**BULETIN FARMATERA**

## ORIGINAL ARTICLE

# Comparison of Antioxidant and Anti-Tyrosinase Activity between Black Soybean (*Glycine max* (L.) Merr.) and *Daidzein*

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**Abstract:** Free radicals in the body will increase with excessive ultraviolet (UV) light exposure, induce oxidative stress with the formation of Reactive Oxygen Species (ROS). A visible effect on skin tissue known as photoaging, including the process of melanogenesis catalyzed by the tyrosinase enzyme, risked for pigmentation or melanoma disorder.

The use of natural ingredients has been widely used by the community to prevent the aging process. Natural compounds from a plant can be a source of antioxidants and have anti-aging abilities through inhibition of the tyrosinase enzyme. Black soybean (*Glycine max* (L.) Merr.) are high in the isoflavone compound, one of which is *daidzein*.

This study evaluates the antioxidant and anti-aging potential of black soybean extract with *daidzein*. Antioxidants using the DPPH method and anti-aging tests carried out, namely the inhibition of the tyrosinase enzyme, are very important in the aging process.

*Daidzein* has an $IC_{50}$ value of DPPH scavenging activity around 109.34±2.80 µg/mL lower than black soybean extract with 116.52±2.50 µg/mL. Results on tyrosinase enzyme inhibition activity, black soybean extract had an $IC_{50}$ value of 70.71±1.83 µg/mL lower compared to daidzein with 72.65±2.81 µg/mL. In contrast to the better antioxidant activity of *daidzein*, the black soybean extract is more potential to inhibit the enzyme tyrosinase.

**Keywords:** black soybean, *daidzein,* antioxidant, anti-aging, anti-tyrosinase

## INTRODUCTION

The aging process is a physiological process that will occur in every living thing and the skin is a body tissue that gives a visual image of aging. Skin aging is a complex process influenced by genetic, environmental and nutritional properties.[1] Excessive ultraviolet (UV) light exposure increases the contribution of free radicals known as Reactive Oxygen Species (ROS). This certainly influences the process of melanogenesis on the skin catalyzed by the

enzyme tyrosinase. This enzyme regulates skin pigmentation through the synthesis of melanin. Increased UV radiation will increase the synthesis of melanin which causes a risk of pigmentation or melanoma disorders. [2]

Various types of modern medicine have been applied to prevent premature aging, one of which is cosmetic. Most cosmetics have side effects and are not safe, so natural ingredients are needed. [3] Currently, natural herbal plants are getting attentions to solve problems. The use of traditional material has long been used by the community as an alternative treatment, for disease prevention, healing, health recovery and improving health status. Based on WHO data, mentioning Africa, Asia and Latin America using traditional materials as a complement to their primary treatment. [4]

The use of natural materials as traditional ingredients has also been carried out by Indonesia since the days of our ancestors. The tendency of the use of traditional ingredients in the world is caused by plants containing compounds that are efficacious in medicine known as phytochemical compounds, namely a group of natural compounds that can be used to maintain health and treat diseases due to their antioxidant activity. [5]

In Indonesia, black soybeans are one of the plants which are the main food commodities after rice and corn. [6] Black soybeans contain high carbohydrates, vitamins, minerals, and proteins. [7] Besides, the advantages of black soybean plants have the most content of isoflavones (one of them

is *daidzein*) which acts as an antioxidant, only produced from plants and found in legumes. [8] Lately, antioxidants have become somewhat interesting in the medical world, known to have the effect of preventing premature aging (anti-aging) in fighting free radicals. [9]

It is necessary to conduct a study of the activity of black soybean extract as an antioxidant and anti-tyrosinase with the *daidzein* comparison compound. This needs to be done to prove the effectiveness of the black soybeans so that black soybeans can be used as natural food to slow down the aging process.

## METHODS

### Preparation of Black Soybean (*Glycine max* (L.) Merr.) Extract

Black soybean seeds (*Glycine max* (L.) Merr.) are washed and dried. *Glycine max* (L.) Merr.) were milled and extracted using 70% ethanol by maceration method for 3 days at room temperature. The filtrate was evaporated with a rotary evaporator so that a thick extract was obtained in paste form. [10]

### Qualitative Phytochemical Screening Assay
### Flavonoid Identification

The test tube containing HCl 2N was dissolved by mixing 10 mg *Glycine max* (L.) Merr.) into it. Mg/Zn is added enough and heated for 10-15 minutes. Amyl alcohol 1 ml added to the test tube has been cooled and filtered. The presence of a red/orange

color indicates the sample contains a flavonoid compound. [11]

