2 and B3 with different glycerine concentrations are used 20%, 25% and 30%. Glycerine which functions as a humectant and emollient in cosmetic preparations.¹⁵ DMDMH functions as a preservative with a broad antimicrobial spectrum.¹⁶

The smaller the IC₅₀ value, the better the ability to inhibit the tyrosinase enzyme. Whereas the greater the IC₅₀ value the weaker the inhibition of the tyrosinase enzyme.^{12,18} From these results, it was found that the potential for tangerine peel extract against whitening effect is very weak when compared to kojic acid (Figure 7). The IC value of inhibition of tyrosinase the result IC₅₀ tangerine peel extract is 30.000 ppm with weak category and kojic acid 81,10 ppm with strong category (Table 4 and Table 5). The IC₅₀ tangerine peel extract is higher than kojic acid, which is 30.000 ppm (tangerine) and 81,10 ppm (kojic acid). So, the lightening activity possessed by tangerine peel extract is lower than kojic acid (Figure 5).

The tyrosinase enzyme plays a role in giving color pigments to the skin, so the skin becomes more brown or dark spots are formed.¹⁹ That compounds can inhibit melanin formation process are tyrosinase inhibitors, the mechanism of action is to reduce substances that can cause oxidation of dopaquinone and work competitively and non-competitive with tyrosinase substrate, L-tyrosine and L-DOPA, and specifically will bind covalently to the tyrosine enzyme.²⁰ so that the enzyme becomes competitive and non-competitive with tyrosinase substrate, namely L-tyrosine and L-DOPA and specifically will bind covalently to the tyrosine enzyme

so that the enzyme becomes competitive not active during the catalytic reaction.^{13,14}

The splash mask formula has a brown color, because the extract of tangerine has a brownish yellow color and the odor of the splash mask formula has a distinctive odor that is orange but is more concentrated, because the splash mask preparation formula does not add perfume or perfume so it smells odorless, as well as for the texture or form of splash mask preparations that is liquid resembles water, is a bit sticky because the tangerine peel extract is used a lot so it forms a concentrate. During storage there were no significant changes in the preparation of the splash mask.

CONCLUSION

The results of this study indicate the value of inhibition of the tyrosinase enzyme from tangerine peel extract is included in the weak category. This is indicated by the IC₅₀ value of 30,000 ppm.

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Conflict of interest: None declared

Ethical approval: The study was approved by the institutional ethics committee

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