Comparison of Antioxidant and Antiaging Activities Between Dragon Fruit
(*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose) Rind Extract
and Kaempferol

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Abstract

Aging involves progressive physiological changes of the body, including skin dysfunction and death which can be accelerated by the presence of free radicals. Compounds with potential antioxidants and antiaging are contained in various fruits, including dragon fruit (*Hylocereus polyrhizus* (F.A.C. Weber) Britton & Rose). This study aimed to compare the antioxidant and antiaging activities of dragon fruit rind extract (DFRE) and Kaempferol. The antioxidant and antiaging activities were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenger and inhibitory activity assay of tyrosinase, respectively and was conducted at the Biomolecular and Biomedical Research Center, Aretha Medika Utama, from August to November 2018. Data acquired were then analyzed using ANOVA, followed by Post Hoc Test using Turkey HSD test with a confidence level of 95% (α=0.05). Phytochemical assay was also conducted to determine the content of active ingredients in the dragon fruit rind extract. Phytochemical test result showed that DFRE contained flavonoids, tannins, phenols, triterpenoids, and alkaloids compounds. Both DPPH scavenging and tyrosine inhibition assay indicated that Kaempferol compound also had antioxidant and antiaging activities. The IC₅₀ values of DFRE and Kaempferol antioxidant activities were 164.98 μg/mL and 83.30 μg/mL, respectively, while the IC₅₀ values of tyrosinase inhibition activity of DFRE and Kaempferol were 88.46 μg/mL and 59.34 μg/mL, respectively. DFRE had antioxidant activities and tyrosinase inhibition activities thus can be used as an alternative antiaging and antioxidant agents.

Key words: Antiaging, antioxidant, anti-tyrosinase, DFRE, DPPH


Abstrak

Aging atau penuaan adalah proses kompleks yang ditandai dengan penurunan progresif fungsi fisiologis tubuh, termasuk kulit yang dialiri oleh disfungsi dan kematian kulit. Faktor-faktor yang dapat mempercepat proses penuaan dini di antaranya radikal bebas dan gaya hidup yang tidak sehat. Buah-buahan banyak mengandung senyawa yang berpotensi sebagai antioksidan dan antiaging, salah satu di antaranya adalah buah naga. Penelitian ini bertujuan mengetahui potensi antioksidan dan antiaging ekstrak kulit buah naga (EKBN) yang dibandingkan dengan senyawa kaempferol (SK) sebagai pembanding. Penelitian ini dilakukan meliputi uji fitokimia untuk mengetahui kandungan bahan aktif pada kulit buah naga, uji pemerangkapan DPPH, dan uji penghambatan tirosinase. Data lalu dianalisis menggunakan analisis varians (ANOVA) satu arah (one way). Hasil penelitian menunjukkan EKBN mengandung banyak senyawa, flavonoid, tanin, fenol, triterpenoid, dan alkaloid serta tidak mengandung gula sederhana. Senyawa kaempferol memiliki aktivitas antioksidan dan antiaging lebih baik dibandingkan dengan EKBN. Aktivitas antioksidan dan antiaging lebih baik dibandingkan dengan EKBN. Aktivitas antioksidan dilihat dari nilai IC₅₀ EKBN sebesar 164.98 μg/mL dan SK sebesar 83.30 μg/mL. Aktivitas penghambatan tirosinase memiliki nilai IC₅₀ EKBN masing-masing sebesar 88.46 μg/mL dan IC₅₀ SK sebesar 59.34 μg/mL. EKBN memiliki aktivitas antioksidan dan penghambatan tirosinase sehingga dapat digunakan sebagai alternatif bahan antiaging dan antioksidan.

Kata kunci: Antiaging, antioksidan, DPPH, Ekstrak kulit buah naga

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Introduction

Aging involves progressive physiological changes of the body, including the skin, that may take the form of skin dysfunction and death. Factors that can accelerate the aging process are the presence of free radicals and unhealthy lifestyles. Many factors are said to be involved in the aging process, which are divided into internal factors and external factors. Some internal factors include free radicals, tyrosinase enzymes, elastase enzymes, collagenase enzymes, reduced hormones, glycosylation processes, methylation, apoptosis, decreased immune system, and genes. Human being already has a defense system to defeat oxidants coming from inside or outside of the body. Defense system from the body, such as peroxidase enzymes, catalase, glutathione, and histidine-peptidin, is often lacking due to environmental influences and poor diet. In this condition, antioxidant compounds need to be obtained from food.

