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 *Anana scomosus*. *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 H2O2 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 IC50 value of H2O2 scavenging activity of around 24.12±3.13 μg/ml, which was better than CPE with IC50 of 304.56±3.76μg/mL. The results of hyaluronidase inhibition activity assay showed that luteolin compound had a lower IC50 value of 67.38±3.99 μg/mL when compared to PCE with an IC50 value of 161.15±1.05 μg/mL. Hence, luteolin has higher antioxidant and anti-hyaluronidase activities than PCE

**Key words:** Antioxidant, Luteolin, anti-hyaluronidase, pineapple core extract

Perbandingan Aktivitas Antioksidan dan Anthihialuronidase 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 anti-hialuronidase untuk mencegah proses penuaan telah banyak diketahui. *Ananascomosus* merupakan salah satu tanaman yang memiliki potensi sebagai antioksidan 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 H₂O₂. Uji antipenuaan dilakukan melalui uji penghambatan aktivitas hialuronidase, salah satu enzim penting dalam proses penuaan. Hasil penelitian menunjukkan bahwa Luteolin memiliki nilai IC₅₀ aktivitas pemerringkapan H₂O₂ lebih rendah sekitar 24.12±3.13 μg/ml lebih baik daripada EBN dengan IC₅₀ sebesar 304.56±3.76μg/mL. Hasil uji aktivitas penghambatan hialuronidase, senyawa luteolin memiliki nilai IC₅₀ lebih rendah, yaitu sebesar 67.38±3.99 μg/mL dibanding dengan EBN nilai IC₅₀ 161.15±1.05μg/mL. Hasil ini menunjukkan bahwa senyawa pembanding luteolin memiliki aktivitas antioksidan dan antihialuronidase yang lebih tinggi daripada EBN.

**Kata kunci:** Antioksidan, antiaging, luteolin, ekstrak bonggol nanas

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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. Ultraviolet exposures and oxidants from the air can accelerate aging. 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.

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.

It has been known that natural products, such as plant extracts, have good abilities to inhibit aging. 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, β-carotene, catechins, eugenol, hesperidin, and myricetin 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. 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.

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. 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. 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 μL 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:

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

When $S$ is sample absorbance and $C$ is control absorbance.

Hyaluronidase Inhibitory Activity Assay was conducted by a method developed by Thring et al. and Widowati et al. Twenty five (25) 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:

\[ \text{Hyaluronidase Inhibition} (\%) = \left( \frac{C - S}{C} \right) \times 100\% \]

When C is control absorbance and S is sample absorbance.\(^6,8,13,15\)

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\(_{50}\)) values of antioxidant and anti-aging activity were calculated.

**Results**

The H\(_2\)O\(_2\) scavenging activity assay showed that the luteolin had a better H\(_2\)O\(_2\) scavenging activity than PCE. The results of H\(_2\)O\(_2\) scavenging activity were shown in Figure 1. The H\(_2\)O\(_2\) scavenging activities of PCE and luteolin increased with the increasing of the sample concentrations (concentration dependent manner). Luteolin had a lower IC\(_{50}\) value of 24.12±3.13 µg/mL than PCE with IC\(_{50}\) value around 304.56±3.76 µg/mL (Table 2), which means that luteolin had a better antioxidiant 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 1 Solution Composition Used in H\(_2\)O\(_2\) Radical Scavenging Activity Assay**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Control</th>
<th>Test Sample</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous Ammonium Sulfate (1 mM)</td>
<td>12 µL</td>
<td>12 µL</td>
<td>-</td>
</tr>
<tr>
<td>DMSO</td>
<td>63 µL</td>
<td>-</td>
<td>90 µL</td>
</tr>
<tr>
<td>H(_2)O(_2) (5 mM)</td>
<td>-</td>
<td>3 µL</td>
<td>-</td>
</tr>
<tr>
<td>1,10-phenanthroline (1 mM)</td>
<td>-</td>
<td>75 µL</td>
<td>75 µL</td>
</tr>
</tbody>
</table>

