Antioxidant and Anti-Hyaluronidase Activities of Dragon Fruit Peel Extract and Kaempferol-3-O-Rutinoside

Aktivitas Antioksidan dan Anti-Hyaluronidase Ekstrak Kulit Buah Naga dan Kaempferol-3-O-Rutinoside

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ABSTRACT

Aging is a natural process in human life and is triggered by the presence of free radicals (ROS). The use of antioxidants from natural ingredients is one of the breakthroughs to overcome aging and counteract the harmful effects caused by the free radicals. This study aimed to determine and compare the antioxidant activity of H₂O₂ scavenging and hyaluronidase inhibition of red dragon fruit peel extract (DFPE) and kaempferol-3-o-rutinoside (KOR) compounds. Dragon fruit peel extract (DFPE) is obtained through extraction by maceration method using 70% ethanol solvent. The design of this study included antioxidant and anti-aging activity assay of EKBN and KOR at the series concentration of 15.63; 31.25; 62.50; 125; 250; 500 µg/mL through H₂O₂ scavenging, as well as the DFPE and KOR hyaluronidase inhibition assay at the series concentration of 5.21; 10.42; 20.83; 41.7; 83.33; 166.67 µg/mL. EKB shows that the average activity of H₂O₂ scavenging is lower than KOR. In addition, the IC₅₀ values of KOR for H₂O₂ scavenging is lower (351.46±2.30µg/mL) than DFPE (409.64±23.17µg/mL). While, KOR also has higher values of inhibitory activity than of the DFPE. However, the IC₅₀ value of KOR for hyaluronidase inhibition was 84.07±10.46µg/mL, equivalent to the IC₅₀ value of DFPE (85.32±10.24µg/mL). The presence of antioxidant and anti-aging activity in the EKBN is probably caused by betalain and the KOR compound itself contained in red dragon fruit. The results of the paired-samples T-test on antioxidant activity and anti-aging of DFPE and KOR showed non-significant difference. Thus, DFPE has an equivalent antioxidant and anti-aging through H₂O₂ scavenging and hyaluronidase activity as possessed by the KOR compound.

Keywords: Aging, anti-hyaluronidase, dragon fruit peel extract, kaempferol, H₂O₂ scavenging

ABSTRAK

Penuaan merupakan proses alamiah dalam kehidupan manusia yang salah satunya dipicu oleh keberadaan radikal bebas (ROS). Penggunaan antioksidan dari bahan alami merupakan salah satu upaya untuk mengatasi penuaan dan menangkal efek bahaya yang ditimbulkan oleh radikal bebas. Penelitian ini bertujuan untuk menentukan dan membandingkan aktivitas antioksidan pemerangkapan H₂O₂ dan anti-aging penghambatan hialuronidase ekstrak kulit buah naga merah (EKBN) dan senyawa kaempferol-3-o-rutinoside (KOR). Ekstrak kulit buah naga merah (EKBN) didapatkan melalui ekstraksi yang dilakukan menggunakan metode maserasi dengan pelarut etanol 70%. Desain penelitian ini meliputi uji aktivitas antioksidan EKBN dan KOR pada seri konsentrasi 15,63; 31,25; 62,50; 125; 250; 500 µg/mL melalui pemerangkapan H₂O₂, serta uji penghambatan hyaluronidase EKBN dan KOR pada seri konsentrasi 5,21; 10,42; 20,83; 41,7; 83,33; 166,67µg/mL. EKBN menunjukkan rata-rata aktivitas pemerangkapan H₂O₂ yang lebih rendah dibandingkan KOR. Selain itu, perhitungan nilai IC₅₀ menunjukkan pemerangkapan H₂O₂ KOR lebih rendah (351,46±23.09µg/mL) dibandingkan EKBN (409,64±23.17µg/mL). Sementara KOR juga mempunyai rata-rata aktivitas penghambatan yang lebih besar dibandingkan EKBN. Namun, nilai IC₅₀ KOR pada uji penghambatan hialuronidase adalah 84,07±10,46µg/mL setara dengan nilai IC₅₀ EKBN (85,32±10,24µg/mL). Adanya aktivitas antioksidan dan anti-aging pada EKBN diduga disebabkan oleh betalain dan senyawa KOR itu sendiri yang terkandung pada buah naga merah. Hasil uji paired-samples T-test terhadap aktivitas antioksidan dan anti-aging EKBN dan KOR menunjukkan perbedaan aktivitas yang tidak signifikan. Dengan demikian, EKBN mempunyai kapasitas antioksidan pemerangkapan H₂O₂ dan anti-aging penghambatan aktivitas hialuronidase yang setara dengan senyawa KOR.

