

## Comparison of Antioxidant and Anti-hyaluronidase Potentials of Pineapple Core Extract (*Ananas comosus* (L.) Merr.) and Luteolin

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### Abstract

Skin aging processes are divided into chronological aging and premature aging. Premature aging is generally caused by free radicals, from both air pollution and photoaging. Natural compounds from plant extracts are among sources of antioxidants and anti-hyaluronidase which have the ability to prevent antiaging. One of the potential fruits related to antioxidant and antiaging activities is *Ananas comosus*. *A. comosus* has a number of phenolic compounds with biological activities. One of the main phenolic compounds in *A. comosus* is luteolin. The aim of this study was to evaluate the antioxidant and antiaging potentials of pineapple core extract (PCE). This study was conducted at the Biomolecular and Biomedicine Research Center, Aretha Medika Utama from August to November 2018. Analysis of antioxidants from PCE and luteolin was carried out using H<sub>2</sub>O<sub>2</sub> scavenging activity assay. The antiaging assay was carried out through inhibition of hyaluronidase enzyme, one of the important enzymes in the aging process. Luteolin had lower IC<sub>50</sub> value of H<sub>2</sub>O<sub>2</sub> scavenging activity of around 24.12±3.13 µg/ml, which was better than PCE with IC<sub>50</sub> of 304.56±3.76 µg/mL. The results of hyaluronidase inhibition activity assay showed that luteolin compound had a lower IC<sub>50</sub> value of 67.38±3.99 µg/mL when compared to PCE with an IC<sub>50</sub> value of 161.15±1.05 µg/mL. Hence, Luteolin has higher antioxidant and anti-hyaluronidase activities than PCE

**Key words:** Antiaging, antioxidants, anti-hyaluronidase, luteolin, pineapple core extract

## Perbandingan Aktivitas Antioksidan dan Antihialuronidase Ekstrak Bonggol Buah Nanas (EBN) dengan Senyawa Luteolin

### Abstrak

Terdapat dua jenis proses penuaan kulit, penuaan kronologis dan penuaan dini. Penuaan dini umumnya disebabkan oleh radikal bebas, baik dari polusi udara maupun *photoaging*. Penggunaan antioksidan dan antihialuronidase untuk mencegah proses penuaan telah banyak diketahui. *Ananascomosus* merupakan salah satu tanaman yang memiliki potensi sebagai antipenuaan dan antioksidan karena mengandung sejumlah senyawa fenolik yang memiliki berbagai aktivitas biologis. Salah satu senyawa fenolik utama dalam *A. comosus* adalah *luteolin*. Tujuan penelitian ini adalah untuk mengevaluasi potensi antioksidan dan *anti-aging* dari Ekstrak Bonggol Nanas (EBN) dan senyawa pembandingnya yaitu *luteolin*. Penelitian ini dilakukan di Biomolecular and Biomedicine Research Center, Aretha Medika Utama dari Agustus hingga November 2018. Pengujian antioksidan dari EBN dan *luteolin* dilakukan menggunakan uji pemerangkapan H<sub>2</sub>O<sub>2</sub>. Uji antipenuaan dilakukan melalui uji penghambatan aktivitas hialuronidase, salah satu enzim penting dalam proses penuaan. Hasil penelitian menunjukkan bahwa Luteolin memiliki nilai IC<sub>50</sub> aktivitas pemerangkapan H<sub>2</sub>O<sub>2</sub> lebih rendah sekitar 24.12±3.13 µg/mL lebih baik daripada EBN dengan IC<sub>50</sub> sebesar 304.56±3.76 µg/mL. Hasil uji aktivitas penghambatan hialuronidase, senyawa *luteolin* memiliki nilai IC<sub>50</sub> lebih rendah, yaitu sebesar 67,38±3,99 µg/mL dibanding dengan EBN nilai IC<sub>50</sub> 161,15±1,05 µg/mL. Hasil ini menunjukkan bahwa senyawa pembanding *luteolin* memiliki aktivitas antioksidan dan antihialuronidase yang lebih tinggi daripada EBN.

