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Antioxidant and Anti-Collagenase Effectivity of Red Dragon Fruit Peel and Kaempferol 3-0-Rutinoside

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Abstract

Aging is a natural process in human life, which is closely related to various degenerative processes. Skin aging can be caused by internal factors that are influenced by the enzyme collagenase and elastase while external factors are influenced by the environment such as sunlight and chemicals. Free radicals are one of the aging factors that cause oxidative stress and cell apoptosis. Antioxidant compounds can fight oxidation by neutralizing free radicals. Natural antioxidants from the peel of red Dragon fruit as antiaging agents. **Aims**: To investigate the antioxidant and anti-aging activities of ethanol extract of Red Dragon fruit peels and kaempferol-3-O-rutinoside compounds. **Methods**: antioxidant assay was measured by the FRAP method and anti-aging property was measured through inhibitory activities of collagenase. **Results:** Red dragon fruit peel extract had the highest FRAP antioxidant activity at a concentration of 50 ug/ml which FRAP activity was 102.35 µg Fe while the highest FRAP antioxidant activity in kaempferol was 202.57 µg Fe. Anticolagenase activity by the highest red dragon fruit peel extract at a concentration of 250ug / ml was 74.36 ± 0.56% while kaempferol was 72.79 ± 1.02% Collagenase inhibition activity can be seen from the IC50 value. IC50 extracts of red dragon fruit skin 109.84 ug/ml and kaempferol at 44.32 ug/ml. **Conclusion**: Overall, Kaempferol -3-O-rutinoside compound has higher antioxidant and anti-aging activity compared to red dragon fruit peel extract.

Keywords: antioxidant; anti collagenase; red dragon fruit peel extract; kaempferol 3- O-rutinoside.

1. Introduction

Aging is a process of the reduced ability of tissue functions that will occur in all organs of the body both internal organs and external organs such as the skin, is a natural process in human life(1). Aging of the skin can be caused by internal factors and external factors.

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Internal factors such as changes in skin elasticity, which are influenced by the enzyme collagenase and elastase enzyme, which results in wrinkles, dry skin and accelerated premature aging. While external factors are influenced by the environment, such as sunlight, air, chemicals, and the surrounding environment (2). Some people experience aging according to their age, but some of these processes take place earlier called premature aging(3). Free radicals are one of the aging factors because they can damage biological molecules in the body such as tissues and cells, and cause oxidative stress and cell apoptosis (4). Another aging factor, photoaging by exposure to UV light also affects the elasticity of the skin which is degraded by the enzyme elastase in the extracellular matrix which causes wrinkles (5). Free radicals can increase the levels of collagenase enzymes and increase the activity of the elastase enzyme which increases collagen degradation which causes skin shrinkage accelerates the aging process (6). Antioxidant compounds can fight oxidation in the body (7). Exogenous antioxidants play a role in neutralizing free radicals exogenous antioxidant intake from natural products such as fruits, vegetables, and seeds (8). Red dragon peels (*Hylocereus polyrhizus*) which is a dry tropical climate has antioxidant content (9). Antioxidant and anti-aging activity is known from IC_{50} value, meaning the concentration of antioxidant or anti-aging which can cause a 50% loss of free radical activity (10) The aims of this study was to evaluate the antioxidant and antiaging potential of extract Red Dragon fruit peels and kaempferol-3-O-rutinoside compounds through antioxidant activity FRAP assay and collagenase inhibitory activity assay.