Kata Kunci: Antihiperoksidan, ekstrak kulit buah naga, kaempferol-3-O-rutinoside, pemerangkapan H₂O₂

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INTRODUCTION

Aging is a natural process in human life and closely related to various regenerative processes (1). Intrinsic and extrinsic factors influence the aging process in skin tissue. Intrinsic aging is an aging process that cannot be prevented and occurs over time at cellular level. Intrinsic aging is affected by changes in sex hormone production associated with increased age. The biological process plays a role in determining the amount of multiplication in each cell until the cell stops dividing and then dies. Extrinsic aging is caused by an exposure to external factors, especially UV light. Thus, it is often referred to as photoaging. The other factors of extrinsic aging include pollution, smoking habits, and unbalanced nutrition (2,3).

The existence of free radicals due to oxidative processes is a mechanism mostly used to explain the occurrence of the aging process. Free radicals are molecules that have one or more unpaired electrons in their outer orbit, so they are relatively unstable or often referred to as Reactive Oxygen Species (ROS) (4,5). These molecules have an action mechanism to take electrons from various vital components of cells, such as DNA, cytoskeleton, protein, and cell membranes, and cause damage at the cellular level and accelerate the aging process (6).

Currently, many research are conducted to prevent skin aging. The use of antioxidants is one of the efforts to overcome skin aging besides the use of anti-aging cosmetics because antioxidants can counteract the harmful effects caused by free radicals. In addition to antioxidants, some inhibitor compounds for extracellular matrix enzymes degradation such as elastase, hyaluronidase, and collagenase have been known to play an important role in preventing skin aging (7). Hyaluronidase inhibitors work by preventing hyaluronidase activity which degrades hyaluronic acid. Hyaluronic acid is an important component of Extra Cellular Matrix (ECM) that plays a role in maintaining skin moisture and its elasticity. There are many cosmetics containing synthetic hyaluronic acid to prevent skin aging available world-wide. However, people or scientist tend to develop natural cosmetics due to their minimum safety risks and side effects compared to the synthetic one (8).

Biodiversity of Indonesia provides variety of products that contain antioxidant activities, red dragon fruit (Hylocereus polyrhizus) is one of them. Besides utilizing the fruit, it turns out that its peel can also be processed into basic ingredients for cosmetics making and natural food coloring. This is because the dragon fruit peel contains many compounds that can be used as antioxidants including betalain and anthocyanin. In addition, dragon fruit peel also contains vitamin C, Vitamin E, vitamin A, alkaloids, terpenoids, flavonoids, thiamine, nicacin, pyridoxine, cobalamin, phenolic, carotene, and phytoalbumin (9). Kaempferol-3-o-rutinoside (KOR) is one of the flavonoids found in dragon fruit (10). Many studies have been conducted to determine the benefits of KOR such as antioxidants, anti-inflammatory, antimicrobial, anticancer, heart disease prevention, neurological diseases, antiabetic, antiosteoporosis, antiastrogenic, analgesic, and hypo-allergenic. In addition to antioxidants compounds, red dragon fruit also found to have anti-aging properties. KOR is also a strong compound that can inhibit hyaluronidase enzymes (11).

This study aimed to determine the antioxidant activity and anti-aging of dragon fruit peel extract and make a comparison with KOR compound through the analysis of H₂O₂ scavenging and hyaluronidase inhibition assay. Thus, DFPE which has been a waste can be utilized as an antioxidant and anti-aging agent derived from natural products.

METHOD

Preparation of Dragon Fruit Peel Extract (DFPE)

The production of ethanol extract was carried out by the maceration method using 70% ethanol solvent. An amount of 500g of dried red dragon fruit peel that has been sliced and placed into a glass container was added with 3.75L ethanol 70% and then covered using the lid. The sample was left for five days and protected from light. It was then wrapped using flannel cloth and stirred until a liquid extract solution was obtained. The filtered pulp was macerated again with 1.25L ethanol solution. Then, the second step of filtration was conducted, and the filter result was combined with the first result. The result obtained was concentrated with a rotary evaporator until all the solvents evaporated and followed by the evaporation process above water bath until obtaining a paste-form extract of dragon fruit peel (12-14).