**Kata kunci:** Antihialuronidase, antioksidan, antipenuaan, ekstrak bonggol nanas, *luteolin*

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## Introduction

Ultraviolet (UV) irradiation and air pollution exposure cause various diseases in humans today. UV irradiation and air pollution are the main source of exogenous oxidants in the form of free radicals originating from outside the human body.<sup>1</sup> Ultraviolet exposures and oxidants from the air can accelerate aging.<sup>2</sup> The age and the continuous exposure of UV light will constantly change the structure of the skin, especially the dermis. This layer contains many collagen, elastin, and proteoglycan fibers, one of which is hyaluronic acid. Hyaluronic acid normally functions as a binding site for collagen and elastin fibers. The increasing of hyaluronidase enzyme due to induction from ultraviolet light and exogenous oxidant will cause signs of aging, such as wrinkles.<sup>3</sup>

Antioxidant is a molecule that has the ability to delay the aging process by maintaining oxidant levels with or without free radical activities. Antioxidants can be given in the form of food supplements for the body or used in skin cares as cosmetic products that act as anti-aging molecules because antioxidants prevent free radicals.<sup>4-6</sup>

It has been known that natural products, such as plant extracts, have good abilities to inhibit aging.<sup>7</sup> Plant extracts have been known having antioxidant activities and are able to inhibit aging-related enzymes that damage extracellular matrix in the skin tissue. Antioxidant and anti-aging activities in plant extracts are caused by natural compounds in the plant extract. The results showed various compounds isolated from plants such as, gallic acid,  $\beta$ -carotene, catechins,<sup>7</sup> eugenol, hesperidin,<sup>6</sup> and myricetin<sup>8</sup> have good antioxidant and anti-aging activities.

Pineapple fruit is a tropical plant containing various bioactive compounds, such as luteolin, quercetin, myricetin. Luteolin is a bioactive flavone compound.<sup>9</sup> Luteolin can also be found in various vegetables such as celery, thyme, green peppers, tea and citrus fruits, grapes, and lemons. This compound can act as an antioxidant that has the ability to fight free radicals.<sup>10,11</sup>

Therefore, it is needed to evaluate the potential of antioxidant and anti-hyaluronidase activity of pineapple core extract (PCE) compared to luteolin compounds through antioxidant activity ( $H_2O_2$  scavenging activity assay) and hyaluronidase inhibitory activity assay.

## Methods

This study was conducted at Biomolecular and Biomedicine Research Center, Aretha Medika Utama from August to November 2018. Pineapple core was obtained from Tambaksari Village, Subang Regency. This study was limited to only in vitro test. The plants were identified at Herbarium Bandungense, Department of Biology, School of Life Science and Technology, Bandung, West Java.

Pineapple core that had been removed from the flesh was dried in a food dehydrator at 70°C for 48 hours then the dried simplicial of pineapple core was made into powder. The powder of pineapple core simplicial was then extracted with 70% distilled ethanol for 24 hours using maceration technique. Ethanol filtrate was filtered and re-macerated in triplicates, and then, the filtrate was evaporated with a vacuum rotary evaporator heater at 70°C.<sup>6,12,13</sup> The yield of Pineapple Core Extract (PCE) was 10.08 g. The luteolin used in this study was purchased from Chengdu Biopurify Phytochemical (Chengdu, PB0896). Antioxidant and anti-hyaluronidase potential of PCE and Luteolin were assessed using in vitro assay. The antioxidant potential was measured using  $H_2O_2$  scavenging activity assay and the anti-aging potential of the figure was assessed using anti-hyaluronidase assay.

