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Ferric Reducing Antioxidant Power (FRAP) and Inhibition of Collagenase Enzyme Activity from Ethanol Extract of Pineapple (*Ananas cosmus* (L.) Merr) Core

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Abstract

Reactive Oxygen Species (ROS) is one of aerobic respiration products in cells which cause aging. Pineapple core is able to protect skin healthy by reducing reactivity from ROS, inhibiting oxidation, absorbing UV light, suppressing enzyme activity (such as collagenase), reducing the formation of wrinkles on the skin and protecting the skin from aging. This study aimed to investigate antiaging effect of pineapple core by their antioxidant and anti-collagenase activity. Determination of Antioxidant activity was using FRAP Methods while Inhibition of Collagenase assay was using Collagenase Enzyme from *Clostridium histolyticum.* The result of this study was expressed by Mean ± SD and analysed by One-Way ANOVA test and followed by the Post Hoc Test with the Tukey HSD test while Inhibition of collagenase were followed to analyse by simultaneous analysis of linear regression then determined the value of Inhibition Concentration 50 (IC_{50}). At the highest concentration (50 µg/ml) from each sample were shown highest FRAP activity were 149.92±0.75 µg Fe^{2+} for ethanol extract of pineapple core and 255.05±1.04 µg Fe^{2+} for luteolin. Same as FRAP Activity, Inhibition of Collagenase assay at the highest concentration (250 µg/ml) from each sample were shown highest percent of collagenase inhibition were 64.62 ± 0.73% for ethanol extract of pineapple core and 81.22 ± 1.00%for luteolin while IC_{50} value of collagenase inhibition activity are shown that ethanol extract of pineapple core has moderate inhibition activity. Whether FRAP activity or Inhibition of Collagenase activity aren't potent as luteolin compound as comparison.

Keywords: Collagenase Inhibition; FRAP; Pineapple Core; Luteolin.

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1. Introduction

Aging is a natural process which cannot be avoided. Instead of increasing age, signs of aging begin to appear on the face. Like wrinkles or fine lines which appear in the corner of the eye, forehead, and around the lips. When fine lines begin to appear, it mean the face requires more care [1]. Reactive Oxygen Species (ROS) is one of aerobic respiration products in cells which cause aging. It involves exposure to heavy metals, ionizing radiation and oxidants. There are some antioxidants which are able to eliminate ROS including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR). The balance between antioxidants and ROS affects aging of the skin at the cellular level [2]. Some natural sources can be used to prevent especially aging caused by sun exposure. Luteolin is one of the bioactive compounds in nature that can be found in chili, onion, broccoli, celery, carrots and pineapple. This compound is reported to have an in vitro anti-photoaging effect. The production of enzymes such as elastase and collagenase is produced by the skin under normal conditions. The acceleration of the process of activation of collagenase enzymes can occur if reactive oxygen species (ROS) or exposure to UV light occur excessively [3]. Other natural sources which have similar effects are pineapple core. For a long time, pineapple is often used as part of the fruit, while pineapple core are wasted which has litlle or no benefits. Several studies have reported the presence of flavonoids and phenolic compounds which can act as antioxidants in the skin and flesh of pineapple [4]. They are able to protect skin healthy by reducing reactivity from ROS, inhibiting oxidation, absorbing UV light, suppressing enzyme activity (such as collagenase), reducing the formation of wrinkles on the skin and protecting the skin from aging [5]. Based on those information is required to explore pineapples core benefits. So this study is aimed to investigate antiaging effect of pineapple core by their antioxidant and anti-collagenase activity.