## Saponin Identification

*Glycine max* (L.) Merr.) (10 mg) was dissolved using ddH$_2$O in a test tube, simmer for 5 minutes. HCl 1N is added to the solution which has been filtered and shaken strongly. The presence of foam that is still present and remained stable shows a sample containing a saponin compound. [11]

## Phenol Identification

*Glycine max* (L.) Merr.) 10 mg dissolved in 5 ml ddH$_2$O, added 500 µl of FeCl$_3$ 1% solution. The presence of green/red/purple/blue/black color indicated *Glycine max* (L.) Merr.) contains phenol compounds. [11]

## Tannin Identification

HCl 2N around 2 mg was dissolved with 10 mg of *Glycine max* (L.) Merr.) in a test tube, heated in a water bath for 30 minutes. Amyl alcohol 500 µl is added after the solution cooled down. Orange/red color in the amyl alcohol layer indicated a tannin compound. [11]

## Alkaloid Identification

*Glycine max* (L.) Merr.) 10 mg dissolved in 5 ml ddH$_2$O was evaporated in the water bath. The resulting residue is added 5 ml HCl 2N. The solution obtained is divided into 2 test tubes. The first tube is added 3 drops of HCl 2N which functions as a blank. The second tube solution was transferred as much as one drop to the drip plate, then added 3 drops of Dragendorff reagent. The orange deposits formed indicate the presence of alkaloids. [11]

## Steroid/Triterpenoid Identification

The drip plate contains 10 mg *Glycine max* (L.) Merr.), added glacial acetic acid until submerged, left for 10-15 minutes and added with a drop of concentrated H$_2$SO$_4$. The presence of a blue-green color indicated the sample contains a steroid compound, whereas if it shows purple/red/orange color, the sample contains the triterpenoid compound. [11]

## Terpenoid Identification

Vanillin is added enough to the drip plate containing 10 mg of *Glycine max* (L.) Merr.) Addition of one drop H$_2$SO$_4$ into it and homogenize. The presence of purple color contains the terpenoid compound. [11]

## DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) Assay

DPPH 0.077mmol 200 µl in methanol was added to each 50 µl of *Glycine max* (L.) Merr.) extract and *daidzein* respectively on the microplate. The mixture was incubated at room temperature for 30 minutes. DPPH 250 µl for negative control. The absorbance value is measured at a wavelength of 517 nm using a microplate reader. [12] DPPH methodantioxidant activity (%):

$$DPPH\ scavenging\ activity\ (\%) = 1 - \frac{sample}{control} \times 100$$

## Tyrosinase Inhibitor Assay

The mixture consisted of 20 µl*Glycine max* (L.) Merr.), 20 µlTyrosinase from Mushroom (125 U/ml) enzyme, and 140 µl potassium phosphate buffer (20 mM, pH 6.8) were incubated at room temperature for 15 minutes. The control contained 20 µl enzyme and 160 µl phosphate buffer, then added 20 µl of L-DOPA substrate (1.5 mM) and incubated again at 10 minutes. The absorbance is measured using a wavelength of 470 nm. [13] The percentage of tyrosinase inhibitor activity is calculated using the formula:

$$scavenging\ activity\ (\%) = \frac{control - sample}{control} \times 100$$

## RESULTS

## Phytochemical Screening of *Glycine Max* (L.) Merr.)

Phytochemical screening of black soybean seed extract (*Glycine max* (L.) Merr.) includes alkaloid, tannin, steroid/triterpenoid, phenol, saponin, flavonoid, and terpenoid. Some of this substrate or component can affect the ability of antioxidants, such as alkaloid and terpenoid. The phytochemical test results provide an overview of the classes of compounds contained in *Glycine max* (L.) Merr.) which is saponin, tannin, triterpenoid, terpenoid, alkaloid, and can be seen in Table 1.

## DPPH Scavenging Activity

The result of the antioxidant activity test using the DPPH method in various concentrations can be seen in Table 2 and Figure 1 with Tukey HSD statistical analysis. In the process of analyzing whether Glycine max (L.) Merr). and *the daidzein* compound has antioxidant activity, it can be seen as a linear regression equation to find $IC_{50}$ values in Table 3.