Tyrosinase is an enzyme that regulates the tyrosine conversion into melanin. Therefore, tyrosine inhibitors can be used in hyperpigmentation therapy. Tyrosinase inhibitors are widely used as whitening or skin lightening agents. UV radiation’s involvement in skin aging has become the main topic of research, especially regarding its pathogenesis and mechanism. Reactive oxygen species (ROS) also has the ability to stimulate skin inflammation by inducing the activation of transcription factors that regulate the degradation of skin collagen and extracellular matrix. Free radicals (ROS) are molecules that have one or more unpaired electrons. These unpaired electrons cause free radicals to become compounds that are highly reactive to cells by binding electrons to cell molecules. This reaction is often referred to as oxidation. ROS is usually removed from body using an antioxidant defense system.

Antioxidant compounds can be used against oxidation in the body. Fruit is one of the food sources that contains rich antioxidant properties. One of the fruits that are currently popular in Indonesia is dragon fruit (Hylocereus undatus (Haw.); Family Cactaceae). Dragon fruit is currently being cultivated in Indonesia. One of the type of the dragon fruits is the red dragon fruit, which is a tropical plant. Red dragon fruit has a higher anti-radical ability than white dragon fruit even though all dragon fruit has antioxidants such as vitamin C, vitamin E, vitamin B, carotenoids, flavonoids, polyphenols, and anthocyanins. This antioxidant properties against free radicals are not only found in the flesh of the dragon fruit, it is also found in the rind of red dragon fruit. The use of dragon fruit rind extract as various functional foods that are beneficial for health has been started recently by properly extracting and processing it. However, studies on antioxidant activities of fruit peels and seeds are very limited, which may relate to their low popularity and commercial applications. Therefore, it is necessary to perform a study on antioxidant and antityrosinase activities in dragon fruit rind extract (DFRE) to determine its potentials as an alternative antioxidant.

Methods

Dragon fruits for this study were collected from Cijambe Village, Cijambe Subdistrict, Subang District, West Java, Indonesia. The plants were identified by a herbarium staff member of the Department of Biology, School of Life Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The extraction, phytochemical screening assay, antioxidant activity assay, and antiaging activity assay was conducted at the Biomolecular and Biomedicine Research Center, Aretha Medika Utama, from August to November 2018.

Extraction was performed on 200 g dried simplicia of dragon fruit rind with 70% distilled ethanol (2,000 mL) using maceration technique. Ethanol filtrate was filtered, then waste was re-macerated in triplicates. Macerates were concentrated using 50°C rotavapor to obtain the extract. The yield of dragon fruit rind extract (DFRE) was 21.05 g and it was stored at -20°C. DFRE was used as the experiment. Kaemferol (Chengdu Biopurify Phytochemicals Ltd BP0823) was used as the standard compound.

The content of DFRE was evaluated by phytochemical screening assay with modified Farnsworth method. The phytochemical screening aimed to identify the qualitative presence of flavonoids, saponins, phenols, tannins, steroids/triterpenoids, terpenoids, and alkaloids.

For flavonoid identification, 10 mg of sample was added into a test tube then Mg [Merck EM105815, USA] and HCl 2N were added to the sample. The mixture was heated for 5 to 10 min then filtered after it was cooled down. Subsequently, amyl alcohol was added into the filtrate. The positive reaction was shown by the formation of red or orange color.

For saponins identification, approximately
10 mg of sample was put into the test tube with some water and boiled for 5 min. The tube was then shaken vigorously and saponin content was indicated by the presence of persistent froth on the surface.6,9

For phenols identification, 10 mg of sample was placed on a dropping plate then 1% FeCl₃ [Merck 1.03861.0250, USA] was added into the sample. The color formation of green/red/purple/blue/black showed the presence of phenol.6,9

For tannins identification, 10 mg of samples was added with 2 mL of HCl 2N [Merck 1003171000] in the test tube, then heated in a the water bath for 30 min. The mixture was cooled down and filtered, the filtrate was added with amyl alcohol [Merck 10979, USA]. Purple colour formation indicated a positive reaction for tannins.6,9

Steroids/Triterpenoids identification was done using the following method. First, 10 mg of samples was added with 2 mL of HCl 2N [Merck 1003171000] in the test tube. Then, the test tube was heated on a the water bath for 30 min. The mixture was cooled down and filtered, the filtrate was added with amyl alcohol [Merck 10979, USA]. Purple colour formation indicated a positive reaction for tannins.6,9

