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Inhibitory Activity of Tyrosinase Enzyme on Lotion Contains Pear (*Pyrus pyrifolia* (Burm.F) Nakai) Rind Extract

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Abstract-**Objectives: The purpose of this study was to make lotion preparations containing ethanol extract of pear rind and to test the inhibitory activity of the tyrosinase enzyme from pear rind ethanol extract lotion preparations. Method: Determination of tyrosinase enzyme inhibitory activity was carried out using a microplate reader instrument. The formula used is ethanol extract of pear rind, stearic acid, cetyl alcohol, triethanolamine, liquid paraffin, glycerin, sodium benzoate, essense pear and distilled water. Results and Discussion: Ethanol extract of pear rind has tyrosinase enzyme inhibiting activity which is indicated by IC_{50} values of 520.45 µg/mL, and in lotion preparations it has tyrosinase enzyme inhibitory activity which is indicated by IC_{50} values of 599.96 µg/mL. Conclusion: From the results of tyrosinase inhibitory activity test, lotion containing ethanol extract of pear rind has the potential to be a skin lightening.**

Keywords: pears (*Pyrus pyrifolia *(Burm.f.) Nakai), skin lightening, lotion, tyrosinase enzyme

I. INTRODUCTION

Indonesia is a tropical country that is rich in sun exposure. Exposure to UV light from the sun in the tropics is a source of free radicals that cause skin aging [2] and increases the activity of the enzyme tyrosinase synthesizing melanin pigment so that the skin color becomes increasingly brown [3]. Melanin is a human skin pigment formed by melanocyte cells located in the epidermis of the skin. The mechanism of melanin formation begins with the oxidation process of tyrosine amino acids by involving tyrosinase. The formation of excess melanin can cause darkening of the skin and black spots on certain skin areas [5].

Based on the fact the number of side effects due to the use of skin lightening cosmetics, it is necessary to look for other alternatives by using natural ingredients as lightening cosmetics that do not trigger skin damage and are not harmful to the body. One of ingredient that can be used as a skin lightener and safe is beta arbutin contained in pear rind. According to the Central Statistics Agency (BPS), imports of pears annually increase by 25%, so that the pears can be extracted as potential natural skin lightening ingredients that produce arbutin.

In research on chemical studies and HPLC it has been revealed that pears contain phenolic compounds such as chlorogenic acid, routine, procyanidin and beta arbutin [7-8]. Research conducted shows that beta arbutin can inhibit tyrosinase enzyme activity in melanoma rats with an IC50 value of 4.8 µg/mL and in mushroom tyrosinase of 8.4 µg/mL [12]. The highest beta arbutin content is found in the rind of fruit which is 1.20 mg/g compared to that found in fruit flesh that is equal to 0.29 mg/g and the average concentration of beta arbutin in pear cultivars oriental is greater that is 0.164 mg/g compared with that found in occidental pear cultivar by 0.083 mg/g, so that fruit flesh is equal to 0.29 mg/g and the average concentration of beta arbutin in oriental pear cultivar is greater that is 0.164 mg/g compared with that found in occidental pear

cultivar at 0.083 mg/g [6], so in this study used oriental pear cultivar, Pyrus pyridolia.

Lotion preparations have the advantage of being spread thin compared to preparations of creams or ointments and are quickly adsorbed on a wider surface of the skin. Lotion is a cosmetic preparation in the form of an emulsion that contains more water than oil and has properties as a source of moisturizer for the skin, soft and easily applied [1].

This research was conducted to provide information on natural ingredients producing beta arbutin derived from pear rind as skin lightening and its inhibitory activity against the enzyme tyrosinase in the formation of melanin in the skin made in lotion preparations.

II. METHODOLOGY

Making pear skin extract by maceration method using 80% ethanol solvent until submerged. Maceration process is carried out for 3x24 hours, then filtered. The results of maceration in the form of liquid extracts are evaporated in order to remove the remaining solvent so that a thick extract is obtained, then the extract yield is calculated [11].

The ethanol extract of pear rind was dissolved in DMSO (dimethyl sulfoxide) and then added to phosphate buffer (pH 6.8) to a volume of 10 mL. The extract solution was tested at concentrations of 500 ppm, 250 ppm, 125 ppm, 62.5 ppm and 31.25 ppm. Beta arbutin as a positive control was tested at the same concentration. Inside the plate drops 96 wells. As much as 70 µl of each of these dilution extracts was added with 30 µl of the tyrosinase enzyme (Sigma 250 units/ml in phosphate buffer pH 6.8), after which incubation was carried out at room temperature for 5 minutes. Then added 110 µl of L-tyrosine substrate in a multi-well plate that has been determined, the solution was incubated for 30 minutes at room temperature then measured using a microplate reader at a wavelength of 477 nm, this aims to determine the inhibition concentration of 50 % (IC50) [4].

Table 1 Lotion Formula

Material	Concentration (%)	
	Base	Formula
Extract	-	0.5
Setil alkohol	2	2
Asam stearat	2.5	2.5
Trietanolamin	1	1
Paraffin cair	8	8
Gliserin	15	15
Natrium benzoat	0,5	0,5
Essence pear	4 qtt	4 qtt
Akuades	Ad 100	Ad 100

Lotion prepartion process begins with weighing the materials needed, the materials used are separated from the oil phase and the water-soluble material. Oil-soluble ingredients namely stearic acid, cetyl alcohol and liquid paraffin are put into the vaporizer cup. Water-soluble ingredients are triethanolamine, glycerin and distilled water. The oil phase and the water phase are heated and stirred at 70-75ºC separately until homogeneous. The mixing process of the two preparations was carried out at a temperature of 70ºC. The stirring process was carried out until both homogeneous phases reached 4°C. then sodium benzoate, essence pear and ethanol extract pear rind are put into the mixture at a temperature of 35ºC and then stirred for approximately one minute.