2. Materials and methods

1. Materials

Materials that used in this study are red dragon fruit (*Hylocereus Polyrhizus*), ethanol 70%, 2,4,6-Tripyridyl-s-Triazine (TPTZ) (Sigma-Aldrich, 3682-35-7), Ferrous (III) Chloride ($FeCl_3$) (Sigma-Aldrich, 12322-2.5L), Dymethilsufoxide (DMSO) (Merck 1029522500), Sodium Acetate (Merck 1062681000), Hydrochloride Acid (Merck 1090631000) Kaempferol 3 rutinocide (Chengdu BP0823), Acetate buffer (pH 3,6), N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (FALGPA) (Sigma F5135), Collagenase from *Clostridium hystolyticum* (Sigma C8051), Tricine (Sigma SA10377), calcium chloride (Merck 1023821000), Sodium chloride (Merck 106406), distilled water, (Merck 1.02931.1000), Hydrochloric acid solution (Merck 109057),

2. Instrumentation

Instruments that used in this study are Micropipette (1-10 µL; 50-200µL; 100-1000 µL), Microplate Reader, 96 well, Tube 15 ml, Tube 50 ml, Pipette Tips (1-10 µl, 50- 200 µl, 100-1000 µl), Multichannel Pipette 30-300 µL, Incubator, analytical balance, vortex, tube Eppendorf 1,5 ml, pH meter, beaker glass.

2.1. Preparation of red dragon fruit (Hylocereus Polyrhizus) extract

The experiments of fruits *Hylocereus Polyrhizus* were collected from Bandung, West Java, Indonesia. The plants were identified by the herbarium staff, Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, Indonesia. *Hylocereus Polyrhizus*'s fruits were washed and extracted using ethanol 70% with the maceration method. Every 24 hour the filtrate would be filtered and collected until it was colorless. The extract evaporated until it becomes a paste form [11]. Extraction using the maceration technique with 70% ethanol solvent every 24 hours the filtrate is collected, until the ethanol filtrate

is colorless then 70% ethanol filtrate is evaporated until 70% ethanol extract it into a concentrated form. The maceration method is a simple method of extraction which is done by soaking the simplicia powder in a liquid for several days at room temperature and protected from light (12-17).

2.2. FRAP assay

The Ferric reducing antioxidant power assay (FRAP) was estimated by using the modified method from Widowati and his colleagues (2018) studies. The FRAP reagent was prepared by adding 2,4,6-Tripyridyl-s-Triazine (TPTZ) (Sigma-Aldrich, 3682-35-7) and ferrous (III) Chloride ($FeCl_3$) (Sigma-Aldrich, 12322-2.5L), forming the Fe 3+ TPTZ complex. In 96- well microplate, 7,5 µl of samples were mixed with 142, 5 µl FRAP reagent then incubated for 30 min at 37°C. The absorbance value was measured at 593 nm with a microplate reader (Multiskan TM Go Microplate Spectrophotometer, Thermo Scientific). The results of the samples were expressed in µM Fe (II)/ µg extract. (18-20)

2.3. Collagenase Assay

Collagenase's Principe :

Collagenase is one of the enzymes in the group of metalloproteinases that play an important role in degrading collagen, one of the structural proteins that make up ECM in the connective tissue of animals.

$$\text{FALGPA} \xrightarrow{\text{collagenase}} \text{FAL} + \text{Gly} - \text{Pro} - \text{Ala}$$

The method used is by reacting the collagenase enzyme (from the bacterium *Clostridium hystolyticum*) with a substrate, FALGPA (N- (3- [2-Furyl] acryloyl) -Leu-Gly-Pro-Ala), a synthetic peptide that mimics the structure of collagen. Collagenase cuts the X-Gly bond so that the FALGPA breakdown reaction by collagenase becomes FAL (N- (3 [2-Furyl] acryloyl) -Leu) and Gly-Pro-Ala sequence. The measurement of the enzymatic activity of collagenase can be detected by spectrophotometry at λ 340 nm which is by decreasing the absorbance of the substrate after reacting with the enzyme. (20,21) The mixture of the 10 µl of collagenase from *Clostridium hystolyticum* (0.1 mg/ml), 30 µl of red dragon fruit peel and 60 µl of tricine buffer (50 mM tricine, 10 mM calcium chloride, 400 mM sodium chloride, pH 7.5) were incubated at 37°C for 20 minutes. Add 20 µl FALGPA 1 mM substrate into the mixture. The absorbance was measured using the microplate reader with 335 nm wavelength [20].