2. Methods

2.1. Materials

Pineapple Core (*Ananas cosmosus (L.) Merr*), ethanol 70%, luteolin compound, 2,4,6-*Tripyridyl-s-Triazine (*TPTZ) (Sigma-Aldrich, 3682-35-7), $FeCl_3$ (Sigma-Aldrich, 12322-2.5L), Dimethyl sulfoxide (DMSO) (Merck, 109) (1029522500), Sodium Acetate (Merck 1062681000), Hydrochloride Acid (Merck 1090631000), Asetate buffer (pH 3,6), *Collagenase* from *Clostridium histolyticum* (Sigma C8051), N-[3-(2-Furyl)acryloyl]-leu-gly-pro-ala (FALGPA) (Sigma F5135), Tricine (Sigma SA10377), *Sodium chloride* (Merck 106406), *Calsium Chloride* (Merck 1023821000), *Dymethilsulfoxide* (Merck 1.02931.1000), *Hydrocholic acid solution* (Merck 109057), Aquadest

2.2. Preparation of Samples

Pineapples core as sample were obtained from Desa Tambaksari, Jalan Cagak District, Subang Regency, and West Java. The sample was sorted and cleaned, and dried using food dehydrator to obtain simplicia. Then simplicia was mashed and weighed 200 grams. It was macerated using 70% ethanol as solvent. Every 24 hours the filtrates were collect until the ethanol filtrate became colourless for 3 days. Then the filtrate was evaporated using rotary evaporator at 50^{o}C until the filtrate became concentrated [6, 7, 8, 9].

2.3. Antioxidant Assay Using FRAP Methods

Added 30 µl sample into each of well on the 96 well micro-plates, then added 30 µl FeCl (3 mM in 5 mM citrate acid) and 240 µl TPTZ (1 mM in 0.05 M HCl). The Absorbance was measured using micro-plates reader (λ = 615 nm) for 15-30 minutes. FRAP activity were gotten using eqution which was gotten from plotting FeSO4.7H2O (Ferrous sulfate) in various concentration as samples. So FRAP Activity would be gotten in equivalent µM Fe^{2+}.

2.4. Inhibition of Collagenase Assay using Collagenase Enzyme from Clostridium histolyticum

Added 30 µl sample into each of well on the 96 well micro-plates, then add 10 µl collagenase from *Clostridum histolyticum* (0.01 U/ml in cold aquadest) and 60 µl Tricine buffer (50 50mM, pH7.5 [10mM CaCl2 and 400mM NaCl]). These were incubated in 37°C for 20 minutes. After that, add 20 µL N- [3- (2-Furyl) acryloyl] -leu-gly-Pro-Ala [Sigma F5135, AS](1mM in buffer theTricine) as a substrate. The absorbance was measured at 355 nm wavelength. Percent of collagenase inhibition was measured using following formulation. Others than percent of inhibition, Median Inhibitory Concentration (IC_{50}) was also measured[7].

$$\%Inhibition = [(Abs_{control} - Abs_{sample})/Abs_{control} \times 100\%] \quad (1)$$

2.5. Data Analysis

Data was analysed by One-Way ANOVA test and followed by the Post Hoc Test with the Tukey HSD test with a confidence level of 95% (α = 0.05). Inhibition of collagenase were followed to analyse by simultaneous analysis of linear regression then determined the value of Inhibition Concentration 50 (IC_{50}).

3. Result and Discussion

To determine FRAP activity from the sample, the equation obtained from the standard curve of $FeSO_4$ as shown in Figure 1 below.

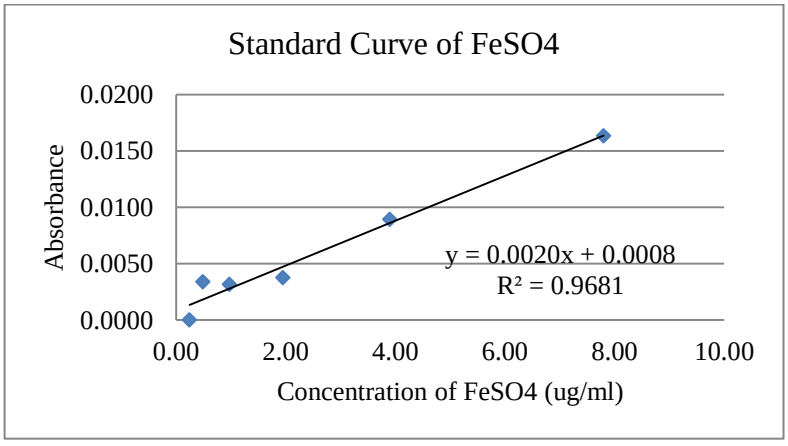

Figure 1: Standard Curve of FeSO4

The average of FRAP activity which was gotten in percent prior was analysed using Post Hoc Test Turkey HSD as show in table 1 below.

