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Potency of Black Soybean (*Glycine max* (L.) Merr) Extract and Daidzein as Antioxidant and Antihyaluronidase

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Abstract

Black soybean (*Glycine max* (L.) Merr.) is a plant that is widely planted and consumed in Indonesia. In addition, black soybean has unique content of isoflavones, such as *daidzein*, which is one of the active compounds that have the effect of fighting free radicals and can inhibit the aging process. The purpose of this study is to analyze the antioxidant potency possessed by black soybean extract (BSE) and daidzein inhibiting aging of the skin. The method used is a colorimetric test. The type of antioxidant test used is H₂O₂ scavenging and inhibiting the activity of the hyaluronidase enzyme for *antiaging*. BSE has better effectiveness of H₂O₂ scavenging (IC₅₀: 286.24±11.16 (µg/mL)) than daidzein compound (IC₅₀: 366.16±2.54 (µg/mL)). In the inhibition of hyaluronidase enzyme, the daidzein has more effective activities (IC₅₀: 95.80±3.98 (µg/mL)) compared to BSE (IC₅₀: 152.56±13.98 (µg/mL)). The antioxidant and anti-aging activities possessed by BSE make it possible to be used as a cosmetic ingredient for skin aging therapy.

Keywords

Antioxidant; antiaging; Glycine max (L.); Hyaluronidase; H2O2 scavenging

Full Text:



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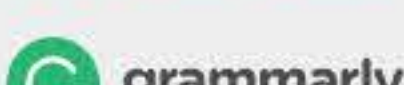
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






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COX-2 *Cynara scolymus* L., *Artemisia china* L., *Borreria repens* DC., *Polygala paniculata* L. DPPH, antioxidant *Eurycoma longifolia* GC-MS *Sterculia quadrifida* anti-inflammatory antioxidant activity antioxidants collagen croscarmellose sodium crospovidone fast disintegrating tablet flavonoid flavonoids gel kulit manggis macrophage nanoemulsion radical scavenging *Tinospora crispa* (L.) Miers, DPPH toxicity

Potency of Black Soybean (*Glycine max* (L.) Merr) Extract and Daidzein as Antioxidant and Anti-hyaluronidase

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ABSTRACT

Black soybean (*Glycine max* (L.) Merr.) is a plant that is widely planted and consumed in Indonesia. In addition, black soybean has unique content of isoflavones, such as *daidzein*, which is one of the active compounds that have the effect of fighting free radicals and can inhibit the aging process. The purpose of this study is to analyze the antioxidant potency possessed by black soybean extract (BSE) and daidzeinin inhibiting aging of the skin. The method used is a colorimetric test. The type of antioxidant test used is H₂O₂ scavenging and inhibiting the activity of the hyaluronidase enzyme for *antiaging*. BSE has better effectiveness of H₂O₂ scavenging (IC₅₀: 286.24±11.16 (µg/mL)) than daidzein compound (IC₅₀: 366.16±2.54 (µg/mL)). In the inhibition of hyaluronidase enzyme, the daidzein has more effective activities (IC₅₀: 95.80±3.98 (µg/mL)) compared to BSE (IC₅₀: 152.56±13.98 (µg/mL)). The antioxidant and anti-aging activities possessed by BSE make it possible to be used as a cosmetic ingredient for skin aging therapy.

Keywords: Antioxidant, antiaging, *Glycine max* (L.), Hyaluronidase, H₂O₂ scavenging

INTRODUCTION

People who live in the modern era such as the present day have a very high desire to treat skin health so they could look young. This has triggered the cosmeceutical industry where cosmetics are one of the products used to treat skin health. The high demand in skin health care has increased research on ingredients to inhibit skin aging which are effective for use in cosmetic ingredients. Cosmetic chemicals used often have a harmful effect on health. Thus, natural ingredients are needed to overcome the adverse effects of the use of cosmetic chemicals and skin care (Jadhav, Dhande, & Kadam, 2016).

Aging of the skin is influenced by various intrinsic and extrinsic factors such as the sun exposure, lifestyle, smoking, genetic, and hormone that are not stable. Ultraviolet rays of the sun are a major factor causing skin aging or commonly called photoaging (Garg, Khurana, & Garg, 2017; Widowati et al., 2018). The extracellular matrix of the skin consists of collagen, elastin and hyaluronic acid to maintain skin moisture and elasticity. The formation of free radicals by ultraviolet light such as Reactive Oxygen Species and oxidative stress affects the enzymes that work in maintaining the balance of the skin, one of which is the enzyme hyaluronidase (Ndlovu, Fouche, Tselanyane, Cordier, & Steenkamp, 2013; Widowati et al., 2018).