**Table 2 Solution Composition Used in H\(_2\)O\(_2\) Radical Scavenging Activity Assay**

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

*Linear equations, coefficient of regression (R2) and IC50 of each sample were calculated; *PCE = pineapple core extract
Hyaluronidase inhibitory activities of PCE and luteolin based on IC50 value were shown in Table 3. The IC50 value of PCE was 161.15 ± 1.05 µg/mL, while the IC50 value of luteolin was 67.38 ± 3.99 µg/mL. The IC50 value for each sample 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, including quercetin, myricetin, and luteolin. This shows that pineapple contains many active compounds with various biological activities, one of which is luteolin. Luteolin is an important component in pineapple with a variety of biological activities.

The results of the study regarding the scavenging activities of H2O2 free radicals by both PCE and luteolin compounds (Table 2 and Figure 1) showed good antioxidant activities. The IC50 value of H2O2 scavenging activity from luteolin was 24.12 ± 3.13 µg/mL, smaller than PCE which had an IC50 value of 304.56 ± 3.76 µg/mL. Widowati et al. classified antioxidant capacity based on IC50.

### Table 3 IC50 Values of Hyaluronidase Inhibitory Activities of PCE and Luteolin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression</th>
<th>R2</th>
<th>IC50 (µg/mL)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCE</td>
<td>Y=0.1345x+9.4135</td>
<td>0.99</td>
<td>162.72</td>
<td>161.15±1.05</td>
</tr>
<tr>
<td></td>
<td>Y=0.323x+9.8993</td>
<td>0.99</td>
<td>160.81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=0.1292x+10.099</td>
<td>0.93</td>
<td>161.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=0.132x+9.8038</td>
<td>0.93</td>
<td>161.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=0.1068x+47.053</td>
<td>0.96</td>
<td>69.21</td>
<td></td>
</tr>
<tr>
<td>Luteolin</td>
<td>Y=0.1103x+47.627</td>
<td>0.92</td>
<td>70.12</td>
<td>67.38±3.99</td>
</tr>
<tr>
<td></td>
<td>Y=0.1094x+47.457</td>
<td>0.93</td>
<td>62.80</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Y=0.1088x+47.379</td>
<td>0.94</td>
<td>67.21</td>
<td></td>
</tr>
</tbody>
</table>

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

![Graph of H2O2 Scavenging Activities of PCE and Luteolin](image)

### Figure 1 H2O2 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 H2O2 scavenging activities of the samples in different concentration were statistically different in α<0.05. *PCE = pineapple core extract.
value. A compound has a very strong antioxidant if IC$_{50}$ is worth <50 ppm, is strong if IC$_{50}$ is worth 50–100 ppm, while IC$_{50}$ is worth 100–150 ppm, and weak if 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.

This study is in line with other studies that indicated that luteolin is a compound that has a good antioxidant activity. The results of this study about H$_2$O$_2$ scavenging activity of PCE indicated that PCE has a very weak antioxidant activity because it has an IC$_{50}$ value more than 200 µg/mL (304.56±3.76 µg/mL). At present, there are no publications regarding the antioxidant activity of PCE. The study from Fidrianny et al. 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 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. H$_2$O$_2$ is a radical compound found in many tissues. The higher the H$_2$O$_2$ trapped by the active compounds, the higher the antioxidant activity has.

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 ± 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 hyaluronidase inhibitory activities of the samples in different concentration were statistically different in α<0.05.

Table 2 showed that PCE had a lower antioxidant activity than luteolin indicated by the 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 ROD* radicals. 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. 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 IC$_{50}$ values of 67.38±3.99 µg/mL for luteolin and 161.15±1.05 µg/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.
stating that the IC_{50} 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, 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. Besides being able to be churned 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.

In conclusion, PCE has a lower antioxidant and antihyaluronidase than luteolin. Based on the IC_{50} 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.

References