Table 1: Percentage of FRAP Activity from Ethanol Extract of Pineapple and Luteolin (Mean, Post Hoc Test Tukey HSD)

Concentration (ug/ml)	Average of FRAP Activity (µg Fe^{2+})	
	Ethanol Extract of Pineapple Core	**Luteolin**
50.00	149.92±0.75^{e}	255.05±1.04^{f}
25.00	113.43 ±5.75^{d}	204.03±1.84^{e}
12.50	68.62±3.37^{c}	142.15±13.21^{d}
6.25	43.40±10.71^{b}	111.27±12.32^{c}
3.13	16.25±1.47^{a}	58.03±6.66^{b}
1.56	10.67±0.51^{a}	29.53±0.68^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < 0.05$

Based on table 1 above, at various concentration luteolin has higher FRAP activity than Ethanol Extract of Pineapple core. However, ethanol extract of pineapple core had a significant different (P value < 0.05) of average FRAP activity in each concentration, excpect two lower concentration. While the luteolin in various concentration had a significant different (P value < 0.05) of average FRAP activity. FRAP activity of ethanol extract of pineapple core or luteolin had a linear model, which mean increment of concentration would increase FRAP activity. At the highest concentration (50 µg/ml) from each sample were shown highest FRAP activity were 149.92±0.75 µg Fe^{2+} for ethanol extract of pineapple core and 255.05±1.04 µg Fe^{2+} for luteolin. The average of Collagenase Inhibition Activity was analysed using Post Hoc Test Tukey HSD as show in table 2 below.

Table 2: Percentage of Collagenase Inhibition from Ethanol Extract of Pineapple and Luteolin (Mean, Post Hoc Test Tukey HSD)

Concentration (µg/ml)	Average Inhibition Collagenase Activity (%)	
	Ethanol Extract of Pineapple Core	**Luteolin**
250.00	64.62±0.73^{e}	81.22±1.00^{e}
125.00	51.02± 0.62^{d}	59.45± 0.41^{d}
62.50	38.44±1.66^{c}	44.53±1.39^{c}
31.25	32.15±0.14^{b}	34.26±0.77^{b}
15.63	33.89±1.03^{b}	33.55±1.39^{b}
7.81	25.82±0.33^{a}	26.99±0.85^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05

Based on table 2 above, luteolin had more potent inhibition of collagenase than ethanol extract of pineapple core. In the lower concentration (31.25 µg/ml and 15.63 µg/ml) in each sample had no significant different at P value < 0.05. However, in other concentration, each samples had a significant different at P value < 0.05. At the highest concentration (250 µg/ml) from each sample were shown highest percent of collagenase inhibition were 64.62 ± 0.73% for ethanol extract of pineapple core and 81.22 ± 1.00%for luteolin. The median inhibition concentration of collagenase enzyme was plotted using Linear regression. Result of IC_{50} value from linear regression were shown in table 3 below.