$H_2O_2$  scavenging activity was measured using the method described by Mukhopadhyay et al.<sup>14</sup> with modifications. The solution mixture was made as shown in Table 1. After adding  $H_2O_2$ , a mixture of control, sample, and blank solutions which were inserted into the 96-well plate was incubated for 5 minutes in a dark room at room temperature condition. Thereafter each mixture of sample and blank was added 75  $\mu$ L 1,10-phenanthroline, then incubated again for 10 minutes in a dark room at room temperature condition. The absorption was then measured using spectrophotometer with 510 nm wavelength. The percentage of scavenging activities was calculated using the equation:

$$\% \text{ scavenging activity} = S/C \times 100\%,$$

When S is sample absorbance and C is control absorbance.

Hyaluronidase Inhibitory Activity Assay was conducted by a method developed by Thring et al.<sup>15</sup> and Widowati et al.<sup>4,8</sup>. Twenty five (25)  $\mu$ L

**Table 1 Solution Composition Used in H<sub>2</sub>O<sub>2</sub> Radical Scavenging Activity Assay**

Reagents	Control	Test Sample	Blank
Sample	-	60 µL	60 µL
<i>Ferrous Ammonium Sulfate</i> (1 mM)	12 µL	12 µL	-
DMSO	63 µL	-	90 µL
H <sub>2</sub> O <sub>2</sub> (5 mM)	-	3 µL	-
1,10-phenanthroline (1 mM)	-	75 µL	75 µL

of PCE from serial concentration (166.67; 83.33; 41.67; 20.83; 10.42; 5.21 µg/mL) was mixed with 3 µL hyaluronidase enzyme from the bovine testis (0.4 U/mL in a 20 mM phosphate buffer, pH 7 in 77 mM sodium chloride and 0.01% serum albumin bovine), and 12 µL phosphate buffer (300 mM, pH 5.35) was incubated for 10 minutes at 37°C. Afterwards, 10 µL hyaluronic acid substrate (0.03% in 300 mM phosphate buffer, pH 5.35) was incubated for 45 minutes at 37°C. The decomposition reaction of hyaluronic acid was stopped by adding albumin acid 100 µL (24 mM sodium acetate, 79 mM acetic acid and 0.1% BSA.) The mixture solution was incubated at room temperature for 10 minutes, then the absorbance was measured at 600 nm wavelength using spectrophotometer. The percentage of enzyme inhibition activity was measured by the equation below:

$$\text{Hyaluronidase Inhibition (\%)} = \frac{(-C/S) \times 100\%}{\text{absorbance.}^{6,8,13,15}}$$

When C is control absorbance and S is sample absorbance.

The results were analyzed using the SPSS program with One-Way ANOVA test and followed by Post Hoc Tukey HSD test. Furthermore,

inhibitory concentration 50 (IC<sub>50</sub>) values of antioxidant and anti-aging activity were calculated.

### Results

The H<sub>2</sub>O<sub>2</sub> scavenging activity assay showed that the luteolin had a better H<sub>2</sub>O<sub>2</sub> scavenging activity than PCE. The results of H<sub>2</sub>O<sub>2</sub> scavenging activity were shown in Figure 1. The H<sub>2</sub>O<sub>2</sub> scavenging activities of PCE and luteolin increased with the increasing of the sample concentrations (concentration dependent manner). Luteolin had a lower IC<sub>50</sub> value of 24.12±3.13 µg/mL than PCE with IC<sub>50</sub> value around 304.56±3.76 µg/mL (Table 2), which means that luteolin had a better antioxidant activity than PCE.