H₂O₂ Scavenging Activity Assay

Radical scavenging activity of H₂O₂ was measured based on a method modified by Mukhopadhyay et al. (15). Previously, Dragon Fruit Peel Extract (DFPE) was obtained from extraction and Kaempferol-3-o-rutinoside (KOR) was purchased from Chengdu Biopurify Phytochemicals Ltd. (Chengdu, BP0823). The mixture of the solution was made by adding 60µL of various concentrations of extract sample and compounds (15.63-500µg/mL) into the sample and blank well. As much as 12µL of Ferrous Ammonium Sulfate (1 mM, Sigma 7783859) was added into the control and sample, H₂O₂ solution (5mM, Merck 1.08597.1000) was added to the sample well only, 63µL 10% DMSO was added into control well, and 90µL 10% DMSO into blank well. The mixture was incubated in a dark room at room temperature for 5 minutes. Each sample and control well was added with 75µL 1,10-phenanthroline (1mM, Sigma 131377). The plate was then incubated for 10 minutes in a dark room and at room temperature. The absorbances were measured using spectrophotometry at 510 nm wavelength (12-14). The percentage of scavenging activities was calculated using this following formula:

\[
\text{H}_2\text{O}_2 \text{ Scavenging activity } = \frac{\text{sample absorbance}}{\text{control absorbance}} \times 100\%
\]

Hyaluronidase Inhibitory Activity Assay

Hyaluronidase inhibitory activity was measured by a modified method from Sigma Aldrich and Tu and Kawata (12-14,16). A mix of 25µL of various concentrations of sample and compound extract (5.21-166.67µg/mL), 3µL hyaluronidase from bovine testes type I-S (Sigma H3506, USA), 12µL phosphate buffer (300 mM, pH 5.35) was incubated for 10 minutes at 37°C. Then, 10µL hyaluronic acid substrate (Sigma H5542, USA) was added and incubated for 45 minutes at 37°C. The reaction was stopped by adding 100µL acidic albumin acid. The mixed solution was then incubated at room temperature for 10 minutes. The absorbance was measured at 600 nm wavelengths (12-14). The inhibition activity was calculated using the following formula:
% Hyaluronidase inhibition = \left(1 - \frac{\text{sample absorbance}}{\text{control absorbance}}\right) \times 100\%

**Statistical Analysis**

Data from the results of H\textsubscript{2}O\textsubscript{2} scavenging and anti-hyaluronidase activities were analyzed using One-Way ANOVA and followed by the post hoc test Tukey HSD test (IBM SPSS 22) to determine the significance between each concentration of the DFPE and KOR. Paired Samples T-test (IBM SPSS 22) also was performed to analyze the significance difference between two different samples.

**RESULTS**

**H\textsubscript{2}O\textsubscript{2} Scavenging Activity**

Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is a free radical that is found in nature. The scavenging activity can be measured by the reaction of ferrous ammonium sulfate and phenanthroline. The complex reaction will produce an orange complex of Fe\textsuperscript{2+}-tri-phenanthroline. The presence of H\textsubscript{2}O\textsubscript{2} inhibits the formation of complex, but in the existence of antioxidants that scavenged H\textsubscript{2}O\textsubscript{2}, the complex will be formed again. Thus, the orange color formation indicates the occurrence of H\textsubscript{2}O\textsubscript{2} scavenging activity (15).

The average percentage of H\textsubscript{2}O\textsubscript{2} scavenging activity of KOR shown in Table 1 was higher compared to the scavenging activity of DFPE. There is a significant difference (p<0.05) between concentrations of both treatments, DFPE and KOR. It implied H\textsubscript{2}O\textsubscript{2} scavenging activity of DFPE and KOR compounds among concentrations of 500-15.63µg/mL were the modes that depend on concentration (Figure 1 and Table 1).