The collagenase inhibition activity was measured using the following formula:

$$\%\ Inhibition\ \frac{C - S}{C} X\ 100$$

C: negative control absorbance

S: sample absorbance

3. Statistical Analysis

All data were expressed as mean ± standard deviation of triplicate measurements and were subjected to statistical analysis using Analysis of Variance (One way ANOVA) followed by Tukey's HSD Post-hoc test. Statistical analysis was performed using SPSS software (version 20.0). A value of $P > 0.05$ was considered as the significant value of the data.

4. Results and discussion

Based on the results of changes of data on wet weight, dry weight and dry weight of samples can be seen in Table 1.

Table 1: Wet and Dry Weight Peels of Red Dragon Fruit Extract

No	Fruit Name	Scientific name	Origin	Wet Weight (kg)	Dry Weight (g)
1.	Dragon fruit	*Hylocereus polyrhizus*	Village Cijambe, Sub-district Cijambe, district Subang	1.03	240

Tabel 2: Initial Sample Weight Data

No	Description	Amount	Information
1	Wet weight	1.03 kg	
2	Dry weight	240 gr	

Tabel 3: The yield of Red Dragon Fruit Peel

Sample	Weight of Simplicia(g)	Ethanol Volume for Macerationi(ml)	Duration of Maceration until Colorless	Filtrate's Volume of ethanol(ml)	Weight Yield of Extract (g)	Yield Extract (%)
Dragon Fruit Peel	200	2000	3 days	1300	41	21.05

5. FRAP Antioxidant Test Results of Red Dragon Fruit Peel Extracts and Kaempferol

Tabel 4: The activity of FRAP dragon fruit skin extract, kaempferol (Average, the Tukey HSD Post Hoc Test Test Results)

Final Concentration (ug/ml)	Average FRAP Activity (%)	
	Dragon Fruit Peel Extract	Kaempferol
50.00	102.35 ± 1.75 [d]	202.57 ± 1.63[f]
25.00	81.52 ±2.62 [c]	150.97 ±1.38[e]
12.50	77. 22 ±2.31 [b,c]	101.75 ±0.70[d]
6.25	69.20 ±6.89 [b]	75.45 ±3.70[c]
3.13	56.72 ±5.30 [a]	59.07 ±5.30[b]
1.56	46.15 ±4.85 [a]	49.50 ±4.85[a]

*) Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05 (Tukey HSD post hoc test).

In the table, the results of the study show that the highest reduction in red dragon fruit peel extract of Fe^{3+} into fe^{2+} activity at a concentration of 50 ug/ml was 102.35 µg Fe while in kaempferol was 202.57 µg Fe. At the lowest concentration of 1.56 ug/ml in red dragon, fruit peel was 46.15 µg Fe while kaempferol was 49.50 µg Fe.

6. Anti-collagenase Test Results of Red Dragon Fruit Peel and Kaempferol

Table 5: Collagenase Inhibition Activity of Red Dragon Fruit Peel and Kaempferol (Average, the Tukey HSD Post Hoc Test Test Results)

Final Concentration (ug/ml)	Average Collagenase Inhibiting Activity (%)	
	Dragon Fruit Peel Extract	Kaempferol
250.00	74.36±0.56[f]	72.79±1.02[e]
125.00	54.65±1.43[e]	58.19±0.47[d,e]
62.50	43.08 ±0.57[d]	54.82±0.50[d]
31.25	37.77 ±0.42[c]	48.69±0.38[c]
15.63	33.33 ±0.57[b]	46.67±1.68[b]
7.81	25.96 ±0.24[a]	44.31±2.94[a]

*) Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05 (Tukey HSD post hoc test).