The hyaluronidase inhibitory activity was performed through a spectrophotometric method to measure hyaluronidase activity and to detect the potential hyaluronidase inhibitor. The hyaluronidase inhibitory activity assay showed that luteolin had a better inhibitory activity than PCE (Figure 2). The hyaluronidase inhibitory activities of PCE and luteolin increased with the increasing of the sample concentrations

**Table 2 Solution Composition Used in H<sub>2</sub>O<sub>2</sub> Radical Scavenging Activity Assay**

Sample	Linear Regression	R2	IC50 (µg/mL)	IC50 (µg/mL)
PCE	y=0.1345x+9.4135	0.95	301.76	304.56±3.76
	y=0.1323x +9.8993	0.94	303.10	
	y=0.1292x+10.099	0.93	308.83	
	y=0.132x+9.8038	0.94	304.52	
Luteolin	y=0.1068x+47.053	0.96	27.59	24.12±3.13
	y=0.1103x+47.627	0.92	21.51	
	y=0.1094x+47.457	0.93	23.24	
	y=0.1088x+47.379	0.94	24.09	

\*Linear equations, coefficient of regression (R2) and IC50 of each sample were calculated; \*PCE = pineapple core extract

**Table 3 IC<sub>50</sub> Values of Hyaluronidase Inhibitory Activities of PCE and Luteolin**

Sample	Linear Regression	R <sup>2</sup>	IC50 (µg/mL)	IC50 (µg/mL)
PCE	Y=0.1345x+9.4135	0.99	162.72	161.15±1.05
	Y=0.323x+9.8993	0.99	160.81	
	Y=0.1292x+10.099	0.93	161.01	
	Y=0.132x+9.8038	0.93	161.08	
Luteolin	Y=0.1068x+47.053	0.96	69.21	67.38±3.99
	Y=0.1103x+47.627	0.92	70.12	
	Y=0.1094x+47.457	0.93	62.80	
	Y=0.1088x+47.379	0.94	67.21	

\*Linear equations, coefficient of regression (R2) and IC50 of each sample were calculated; \*PCE = pineapple core extract

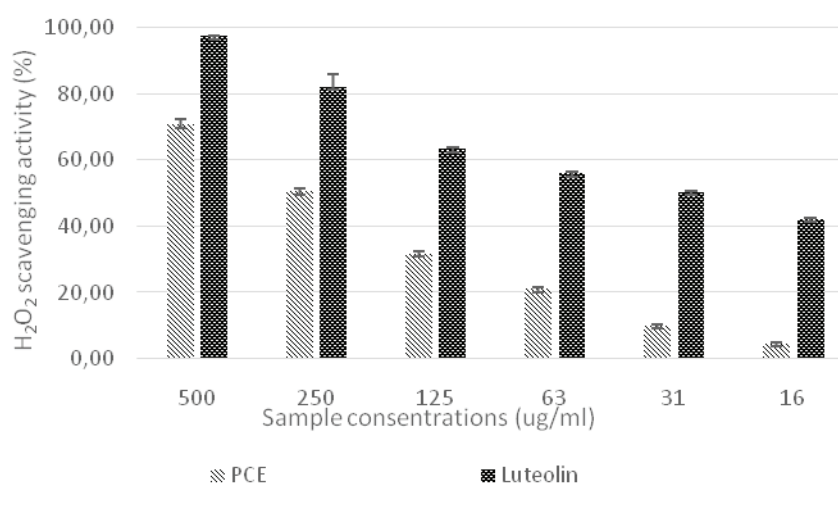
(concentration dependent manner). The hyaluronidase inhibitory activities of PCE and luteolin based on IC<sub>50</sub> value were shown in Table 3. Table 3 showed that luteolin had a lower IC<sub>50</sub> value of 67.38±3.99 µg/mL than PCE with IC<sub>50</sub> value around 161.15 ± 1.05 µg/mL (Table 3). The IC<sub>50</sub> value is the concentration of the sample that inhibits 50% of hyaluronidase activity.