Terpenoids identification was done by adding 10 mg of samples with 2 mL of HCl 2N [Merck 1003171000] in a test tube, then the test tube was heated in the water bath for 30 min. The mixture was cooled down and filtered, the filtrate was added with amyl alcohol [Merck 10979, USA]. Purple colour formation indicated a positive reaction for tannins.6,9

For alkaloids identification, a small amount of sample (10 mg) was introduced into a test tube, then 10% ammonia was added. After chloroform was added to the mixture, two layers of liquid were formed and the bottom layer was collected. HCl 1N was added to the liquid, forming two layers. The upper layer was then collected and added with 1–2 drops of Dragendorf solution. The presence of yellow colour indicated a positive result.6,9

The DPPH assay was conducted using the method from Widowati et al.9 The method was based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical 2,2-diphenyl-1-picrylhydrazine (DPPH-H). In short, 50 μL of various level of samples (50–400 μg/mL for extract and 50–400 μM for compounds in the DMSO) was added to each well in a 96-well micro plate, then followed by addition of 200 μL of DPPH (Sigma D9132, USA) solution (0.077 mmol/L in methanol) into the well. The mixture was then incubated in the dark for 30 min at room temperature. Afterwards, the absorbance was read using a microplate reader (Multiskan™ GO Microplate Spectrophotometer, Thermo Scientific, Waltham, MA, USA) at 517 nm wave length. The radical scavenging activity was measured using the following formula:

\[
\% \text{Scavenging} = \left( \frac{A - A_c}{A} \right) \times 100
\]

\(A_c=\)negative control absorbance (without sample).
\(A_s=\)sample absorbance

Tyrosinase inhibitory activity assay was measured by modification method from Fais et al.10, Tu and Tawata11, and Widowati et al.6 with some modifications. Sample was prepared in a 96 well-plate and potassium phosphate buffer (140 µL, 20 mM, pH 6.8) 20 µL sample with various concentration, and 20 µL tyrosinase enzyme from fungi (125 U/mL in potassium phosphate buffer) were then added. The mixture was incubated at room temperature for 15 minutes. After incubation, the mixture was added by 20 µL L-DOPA (1.5 mM) and incubated at room temperature for 10 minutes. Tyrosinase inhibitory activity was measured by quantifying the DOPA level that formed at 470 nm wavelength using the following formula.

Tyrosinase inhibitory activity (%): \(\left( \frac{A-B}{A} \right) \times 100\)

Statistical analysis was conducted using SPSS software (version 20.0). Data were presented as Mean±Standard Deviation. Significant differences among treatments were determined using the one-way Analysis of variance (ANOVA) with p<0.05 considered statistically significant, along with Tukey honestly significant difference post hoc test and 95% confidence interval.

Results

Phytochemical analysis was performed to determine the content of the compounds class in DFRE. The compound class of DFRE can be seen in Table 1. Phytochemical analysis showed the presence of flavonoids, phenols, tannins, triterpenoids, and alkaloids, but no saponins and terpenoids were identified.

DPPH free radical scavenging activity can be used to determine antioxidant capacity of DFRE and Kaempferol compounds. This assay was based on alcoholic DPPH solution reduction in
Table 1 Qualitative Phytochemical Result of the DFRE

<table>
<thead>
<tr>
<th>Phytochemical Component</th>
<th>Qualitative Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids</td>
<td>++</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Steroids/triterpenoids</td>
<td>+/-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+++</td>
</tr>
</tbody>
</table>

++++: very high content, +++: high content, ++: medium content, +: low content, -: undetected

The presence of a hydrogen-donating antioxidant due to the formation of the non-radical from 2,2-diphenyl-1-picrylhydrazine (DPPH-H). The DPPH samples generally changed from purple colour into a colourless sample when antioxidant molecules quench the DPPH free radicals. The IC_{50} of scavenging activities of DFRE and kaempferol-3-O-rutinoside are listed in Table 2, showing that the IC_{50} value of DFRE was higher (164.36±10.83 μg/mL) than that of kaempferol (83.57±1.28 μg/mL). DPPH free radical scavenging activity increased along with the high concentration of DFRE and kaempferol (Figure 1).

Tyrosinase inhibitory activity assay was conducted to determine the antiaging capacity.