<table>
<thead>
<tr>
<th>Final Concentrations (µg/mL)</th>
<th>The average of H\textsubscript{2}O\textsubscript{2} scavenging activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dragon fruit peel extract</td>
</tr>
<tr>
<td>500.00</td>
<td>57.05±3.39 \textsuperscript{a}</td>
</tr>
<tr>
<td>250.00</td>
<td>36.06±0.48 \textsuperscript{a}</td>
</tr>
<tr>
<td>125.00</td>
<td>27.98±0.49 \textsuperscript{a}</td>
</tr>
<tr>
<td>62.50</td>
<td>18.65±0.31 \textsuperscript{a}</td>
</tr>
<tr>
<td>31.25</td>
<td>11.14±0.54 \textsuperscript{a}</td>
</tr>
<tr>
<td>15.63</td>
<td>5.04±0.44 \textsuperscript{a}</td>
</tr>
</tbody>
</table>

Note: Data were presented as mean ± standard deviation. Different lowercase letters in the same column are significant at p<0.05 (Tukey HSD post hoc test).

The IC\textsubscript{50} value of H\textsubscript{2}O\textsubscript{2} scavenging of DFPE and KOR were shown in Table 2. The results of IC\textsubscript{50} values calculation based on the linear regression equation obtained showed that the IC\textsubscript{50} value of KOR compound was lower (351.46±2.30µg/mL) than the IC\textsubscript{50} value produced by dragon fruit peel extract (409.64±23.17µg/mL). The smaller the IC\textsubscript{50} value, the better the ability of a compound to scavenge the free radicals (17). However, based on paired samples T-test (p<0.05) (Table 3), the whole H\textsubscript{2}O\textsubscript{2} scavenging activities of DFPE and KOR were not significantly different. It suggested, the DFPE had the same H\textsubscript{2}O\textsubscript{2} scavenging activity as it was revealed by the KOR.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Equation</th>
<th>R\textsuperscript{2}</th>
<th>IC\textsubscript{50} (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dragon fruit</td>
<td>Y = 0.0.090x+9.700</td>
<td>0.960</td>
<td>409.64±23.17</td>
</tr>
<tr>
<td>peel extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kaempferol-3-O-</td>
<td>Y = 0.122x+7.123</td>
<td>0.970</td>
<td>351.46±2.30</td>
</tr>
<tr>
<td>rutinoside</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *Linear equations, the coefficient of regression (R\textsuperscript{2}) and IC\textsubscript{50} of each sample were calculated.

**Hyaluronidase Inhibitory Activity**

Hyaluronidase is one of the metalloproteinases matrixes that function in degrading hyaluronic acid (HA), a component in the extracellular matrix (ECM). The mechanism of HA degradation by hyaluronidase is carried out through the catalysis of hyaluronic hydrolysis reactions. The hyaluronidase enzymes activity can be detected by measuring the HA levels. HA can be measured significantly different. It suggested, the DFPE had the same H\textsubscript{2}O\textsubscript{2} scavenging activity as it was revealed by the KOR.

The hyaluronidase inhibition activity of H\textsubscript{2}O\textsubscript{2} scavenging activity between KOR and DFPE are not significantly different (Table 3).

**Table 4. Hyaluronidase inhibition activity of H\textsubscript{2}O\textsubscript{2} DFPE and KOR**

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>The average of hyaluronidase inhibition activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DFPE</td>
</tr>
<tr>
<td>166.67</td>
<td>69.75±1.52\textsuperscript{a}</td>
</tr>
<tr>
<td>83.33</td>
<td>51.90±3.46\textsuperscript{a}</td>
</tr>
<tr>
<td>41.67</td>
<td>42.41±8.01\textsuperscript{a}</td>
</tr>
<tr>
<td>20.83</td>
<td>34.41±4.93\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Figure 1. Effect of various concentrations of DFPE and KOR toward H\textsubscript{2}O\textsubscript{2} scavenging activity. DFPE and KOR were diluted in DMSO 10% to reach the final concentration of 500; 250; 125; 62.50; 31.250; 15.63 (µg/mL)
Based on the results that is shown in Table 3, the hyaluronidase inhibition activity of KOR compound only higher at the highest concentration compared to that of dragon fruit peel extract. Also, there was a decrease in hyaluronidase inhibition activity from high concentrations to low concentrations (Figure 2). Only concentrations between 166.67µg/mL and 83.33µg/mL of both DFPE and KOR compounds had significant differences (p<0.05).