### Discussion

Pineapple (*A. comosus*) is a tropical plant that has various active compounds. Pineapple contains

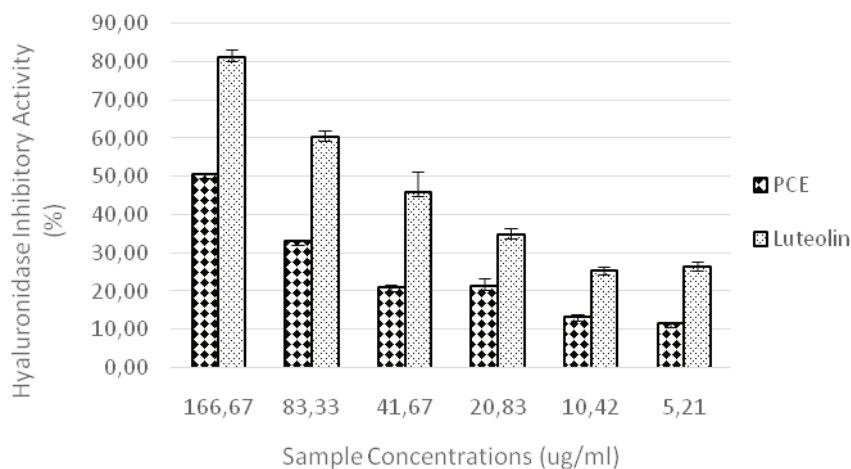
several active compounds such as quercetin, myricetin, luteolin.<sup>9</sup> This shows that pineapple contains many active compounds with various biological activities, one of which is luteolin. Luteolin is an important component in pineapple which has a variety of biological activities.<sup>10</sup>

The results of the study regarding the scavenging activities of H<sub>2</sub>O<sub>2</sub> free radicals by both PCE and luteolin compounds (Table 2 and Figure 1) showed good antioxidant activities. The IC<sub>50</sub> value of H<sub>2</sub>O<sub>2</sub> scavenging activity from Luteolin was 24.12±3.13 µg/mL, smaller than PCE which had IC<sub>50</sub> value of 304.56±3.76 µg/mL. Widowati et al.<sup>8</sup> classified antioxidant capacity based on IC<sub>50</sub>



**Figure 1 H<sub>2</sub>O<sub>2</sub> Scavenging Activities of PCE and Luteolin**

\*The data was presented as mean ± standard deviation. Luteolin and PCE were diluted in DMSO of 10% to reach the final concentration of 166.67; 83.33; 41.67; 20.83; 10.42; 5.21 (µg/mL). The H<sub>2</sub>O<sub>2</sub> scavenging activities of the samples in different concentration were statistically different in α<0.05. \*PCE = pineapple core extract



**Figure 2 Hyaluronidase Inhibitory Activities of PCE and Luteolin. Luteolin had a higher Hyaluronidase Inhibitory Activity than PCE. The Hyaluronidase Inhibitory Activities of PCE and Luteolin Increased with the Increasing of the Concentration of Sample**

\*The data was presented as mean  $\pm$  standard deviation. Luteolin and PCE were diluted in DMSO of 10% to reach the final concentration of 166.67; 83.33; 41.67; 20.83; 10.42; 5.21 ( $\mu\text{g/mL}$ ). The hyaluronidase inhibitory activities of the samples in different concentration were statistically different in  $\alpha < 0.05$ ; \*PCE = pineapple core extract

value. A compound has a very strong antioxidant if  $\text{IC}_{50}$  is worth  $< 50$  ppm, is strong if  $\text{IC}_{50}$  is worth 50–100 ppm, while  $\text{IC}_{50}$  is worth 100–150 ppm, and weak if  $\text{IC}_{50}$  is worth 151–200 ppm or more. Based on this classification, it can be concluded that luteolin has a very high antioxidant activity and PCE has a weak antioxidant activity.<sup>8</sup>