Table 2 IC_{50} value of DPPH Scavenging Activity of DFRE and Kaempferol-3-O-rutinoside

<table>
<thead>
<tr>
<th>Samples</th>
<th>Equation</th>
<th>R^2</th>
<th>IC50 (µg/mL)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFRE (replication 1)</td>
<td>y = 0.1493x + 26.435</td>
<td>0.98</td>
<td>164.51</td>
<td></td>
</tr>
<tr>
<td>DFRE (replication 2)</td>
<td>y = 0.1246x + 27.946</td>
<td>0.96</td>
<td>157.84</td>
<td>164.98±10.47</td>
</tr>
<tr>
<td>DFRE (replication 3)</td>
<td>y = 0.1312x + 28.993</td>
<td>0.97</td>
<td>177.00</td>
<td></td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (1)</td>
<td>y = 0.2819x + 26.704</td>
<td>0.94</td>
<td>83.32</td>
<td></td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (2)</td>
<td>y = 0.2918x + 25.926</td>
<td>0.94</td>
<td>82.64</td>
<td>83.30±1.27</td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (3)</td>
<td>y = 0.2939x + 25.085</td>
<td>0.94</td>
<td>82.50</td>
<td></td>
</tr>
</tbody>
</table>

*Linear equations, coefficient of regression (R^2) and IC50 of each sample were calculated.

Figure 1 DPPH Scavenging Activities of DFRE and Kaempferol-3-O-rutinoside
Table 3  The IC_{50} value of Tyrosinase Inhibitory Activity of DFRE and Kaempferol-3-O-Rutinoside

<table>
<thead>
<tr>
<th>Samples</th>
<th>Equation</th>
<th>R^2</th>
<th>IC50 (µg/mL)</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFRE (1)</td>
<td>y = 0.2764x + 25.503</td>
<td>0.97</td>
<td>88.77</td>
<td></td>
</tr>
<tr>
<td>DFRE (2)</td>
<td>y = 0.2874x + 24.632</td>
<td>0.99</td>
<td>88.40</td>
<td>88.46±0.23</td>
</tr>
<tr>
<td>DFRE (3)</td>
<td>y = 0.2821x + 25.129</td>
<td>0.94</td>
<td>88.23</td>
<td></td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (1)</td>
<td>y = 0.3223x + 30.983</td>
<td>0.96</td>
<td>59.07</td>
<td></td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (2)</td>
<td>y = 0.3129x + 30.974</td>
<td>0.99</td>
<td>60.99</td>
<td>59.34±1.53</td>
</tr>
<tr>
<td>Kaempferol-3-O-rutinoside (3)</td>
<td>y = 0.3409x + 30.294</td>
<td>0.97</td>
<td>57.97</td>
<td></td>
</tr>
</tbody>
</table>

based on the inhibition activity of tyrosinase using DFRE and Kaempferol as comparison. The IC_{50} of tyrosinase inhibitory activities of DFRE and Kaempferol can be seen in Table 3. As shown in Table 3, the IC_{50} value of DFRE was higher (88.46±0.23 µg/mL) than that of Kaempferol-3-O-rutinoside (59.34±1.53 µg/mL). Tyrosinase inhibitory activity increased along with the high concentration of DFRE and Kaempferol-3-O-rutinoside (Figure 2).

Discussion

Dragon fruit is believed to reduce cholesterol level, balance blood sugar level, prevent colon cancer, strengthen kidney and bone function, strengthen the brain’s working power, improve eye sharpness, and work as an antioxidant agent. A phytochemical assay was carried out to determine the chemicals contained in a plant. In the qualitative phytochemical assay (Table 1), DFRE was found to contain high alkaloids (+++), flavonoids (++), low level of phenols, tannins and triterpenoids (+), with no saponins and terpenoids compounds. Unlike the findings in Nuruliyana et al. study, the total content of phenol and flavonoids in the DFRE was high at 1049.18 mg GAE/100 g (gallic acid equivalent) and 1310.10 mg CE/100 g (catechin equivalent), respectively, when compared to dragon fruit extract. Furthermore, Manihuruk et al. stated that DFRE contained tannins and saponins, but did not contain alkaloids. This difference was due to the use of different solvents because this study used ethanol solvents while Manihuruk et al., used hexane solvents. Polar solvents such as ethanol can dissolve more than non-polar solvents such as hexane. The content of phenol and flavonoids in DFRE played a role in...
antioxidant activities.\textsuperscript{14} Red dragon fruit skin was also known to contain betalain color pigments, which have antioxidant activities.\textsuperscript{2} Antioxidant activities in dragon fruit rind is better than in the fruit flesh (preliminary research); thus, it has the potential to be developed as a source of natural antioxidants. Scavenging of DPPH free radicals was used to measure the antioxidant activities.\textsuperscript{15} The active antioxidant compound in the sample was characterized by dark purple color. DPPH changed after receiving protons from antioxidants and reduced the colour of protonated DPPH molecules to 1.1 diffenyl-2-pikrilhidrazine, which was yellowish or pale yellow.\textsuperscript{15}