Under normal circumstances, this enzyme works as a binding site for collagen and elastin. A variety of free radicals and oxidative stress causes an increase in the activity of the hyaluronidase enzyme, causing signs of aging such as wrinkles on the skin. Because of this, antioxidants are needed which play a role in inhibiting the aging process (Ndlovu et al., 2013; Widowati et al., 2016).

Antioxidants are responsible for reducing damage caused by free radicals to avoid damage at the cellular level. Antioxidants also help to inhibit inflammation and provide protection against damage to photoaging and skin cancer. Topical application of sunscreen does not provide complete protection against ultraviolet light damage, while antioxidants play a major role in the prevention and treatment of ultraviolet-induced skin aging and the addition of formulations for sun protection (Ramos-e-Silva, Celem, Ramos-e-Silva, & Fucci-da-Costa, 2013).

Black soybeans are plants that are widely planted and consumed by Indonesian people. Black soybean seeds have various bioactive ingredients including isoflavones, phenols, flavonoids, saponins, and phytosterols (Alghamdi et al., 2018; Gupta, 2017; Zhou, Cai, & Xu, 2017). Isoflavones are secondary metabolites which are mostly found in these nuts, such as genistein and daidzein. These compounds have antioxidant activity that is able to fight free radicals and also acts as an anti-inflammatory, anti-viral, and anti-microbial (D Sumardi et al., 2017; Wójciak-Kosior et al., 2016).

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Hence, in this study, it is needed to reveal the antioxidant and antiaging potential possessed by black soybeans using H₂O₂ scavenging assay and inhibition of hyaluronidase as antiaging test.

METHODOLOGY

Materials

Black soybeans were obtained from Unit Pengelolaan Benih Sumber (UPBS) Balai Penelitian Tanaman Aneka Kacang dan Umbi, Malang, East Java, Daidzein (Chengdu Biopurify, BP0445), *Ferrous Ammonium Sulfate* (Sigma 7783859), DMSO (Merck 1.02952.1000), H₂O₂ (Merck 1.08597.1000), 1,10-phenanthroline (Sigma 131377), *Sodium phosphate monobasic* (Merck 567545), *Hyaluronic acid* (Sigma H5542), *Hyaluronidase from bovine testes type I-S* (Sigma H3506), *Sodium chloride* (Merck 1064040500), *Bovine Serum Albumin* or BSA (Sigma A4503), *Sodium Acetate* (Merck 1062681000), *Acetic Acid* (Merck 100063), *Hydrochloride acid solution* (Merck 109057), *Sodium hydroxide* (Merck 106498), aquades.

Preparation of Black Soybean Extract (BSE)

A total of 250g of dried black soy bean was milled and stored to the maserator for extraction. The solvent used was ethanol 70%. The filtrate was collected every 24 hours and ethanol was added until the resulting filtrate was colorless. The filtrate was then evaporated using a *rotary evaporator* at 50 ° C until a paste extract was formed. The extract from black soybeans was then stored at -20 ° C (Widowati et al., 2018, 2016, 2017).

H₂O₂ Scavenging Assay

60 µL black soybean extract and daidzein in various concentration was added to well test and in well blanks in 96-well plate (TPP 92096). Furthermore, 12 µL of *Ferrous Ammonium Sulfate* (1 mM) was added to the well control and sample wells. 63 µL DMSO was added in the well control and 90 µL in the well blank, followed by 3 µL of H₂O₂ (5 mM) to the well sample. Then, after adding H₂O₂ mixed solution of the controls, samples and blanks into 96-well plates, it was then incubated for 5 minutes in a dark room at RT. Then each mixture of sample and blank was added 75 µL of 1,10-phenanthroline, then incubated again for 10 minutes in a dark room with room temperature. Absorbance was measured at 510 nm using spectrophotometer *Multiskan Go Reader* (Thermo Fisher Scientific 1510).

$$\% \text{ H}_2\text{O}_2 \text{ scavenging Activity} = \frac{\text{Absorbance Sample}}{\text{Absorbance Control}} \times 100$$