Table 4. Hyaluronidase inhibition activity of H2O2, DFPE and KOR

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>The average of hyaluronidase inhibition activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.83</td>
<td>34.41±4.93abc</td>
</tr>
<tr>
<td>10.42</td>
<td>26.48±1.62a</td>
</tr>
<tr>
<td>5.21</td>
<td>24.32±4.59a</td>
</tr>
</tbody>
</table>

Note: Data were presented as mean ± standard deviation. Different lowercase letters in the same column are significant at p<0.05 (Tukey HSD post hoc test).

Figure 2. Effects of various concentrations of DFPE and KOR on hyaluronidase inhibition activity. DFPE and KOR were diluted in DMSO 10% to reach the final concentration of 166.67; 83.33; 41.67; 20.83; 10.42; 5.21 (µg/mL)

Table 5. The IC50 value of hyaluronidase inhibition activity of DFPE and KOR

<table>
<thead>
<tr>
<th>Sample</th>
<th>Equation</th>
<th>R²</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dragon fruit peel extract</td>
<td>Y = 0.272x + 26.63</td>
<td>0.960</td>
<td>85.32 ± 10.24</td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside</td>
<td>Y = 0.345x + 21.15</td>
<td>0.983</td>
<td>84.07 ± 10.46</td>
</tr>
</tbody>
</table>

Note: *Linear equations; the coefficient of regression (R2) and IC50 of each sample were calculated.

The results of calculations using the linear regression equation is shown in Table 4. It showed the IC50 of hyaluronidase inhibition by KOR compound (84.07±10.46µg/mL) was lower than the IC50 value that is produced by DFPE (85.32±10.24µg/mL). However, the difference in IC50 values was relatively small. It also supported by the analysis of paired samples T-test (p<0.05) (Table 3) that the whole hyaluronidase inhibition activities of DFPE and KOR were not significantly different. Therefore, it can be assumed that DFPE had an equal inhibitory activity to its comparative compound, KOR.

**DISCUSSION**

Phenol is one of the compounds highly contained in plants and has been widely studied due to its biological activities such as anti-mutagenic, anticancer, anti-aging, and antioxidant. The DFPE from *H. polyrhizus* is known to contain more phenolic compounds than the other types of dragon fruit plants. The phenolic compounds contained and characterized in DFPE are chlorogenic acid, gallic acid, and quercetin. These three phenolic compounds are widely known as powerful antioxidant agents (18). Its reddish color was one indication of a high content of phenolic compounds and betalain components. Betalain is a pigment that can dissolve in water and consists of betacyanin (violet) and betaxanthin (yellow). Higher antioxidant capacity was found in red dragon fruit plants, presumably because of the high betalain content (19).

*Kaempferol-3-O-rutinoside* (KOR) is a natural flavonol compound which is a derivative of the flavonoid group and commonly found in fruits, vegetables, and other herbs such as grapes, tomatoes, broccoli, and gingko biloba leaves. In addition, KOR is one of the flavonoids found in dragon fruit; therefore KOR was used as a comparative compound in this study. KOR compound has high biological activities such as antioxidants, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities. The flavonoid compound characteristics owned by KOR result in high antioxidant activity (20).

There are two types of antioxidants that are categorized based on its mechanism of action, namely primary and secondary antioxidants. The type of antioxidant capacity examined in this study can be classified into the primary antioxidant group. Primary antioxidants work through free radicals scavenging mechanism and turn them into more stable compounds or products by inhibiting initiation and damaging the propagation chain by transferring hydrogen atoms or electron. Whereas, the mechanism of secondary antioxidant action is through inhibition of free radical formation and protection against oxidation damage, which was not examined in this study because it involves an assay model (21).

Based on the result of H2O2 free radical scavenging activity of DFPE and KOR compounds (Table 4.3), both showed decent primary antioxidant activity. However, based on IC50 values, the antioxidant capacities owned by DFPE (IC50 409.64±23.17µg/mL) and the comparative KOR compound (IC50 351.46±2.30µg/mL) were considered as weak. In accordance with the standards that classified antioxidant capacity based on IC50 values, very strong (IC50 value less than 50ppm), strong (IC50 value between 50-100ppm), moderate (IC50 between 100-150ppm), and weak (IC50 value between 151-200 ppm or more) (13). According to a study conducted by Lourith & Kanlayavattanakul, IC50 values were also influenced by solvents used in the extraction process. The study compared several solvents used in the extraction method. The results indicated that IC50 value of DFPE in the ABTS and DDPH assay resulted in a lower value, indicating a strong antioxidant in the sample dissolved in water compared to the sample dissolved in a polar ethanol solvent and non-polar n-hexane solvent (18).