In the table, it can be seen that the results of this study showed that the highest anti collagenase activity by red dragon fruit peel extract at a concentration of 250ug / ml was 74.36 ± 0.56% while kaempferol was 72.79 ± 1.02%. To determine the IC50 of red dragon fruit peel extract and kaempferol, a linear regression equation is used between concentrations. Results of IC50 anti collagenase values from red dragon fruit peel extract and kaempferol can be seen in table 6.

Table 6: IC50 Collagenase Inhibition of Dragon Fruit Skin Extract, kaempferol

Sample	Equation	R2	IC50 (µg/mL)	IC50 (µg/mL)
Red Dragon Fruit Peels Extract (1st Repetition)	Y = 0.185x + 29.84	0.97	108.22	109.95 ± 1.51
Red Dragon Fruit Peels Extract (2nd Repetition)	Y = 0.722x + 29.24	0.979	111.02	
Red Dragon Fruit Peels Extract (3rd Repetition)	Y = 0.737x + 29.98	0.963	110.61	
Red Dragon Fruit Peels Extract (Average)	Y = 0.184x + 29.69	0.97	109.84	
Kaempferol (1st Repetition)	Y = 0.119x + 43.97	0.979	50.67	44.12 ± 6.16
Kaempferol (2nd Repetition)	Y = 0.105x + 45.46	0.97	43.24	
Kaempferol (3rd Repetition)	Y = 0.109x + 45.81	0.964	38.44	
Kaempferol (Average)	Y =0.111x + 45.08	0.97	44.32	

Collagenase inhibition activity can be seen from IC50 values. IC50 red dragon fruit peel extract at 109.84 ug/ml and kaempferol at 44.32 ug/ml.

Based on FRAP antioxidant test results of red dragon fruit peel extract, antioxidant activity was obtained at 102.35 µg Fe at a concentration of 50 µg / ml while the antioxidant activity of kaempferol was 202.57 µg Fe at a concentration of 50 ug/ml which are the highest concentration. Whereas at the lowest concentration of 1.56 ug/ml red dragon fruit peel extract, antioxidant activity was 46.15 µg Fe and in antioxidant activity of kaempferol was 49.50 µg Fe. Collagenase inhibition testing using modification methods Sigma Aldrich and Wittenauer and his colleagues (2015). The anti-collagenase activity test is used to test the activity of collagenase and detected the potential inhibition of collagenase, using spectrophotometry at a wavelength of 340 nm by reducing the absorbance of the substrate after reacting with the enzyme. The highest anti collagenase activity test for red dragon fruit peel extract results at 250 ug/ml concentration was 74.36% and the lowest at 7.81 ug/ml was 25.96%. While the highest anti collagenase kaempferol activity test results at a concentration of 250 ug/ml were 72.79% and the lowest at a concentration of 7.81 ug/ml was 44. 31%. This study has a similar result was reported by Widowati and her colleagues [16]. Which reported that the IC50 value of the collagenase inhibition of *Oryza sativa* extract was 816.78 µg/mL, the result indicated the *Oryza sativa* extract has lower antiaging activity particularly as collagenase inhibition compared with the other compounds(20). The bioactive compound dramatically induced collagen synthesis as a booster of collagen in the skin and inhibited collagen activity by reducing collagen breakdown (23). The collagenase inhibition activity delays the aging process such as wrinkle formation via delay the forming pre-collagen fibers (24). The polyphenols such as flavonoid which is found in the plant extract have an anti-collagenase activity has been used as a basic material for synthesizing several anti-aging molecules (25)

7. Conclusion

From the result, kaempferol 3-o-rutinoside has potential antioxidant and antiaging activity through collagenase

inhibitor higher red dragon fruit peel extract. The kaempferol -3-O-rutinoside compound has higher FRAP activity and collagenase inhibition than the red dragon fruit peel extract. The compound in the *Hylocereus polyrhizus* responses antioxidant activity through FRAP activity and antioxidant activity particularly as collagenase inhibitor, so *Hylocereus poliovirus* can be developed into antiaging products.

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