Hyaluronidase Inhibition Assay

Inhibition of hyaluronidase enzyme activity was measured based on the method described by Sigma Aldrich and (Tu & Tawata, 2015), with slight modifications (Widowati et al., 2018, 2016, 2017). A mixture of solutions consisted of 25 µL samples (0.78 - 50 µg / mL), 3 µL enzyme *hyaluronidase from IS type bovine testes* (0.02 mg / mL) and 12 µL phosphate buffer (300 mM of NaH₂PO₄ pH5.35 adjusted with HCl and NaOH), then incubated at 37 ° C for 10 minutes. In addition, it was also prepared for controls containing only 3 µL enzymes and 37 µL phosphate buffers and blanks containing only 15 µL phosphate buffers and 25 µL samples. Furthermore, a mixture of 10 µL of *hyaluronic acid* as a substrate was added and re-incubated at 37 ° C for 45 minutes. The stop solution in the form of *acid albumin*, contains 0.1% BSA, *Sodium Acetate* 24 mM and *Acetic Acid* 79 mM, was added as much as 100 µL into the solution and left at RT for 10 min. Absorbance was measured at 600 nm using *Multiskan Go Reader* (Thermo Fisher Scientific 1510).

$$\% \text{ Hyaluronidase inhibition activity} = \frac{(C-S)}{C} \times 100$$

C : absorbance of enzyme activity without sample;
S : absorbance of enzyme activity with the addition of the tested sample

Data analysis

The results in group were analyzed using the SPSS program with One-Way test and followed by Post Hoc Test with Tukey HSD test to see significances among concentration in group. The significances between BSE and Daidzein activities were also analyzed using Mann-Whitney U Test with significance level is 0.05. The test results of H₂O₂ scavenging activity and antihyaluronidase were continued by determining the inhibition concentration 50 (IC₅₀) value.

RESULTS AND DISCUSSION

Human skin aging is one of the complex biological processes. Aging on the skin can be caused by two factors, namely internal and external. Internal factors are impact of aging on the skin caused by a person's genetic changes that cause disruption of physiology, metabolism and cell reproduction (Kenyon, 2010). Unlike internal factors, the causes of external skin aging or from outside the body are usually caused by UV radiation or called *photoaging*.

UV radiation is absorbed directly by the skin, causing an increase of *Reactive Oxygen Species*

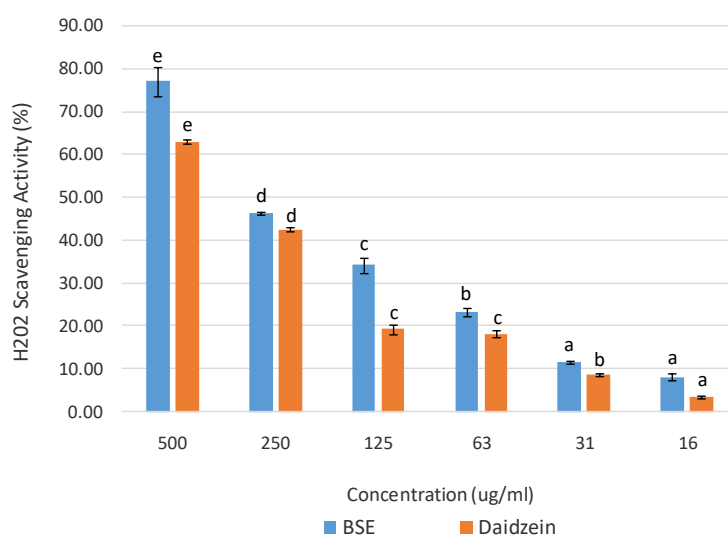


Figure 1. Effect Concentrations of BSE and Daidzein toward H₂O₂ Scavenging Activity. Each value is expressed as the mean ± SD of triplicate determinations. Statistical analysis was performed using one-way ANOVA (p<0.05). Different letters (a, b, c, d, e, f) above the bars in the same group show significant differences at the 0.05 significance level, based on the Tukey HSD Post Hoc Test.

Table I. The IC₅₀ Value of H₂O₂ Scavenging Activity of BSE and Daidzein

| Sample | Linear Equation | R2 | IC ₅₀ (µg/mL) | IC ₅₀ (µg/mL) |
|----------|----------------------|------|--------------------------|--------------------------|
| BSE | Y = 0.1425x + 11.045 | 0.97 | 273.37 | 286.24 ± 11.16 |
| | Y = 0.1323x + 11.345 | 0.95 | 292.18 | |
| | Y = 0.1334x + 10.891 | 0.97 | 293.17 | |
| | Y = 0.1361x + 11.094 | 0.97 | 285.86 | |
| Daidzein | Y = 0.1182x + 6.5597 | 0.96 | 367.62 | 366.16 ± 2.54 |
| | Y = 0.1205x + 5.7014 | 0.96 | 367.62 | |
| | Y = 0.1196x + 6.5574 | 0.96 | 363.23 | |
| | Y = 0.1194x + 6.2728 | 0.96 | 367.52 | |