Based on the DPPH assay, a study conducted by Tatsimo et al., showed that kaempferol was considered to have a strong antioxidant capacity with an IC50 value of 52.48µg/mL. This result suggested that the antioxidant capacity of a certain compound was proportional to IC50.
value and its properties and vary depending on the free radicals that were scavenged (22). This allegation was also supported by findings previously obtained by Velloso et al., that hydrogen peroxide (H₂O₂) was a lesive free radical which can cause severe lesion conditions; therefore specific agents are needed to fight against H₂O₂. Hence, among the 3 compounds that have been examined in the study (quercitrin, iso-quercitrin, and kaempferol), no one could react with H₂O₂ (23). Another study also revealed that anthocyanin and betalain content in DFPE are the best for antioxidative effect. These pigments can reduce free radicals by binding metal ions and formed a more stable pigment-metal ion complex (24).

Extrinsic aging caused mainly by UV radiation can increase the number of free radicals or ROS in the skin which can cause inflammation and oxidative stress. This triggers the skin aging process that is closely associated with the overexpression of matrix metalloproteinase. The decrease of epidermal hyaluronic acid was one of the major changes that occur in skin aging and is found in some skin aging cases. In addition, a decrease in skin elasticity and premature skin aging was significantly correlated with an increase in elastase and hyaluronidase. Therefore, one mechanism to inhibit aging was to reduce various enzyme activities that support matrix degradation from the abundant connective tissues in the skin (25,26).

The antioxidant capacity of a certain compound was closely related to its anti-aging potential. This is due to the ROS mechanism which is one of the main causes of skin aging. Thus, compounds having high antioxidant capacity also have strong anti-aging activity (27). This was in line with research conducted by Tu & Tawata that certain extracts with high DPPH scavenging activity and low IC₅₀ values also showed high hyaluronidase inhibitory activity (16).

Based on the results obtained in this study, the hyaluronidase inhibitory activity by both dragon fruit peel extract and its comparative compound were considered as strong enzyme inhibitors with IC₅₀ values of 85.3±0.124µg/mL and 84.0±0.106.46µg/mL respectively. The results obtained by Tu & Tawata also showed that IC₅₀ value of an extract for anti-hyaluronidase ranging from 50-100ppm was considered as a strong inhibitor (16). Comparative compound kaempferol had generally smaller hyaluronidase inhibitory activity and IC₅₀ value. Thus its capacity as anti-hyaluronidase was higher than that of dragon fruit peel extract. However, the difference of IC₅₀ value of hyaluronidase inhibition owned by DFPE was relatively small. It also supported by the statistical analysis that the whole hyaluronidase inhibition activities of DFPE and KOR were not significantly different. Hence, it had equal anti-aging potential to KOR, especially for hyaluronidase inhibitions.

The hyaluronidase inhibition capacity was caused by phenol and flavonoid contents found in both DFPE and KOR. Free radicals trigger the activation of various degrading enzymes; one of them is hyaluronidase that is known as matrix metalloproteinase (MMP) through the inflammatory process. The inflammation mechanism begins with the induction of various growth factors and proinflammatory cytokines (TNF-A, EGF, IL-1) by free radicals. Furthermore, these cytokines carried signal transduction and activated AP-1 protein which was a transcription factor to increase MMP expression and inhibited the expression of collagen. MMP worked through the degradation of various proteins and components found in ECM and caused the skin to lose its elasticity, wrinkles, hyperpigmentation, and triggers other inflammatory responses. The presence of antioxidant compounds could prevent the ECM destruction process through the prevention mechanism of free radical stabilization by donating hydrogen atom or electron that causes inhibition of MMP production. In addition, antioxidant compounds were found to inhibit MMP work. Thus, they were considered to have anti-aging characteristics (1,5).

This study concluded that DFPE has an equivalent antioxidant and anti-aging through H₂O₂ scavenging and hyaluronidase activity as possessed by the KOR compound. It supported by the statistical analysis that the whole H₂O₂ scavenging and hyaluronidase inhibition activities of DFPE and KOR were not significantly different, although generally KOR had higher activities and lower IC₅₀ in both assays.

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REFERENCES