To find out IC_{50} ethanol extract lotion of pear rind, lotion preparation is dissolved in DMSO (dimethyl sulfoxide) and then phosphate buffer (pH 6.8) is added to a volume of 10 mL. Sample solutions were tested at concentrations of 5000, 2500, 1250 and 625 µg / mL. Lotion containing beta arbutin as a positive control was tested at the same concentration. A total of 70 µL from each of these dilution samples was added with 30 µL of the tyrosinase enzyme (Sigma 250 units / mL in phosphate buffer pH 6.8), after which incubation was carried out at room temperature for 5 minutes. Then 100 µL of L-tyrosine substrate was added to the determined multi-well plate well, the solution was incubated for 30 minutes at room temperature. Then measured using a

microplate reader at a wavelength of 477 nm [4].

III. RESULTS AND DISCUSSION

The extraction process was carried out using the maceration method with 80% ethanol solvent. This method was chosen because the beta arbutin compound is relatively stable at low temperatures of 35-500C and maceration is an extraction process by immersion of simplicia in a solvent by shaking several times and stored at room temperature. The advantage of using this method is easy to do. Yield of pear rind ethanol extract obtained from the weight of simplicia 500 gram and thick extract 64.62 gram obtained yield of 12.92 gram.

The tyrosinase enzyme inhibiting activity test was conducted to determine whether there is inhibition of bioactive compounds found in the ethanol extract of pear rind. Tyrosinase inhibitory activity is indicated by the IC_{50} value, which is the concentration needed to inhibit 50% of tyrosinase activity using a microplate reader. The working principle of the in vitro enzyme tyrosinase inhibition test method is based on a decrease in the amount of doparom which is the result of oxidation of DOPA by the tyrosinase enzyme. Dopakroms that form will be dark orange to red. If tyrosinase enzyme activity is inhibited, the color intensity of dopakrom will decrease so that absorption can be measured (absorbance) using a microplate reader at maximum wavelength. Absorption (absorbance) obtained is used to determine how much the ethanol extract of pear rind in inhibiting the activity of the enzyme tyrosinase.

The method of testing tyrosinase inhibition activity refers to the method that has been done [4]. Optimized variables include three things, namely substrate concentration, incubation time, and maximum wavelength. Optimum conditions used in activity testing are using optimal results in the form of a substrate concentration of 1 mM, incubation time for 30 minutes and scanning absorption (absorbance) using a microplate reader (Infinite M200 Pro®), at a wavelength of 477 nm. The incubation temperature used is a room temperature of 26°C. This temperature is still included in the stable temperature of tyrosinase enzyme activity. Based on research conducted [9], tyrosinase enzyme activity can be maintained at a temperature of 20-600°C and its activity is optimum at a temperature of 25°C -30°C. The buffer pH used is always observed using a pH meter. The buffer solution used is phosphate buffer pH 6.8 which is still the optimum pH of tyrosinase enzyme activity. The tyrosinase enzyme inhibition activity test was carried out by measuring the absorption (absorbance) of ethanol extract of pear rind and beta arbutin standard as a positive control in triplo. The results of tyrosinase resistance testing are stated in IC_{50}.

IC_{50} of pear rind ethanol extract sample was 520.45 µg/mL and IC_{50} of beta arbutin standard was 358.93 µg/mL. IC_{50} determine the level of pear rind ethanol extract as tyrosinase inhibitors to inhibit melanin formation. The smaller the IC_{50}, the more potential the compound is as a tyrosinase inhibitor. Compounds that have an IC_{50} value <1000 µg/mL are classified as having inhibitory activity against the tyrosinase enzyme. Data on IC_{50} of tyrosinase inhibitory activity can be seen in table 2.

The ethanol extract lotion of pear rind was tested for enzymatic inhibition activity of tyrosinase. The test was carried out on the first day in order to determine the effect of base addition on the tyrosinase enzyme inhibitory activity. From the results of tests that have been carried out on pear rind ethanol extract lotion preparations have inhibitory activity against the tyrosinase enzyme which has an IC_{50} of 599.97 µg/mL and standard beta arbutin preparations of 471.40 µg/mL. This shows that there is a slight change in the inhibitory activity of the tyrosinase enzyme after base addition when compared with the IC_{50} results in the extract. This happens because there are components of the substance on the base that provide absorption at the time of measurement.

Table 2 IC_{50} of tyrosinase inhibitory activity in extracts and preparations

Sample	IC_{50} (µg/mL)
Extract	520,45
Beta Arbutin	358,93
Extract in lotion preparations	599,97
Beta Arbutin in lotion preparations	471,40

V. CONCLUSION

The ethanol extract of pear rind has tyrosinase enzyme inhibiting activity which is indicated by IC_{50} values of 520.45 µg/mL, and in lotion preparations it has tyrosinase enzyme inhibitory activity which is indicated by IC_{50} values of 599.96 µg/mL. So it can be concluded that from the results of tyrosinase inhibitory activity test, lotion containing ethanol extract of pear rind has the potential to be a skin lightening

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