(ROS) which results in oxidative stress in cells and damages the cell membrane, damage to mitochondria and DNA. In addition, ROS also plays a very important role in the aging process (Wlaschek *et al.*, 2001). ROS or free radicals has unpaired electrons in their outer orbitals (Clarkson & Thompson, 2000). UV radiation increases ROS results in damage to the formation of collagen fibers. Collagen is an important molecule in the formation of the skin (Mukherjee, Maity, Nema, & Sarkar, 2011).

ROS caused by UV radiation is very dangerous for body. It is needed compounds to balance ROS in the body. Antioxidants are an important compound that can protect damage caused by oxidative stress (Mahdi-Pour, Jothy, Latha, Chen, & Sasidharan, 2012). Antioxidants work by donating one electron, this can balance the ROS that has free electrons outside their orbit.

The hydrogen peroxide (H₂O₂) scavenging activity was measured by the reaction method of

ferrous ammonium sulphate and phenanthroline with a little modification. Ferrous ammonium sulphate was reacted with phenanthroline, forming Fe²⁺-tri-phenanthroline complex (orange in color), the existence of H₂O₂ in the reaction will not form orange complex (Mukhopadhyay *et al.*, 2016). H₂O₂ scavenging activity from BSE and daidzein (Figure 1)

At the highest concentration 500 µg/mL, BSE showed higher H₂O₂ scavenging activity (76.94 %) compared to Daidzein (62.92 %) (Figure 1). Based on (Table I), BSE suggested smaller IC₅₀ value (286.24 ± 11.16µg/mL) than daidzein (366.16 ± 2.54µg/mL), implicating BSE had better antioxidant activity than daidzein. Nevertheless, there is no significant difference between BSE and daidzein at scavenging H₂O₂.

Hyaluronidase is a group of proteases that function in the degradation of hyaluronic acid (HA), one of the extracellular matrix constituents (ECM), by catalyzing hyaluronic hydrolysis

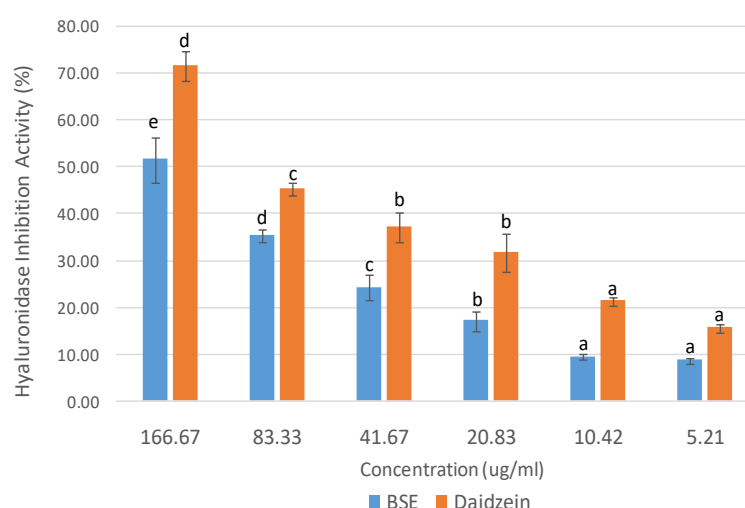


Figure 2. Effect Concentrations of BSE and Daidzein toward H₂O₂ Scavenging Activity. Each value is expressed as the mean ± SD of triplicate determinations. Statistical analysis was performed using one-way ANOVA (p<0.05). Different letters (a, b, c, d, e) above the bars in the same group show significant differences at the 0.05 significance level, based on the Tukey HSD Post Hoc Test.

Table II. The IC₅₀ Value of Hyaluronidase Inhibition Activity of BSE and Daidzein

| Sample | Linear Equation | R2 | IC ₅₀ (µg/mL) | IC ₅₀ (µg/mL) |
|----------|----------------------|------|--------------------------|--------------------------|
| BSE | Y = 0.2651x + 9.7338 | 0.97 | 151.89 | 152.56 ± 13.98 |
| | Y = 0.2405x + 9.8709 | 0.94 | 166.86 | |
| | Y = 0.2873x + 10.087 | 0.96 | 138.92 | |
| | Y = 0.2643x + 9.8972 | 0.96 | 151.73 | |
| Daidzein | Y = 0.3283x + 19.570 | 0.96 | 92.69 | 95.80 ± 3.98 |
| | Y = 0.3235x + 19.457 | 0.93 | 94.41 | |
| | Y = 0.2956x + 20.355 | 0.94 | 100.29 | |
| | Y = 0.315x + 19.79 | 0.96 | 95.65 | |

reactions. Hyaluronidase decreases hyaluronan viscosity thereby increasing ECM and tissue permeability (Widowati *et al.*, 2018, 2016, 2017).