This study is in line with other studies that indicated that luteolin is a compound that has a good antioxidant activity.<sup>11</sup> The results of this study about  $\text{H}_2\text{O}_2$  scavenging activity of PCE indicated that PCE has a very weak antioxidant activity because it has an  $\text{IC}_{50}$  value more than 200  $\mu\text{g/mL}$  ( $304.56 \pm 3.76 \mu\text{g/mL}$ ). At present, there are no publications regarding the antioxidant activity of PCE. The study from Fidrianny et al.<sup>16</sup> that conducted antioxidant assay of the peel, flesh and bractea of pineapple fruit showed that the antioxidant activities of the peel, flesh, and bractea are very strong. The  $\text{IC}_{50}$  value from fruit flesh and the peel extract from Bogor showed a value below 50 ppm. This indicated that PCE is a part of pineapple fruit which has the lowest antioxidant activity. It is expected to compare the antioxidant activities between luteolin and PCE as an active compound which are found in pineapple core.  $\text{H}_2\text{O}_2$  is a radical compound found in many tissues. The higher the  $\text{H}_2\text{O}_2$  trapped by the active compounds, the higher the antioxidant activity has.  $\text{IC}_{50}$  values are used to represent

antioxidant and anti-aging activities on PCE and luteolin. The smaller  $\text{IC}_{50}$  values, the better the ability of the active compounds in the activity of trapping free radicals. Table 2 showed that PCE had a lower antioxidant activity than luteolin indicated by the  $\text{IC}_{50}$  values. The antioxidant activity of natural compound was determined by the amount of hydroxyl group. The hydroxyl group in the phenolic compound will donate the hydrogen ion to the chain carrying  $\text{ROD}^*$  radicals.<sup>6,8</sup> The antioxidant ability of a compound is closely related to its anti-aging potential. This is because free radicals found in skin tissue are the main cause of tissue damage. Therefore, compounds having high antioxidant capacity can prevent both chronological and premature aging.<sup>17</sup> The results of this study indicated that luteolin has a great potential to be developed into compounds for the prevention of premature aging.

The results showed that the PCE inhibitory activity of hyaluronidase and its comparative compounds showed a hyaluronidase inhibition activity with  $\text{IC}_{50}$  values of  $67.38 \pm 3.99 \mu\text{g/mL}$  for luteolin and  $161.15 \pm 1.05 \mu\text{g/mL}$  for PCE. The inhibition activity of hyaluronidase from luteolin was classified into a strong inhibitor while the inhibitory activity of luteolin was classified into a weak inhibitory activity. This was also based on the results obtained by Tu & Tawata,<sup>18</sup>

stating that the IC<sub>50</sub> value of an extract for anti-hyaluronidase ranging from 50–100 ppm is considered as a strong inhibitor.

Hyaluronidase is an enzyme that plays a role in the degradation of hyaluronic acid (HA). Hyaluronic acid (HA) is a key compound in the aging process and a glycosaminoglycan polymer that plays a role in tissues. Hyaluronic acid (HA) maintains water levels in the skin, which means it can act as a molecule that can increase skin moisture. Besides its role mentioned above, HA is also an extracellular matrix (ECM) molecule that maintains the elasticity of the skin,<sup>19</sup> which can be degraded by the hyaluronidase enzyme. In humans, there are 6 types of hyaluronidase enzymes. Hyaluronic acid (HA) degradation occurs through hydrolysis of 1,4-hexosaminidic bonds between N-acetyl-D-glucosamine and D-glucuronic acid which are residues of HA.<sup>20</sup> Besides being able to be crushed by enzymes, HA can also be degraded non-enzymes by free radicals when there are reducing compounds such as ascorbic acid, thiol, iron ions, and copper ions. Therefore, the use of ingredients that have an antioxidant activity can reduce the degradation of HA in tissues.<sup>19</sup>

In conclusion, PCE has a lower antioxidant and antihyaluronidase than luteolin. Based on the IC<sub>50</sub> values, it showed that antioxidant activity and antihyaluronidase from PCE are less effective than luteolin. Hence, luteolin has more potential as anti-aging considering antioxidant and anti-hyaluronidase.

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