Table 3: IC_{50} Value of Collagenase Enzyme from Ethanol Extract of Pineapple Core and Luteolin Compound

Sample	Equation	R^2	IC_{50} (µg/mL) from Equation	IC_{50} (µg/mL) Mean ± SD
Ethanol Extract of Core Pineapple				
1st Repetion	y = 0.1531x + 29.045	0.96	136.81	141.60±4.14
2nd Repetion	y = 0.1476x + 28.847	0.95	143.31	
3rd Repetion	y = 0.1532x + 27.847	0.96	144.60	
Average	y = 0.1513x + 28.58	0.96	141.57	
Luteolin Compound				
1st Repetion	y = 0.2217x + 28.631	0.98	96.39	97.33±1.15
2nd Repetion	y = 0.2227x + 28.039	0.98	98.61	
3rd Repetion	y = 0.2093x + 29.701	0.98	96.99	
Average	y = 0.2179x + 28.79	0.98	97.34	

Based on table 3 above, IC_{50} value of ethanol extract from pineapple core (141.60±4.14%) was higher than luteolin compound (97.33±1.15%). It meant that a higher concentration of ethanol extract from pineapple core was needed to inhibit half of collagenase enzyme than Luteolin compound.

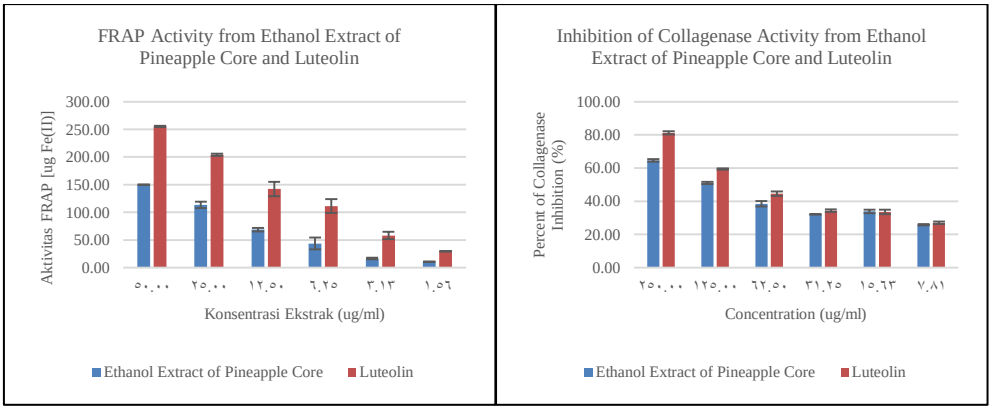

Figure 2: Histogram of FRAP Activity (Left) and Inhibition of Collagenase (Right) from Ethanol Extract of Pineapple Core and Luteolin Compound

Figures 2 shows whether at various concentration of ethanol extract from pineapple core has FRAP activity and inhibition of collagenase enzyme that are less potent than luteolin as a comparison. Based on IC50 value, there

are there categories of inhibition activity. Those are very strong activities (IC_{50} less than 50µg / mL), strong activity (IC_{50} 50 - 100µg / mL), moderate activity (IC_{50} 101 - 150µg / mL), and weak activity (IC_{50} greater than 150µg / mL) [4, 8]. According to IC_{50} value of collagenase inhibition activity, ethanol extract of pineapple core has moderate inhibition activity while luteolin has strong inhibition activity. It is because of the presence of phenolic compounds, flavonoids and triterpenoids which are responsible for protecting the skin. Phenolic and flavonoids have phenol rings in the presence of hydroxyl substituents that can inhibit ROS, reduce metal ions, modulate phosphorylation of proteins associated with inhibition of enzyme activity (collagenase) and inhibition of lipid peroxidation [5, 10]. Luteolin shows anti-photoaging effects in vitro and in vivo and may have potential as a treatment for prevention of skin aging because it can inhibit enzymatic activity [3] The triterpenoid content in pineapple core has the potential to be an antioxidant because it can donate hydrogen atoms (H) so that free radicals can be turned into non-reactive [11]. The presence of triterpenoid compounds in the extract is responsible for protecting the skin due to the presence of hydroxyl subtituents capable of inhibiting ROS, reducing metal ions, modulating protein phosphorylation related to inhibition of enzyme activity and inhibition of lipid peroxidation [5, 10].

4. Conclusion

Pineapple core has a potential antiaging effect espacially from its antioxidant activity than inhibition of collagenase activity, but overall these activity aren't potent as luteolin compound as comparison.

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