This study showed that at highest concentration (166.67 µg/mL), daidzein had (71.44 ± 3.13%) better inhibition activity than BSE (51.50 ± 4.75%), performing daidzein had stronger ability than BSE to inhibit hyaluronidase enzyme (Fig. 2). BSE had higher IC₅₀ value (152.56 ± 13.98 µg/mL) than daidzein (95.80 ± 3.98 µg/mL), suggesting that daidzein showed more effective inhibitory activity of hyaluronidase enzyme compared to BSE (Table II).

The antioxidant activity of black soybeans and daidzein compound was proven by activity tests using the H₂O₂ scavenging activity. Hydrogen peroxide (H₂O₂) is an important compound in the body because it has the ability to penetrate into cell membranes. However, excess hydrogen peroxide in the body can be toxic because it can increase hydroxyl radicals in cells (Gülçin, Huyut, Elmastaş, & Aboul-Enein, 2010). Hydroxyl radicals are *Reactive Oxygen Species* (ROS) which can cause

some damage to cells (Pavithra & Vadivukkarasi, 2015).

BSE had better antioxidant activity than daidzein which can be seen from the concentration of IC₅₀ value. This was presumably because BSE was a *crude extract* which still allowed the potential of other compounds while daidzein is a pure compound. Research conducted by showed that 70% ethanol extract from BSE was able to reduce ABTS as free radicals, showing that BSE had a high antioxidant potential (Prvulović, Malenčić, & Miladinović, 2017).

Even though BSE has better antioxidant activity than daidzein and daidzein has better antihyaluronidase activity than BSE, there is no significance activity between the groups. According to Mann-Whitney U Test, significant level between BSE and daidzein is 0.080 (the significance level of the test is 0.05). Meanwhile, significance level in inhibition of hyaluronidase activity is 0.314 which shows insignificance. It meant that BSE possessed antioxidant and antihyaluronidase activity as well as daidzein, one

of the strongest compound that has antioxidant ability in black soy bean.

The presence of antioxidant activity through H₂O₂ scavenging activity of BSE and daidzein compound had the potential as an aging inhibitor. Aging of the skin is associated with loss of moisture in the skin. Important molecules play a role in the moisture in the skin including glycosaminoglycan, hyaluronic acid and water (Baumann, 2007). Hyaluronic acid is the main molecule in extracellular matrix preparation. The main function of hyaluronic acid is to repair damage that occurs to the skin. Degradation of the extracellular matrix is directly related to enzymes that play a role in skin aging (Longo, 2003; Makrantonaki et al., 2012).

Enzymes playing a role in degrading hyaluronic acid is hyaluronidase. The *antiaging* activity of BSE and daidzein compound was proven by the hyaluronidase inhibition test. The results showed that the two treatments had the ability to inhibit the hyaluronidase enzyme. The daidzein compound had better potential compared to the black soybean extract in inhibiting the activity of hyaluronidase enzymes. Isoflavones were included in phenolic compounds that have antioxidant activity and the most secondary metabolites produced by plants. In addition, isoflavone compounds are important for human health (Kim et al., 2006).

Black soybean (*Glycine max* (L.) Merr.) has two aglicans namely daidzein and genistein compounds and also has two glucosides namely daidzein and genistin (Silva et al., 2013). Daidzein is a natural antioxidant that has two mechanisms to inhibit free radicals. First, in the membrane liposomes, daidzein inhibits lipid oxidation by directly capturing free radicals (Liang et al., 2008). Both mechanisms of antioxidants indirectly by increasing the activity of antioxidant enzymes (Kampkötter et al., 2008).

CONCLUSION

Black soybean extract can be used as an alternative aging therapy material because it contains phenolic compounds such as daidzein which functions as an antioxidant. This is supported by the results of the antioxidant activity test and the inhibition test of the hyaluronidase enzyme which is owned by BSE and daidzein compounds. The lack of side effects from BSE makes this extract and comparative compound as an alternative ingredient in making cosmetics.

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