The DPPH radical scavenging by DFRE presented an IC\textsubscript{50} value of 164.36 ± 10.83 μg/mL with Kaemferol as a comparison, i.e. IC\textsubscript{50} value of 83.57±1.28 μg/mL. This showed that the DPPH scavenging antioxidant activity by DFRE was lower than the comparative compound, which was in line with Lourith & Kanlayavattanakul’s\textsuperscript{16} study that suggested the comparative compound to have a higher antioxidant activity compared to the ethanol extract of dragon fruit peel. Kaemferol is a flavonoid compound and strong antioxidant component.\textsuperscript{16} The antioxidant activity in the DFRE was lower because it contained other active ingredients, unlike Kaemferol which was the active compound.

The presence of phenol compounds and flavonoids in dragon fruit skin reflects the presence of antioxidant activities. The results of the study showed that DPPH free radical scavenging activity increased along with the high concentration of DFRE (dose-dependent manner). This was in line with the findings of Harivaindaran et al. and also Nurliyana et al.\textsuperscript{13} which stated that the high concentration of dragon fruit peel extract led to the increase in DPPH scavenging activities. This was because the higher the content of the hydroxyl group (-OH) in samples is, the higher antioxidant activities.\textsuperscript{17}

ROS is one of the main factors that cause skin pigmentation. Melanocytes produce nitric oxide radicals (NO) which trigger an increase in melanogenesis enzyme, namely tyrosinase. The presence of tyrosinase causes the darkening of the skin. Several studies on the antioxidant potentials have been carried out to reduce the skin pigmentation.\textsuperscript{18} Benzaldehyde and benzoate derivatives isolated from plants were known to be potential as an alternative tyrosinase inhibitor. Benzaldehyde can be isolated from cumin and 2-hydroxy-4-methoxybenzaldehyde can be isolated from the roots of Mondia whitei.\textsuperscript{19} In addition, dragon fruit skin also contained benzaldehyde which can be used as antityrosinase. The results of tyrosinase inhibition activity of DFRE presented an IC\textsubscript{50} value of 88.46±0.23 μg/ml with kaemferol as a comparison achieved approximately 59.34±1.53 μg/ml. This showed that the DFRE inhibition activity was lower than the comparative compound. A previous study from Vijayakumar et al.,\textsuperscript{18} also showed the same thing, that the tyrosinase inhibition activity by dragon fruit peel extract was lower than that of Kojic acid which was 66.29% and 76.20%, respectively.

Tyrosinase inhibition activity increases along with the high DFRE concentration used. This is relevant with the study conducted by Vijayakumar et al.\textsuperscript{18} that stated dragon fruit peel extract also has a tyrosinase inhibitory activity. Similarly, Jiménez et al.\textsuperscript{19} suggested that dragon fruit peel extract has a tyrosinase inhibition activity. The higher the concentration of extract used, the greater the active ingredients that can interfere the melanogenesis process. Therefore, the active ingredients contained in plant extracts were widely used as tyrosinase inhibitors including the dragon fruit rind extract.

Although tyrosinase inhibitors are widely used in cosmetic products as skin lightening agents, many of them do not met clinical efficacy standards and might endanger the consumers. This situation demands the availability of active ingredients derived from plants as skin lightening agents. In addition to vitamin C, the presence of other active ingredients in plants, such as polyphenols and flavonoids, also contributes significantly to tyrosinase inhibition activities as a competitive inhibitor in melanin synthesis.\textsuperscript{20} Therefore, DFRE may be used as an alternative tyrosinase inhibitor. Hence, DFRE contains flavonoids, tannins, phenols, triterpenoids, and alkaloids and does not contain saponin, steroid and terpenoid compounds. Kaemferol compound has an antioxidant activity through DPPH scavenging and antiaging activity through tyrosinase inhibition, which is better than DFRE. DFRE had antioxidant and tyrosinase inhibition activities that enable its use as an alternative antiaging inhibitor and antioxidant agent.

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