**Table 1. Phytochemical Screening of *Glycine max* (L.) Merr.)**

| Contain | Result |
|---|---|
| Flavonoid | - |
| Saponin | + |
| Phenol | - |
| Tanin | + |
| Steroid/triterpenoid | -/+ |
| Terpenoid | + |
| Alkaloid | + |

**Table 2. DPPH Scavenging Activity**

| Concentration (µg/ml) | Average DPPH Scavenging (%) | |
|---|---|---|
| | Glycine max (L.) Merr. | Daidzein |
| 200 | 65.01 ± 2.02[c] | 67.87 ± 1.02[e] |
| 100 | 49.85 ± 0.84[b] | 50.88 ± 0.98[d] |
| 50 | 34.52 ± 1.25[a] | 37.88 ± 0.46[c] |
| 25 | 31.59 ± 1.10[a] | 30.96 ± 0.44[b] |
| 12.5 | 31.97 ± 1.99[a] | 29.24 ± 0.51[a,b] |
| 6.25 | 29.90 ± 3.07[a] | 27.57 ± 1.23[a] |

**Notes:**
Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P < .05$ (Tukey HSD Post Hoc test).
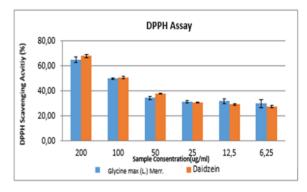


**Figure 1. Various concentrations in DPPH method**

The antioxidant activity test used DPPH scavenging activity parameters as seen in Table 2, where *Glycine max* (L.) Merr). and *daidzein* had the highest antioxidant activity at a concentration of 200 μg/ml, each at 65.01±2.02 % and 67.87±1.02 %. The lower the concentration shows the smaller the percentage of antioxidant activity of Glycine max (L.) Merr). and *daidzein*.

Tabel 3 shows that *Glycine max* (L.) Merr). and *daidzein* with DPPH scavenging activity

obtained IC50 values averaging 116.52±2.50 µg/ml and 109.34±2.80 µg/ml.

**Tyrosinase Inhibitor Activity**

The test results of tyrosinase enzyme inhibition activity in various concentrations can be seen in Table 4 and Figure 2. The test results of tyrosinase inhibition activity in both samples were linear regression equations to find the IC50 value in Table 5.

**Table 3. The IC50 Value of DPPH Scavenging- Activity**

| Sample | | Equation | $R_2$ | IC$_{50}$ (µg/ml) | Average IC$_{50}$ (µg/ml) |
|---|---|---|---|---|---|
| Glycine max | 1 | Y = 0.1735x + 30.066 | 0.97 | 114.89 | |
| | 2 | Y = 0.1766x + 28.914 | 0.97 | 119.40 | 116.52 ± 2.50 |
| | 3 | Y = 0.2123x + 25.526 | 0.99 | 115.28 | |
| | Mean | Y = 0.1875x + 28.168 | 0.98 | 116.44 | |
| Daidzein | 1 | Y = 0.2062x + 27.342 | 0.99 | 109.88 | |
| | 2 | Y = 0.2190x + 26.718 | 0.99 | 106.31 | |
| | 3 | Y = 0.2112x + 26.380 | 0.99 | 111.84 | 109.34 ± 2.80 |
| | Mean | Y = 0.2121x + 26.813 | 0.99 | 109.32 | |
| Glycine max | 1 | Y = 0.1735x + 30.066 | 0.97 | 114.89 | |
| | 2 | Y = 0.1766x + 28.914 | 0.97 | 119.40 | |
| | 3 | Y = 0.2123x + 25.526 | 0.99 | 115.28 | 116.52 ± 2.50 |
| | Mean | Y = 0.1875x + 28.168 | 0.98 | 116.44 | |
| Daidzein | 1 | Y = 0.2062x + 27.342 | 0.99 | 109.88 | |
| | 2 | Y = 0.2190x + 26.718 | 0.99 | 106.31 | |
| | 3 | Y = 0.2112x + 26.380 | 0.99 | 111.84 | 109.34 ± 2.80 |
| | Mean | Y = 0.2121x + 26.813 | 0.99 | 109.32 | |

**Table 4. Tyrosinase Inhibition Assay**

| Concentration (μg/ml) | Tyrosinase Inhibition Activity (%) | |
|---|---|---|
| | Glycine max (L.) Merr. | Daidzein |
| 100 | 60.77 ± 1.12[d] | 57.47 ± 1.16[e] |
| 50 | 42.66 ± 0.66[c] | 44.36 ± 0.97[d] |
| 25 | 31.68 ± 0.57[b] | 36.94 ± 1.43[c] |
| 12.5 | 30.57 ± 0.80[b] | 32.02 ± 0.72[b] |
| 6.25 | 27.10 ± 0.73[a] | 26.20 ± 0.58[a] |
| 3.13 | 27.48 ± 0.72[a] | 24.73 ± 0.42[a] |

**Notes:**
Data were presented as mean ± standard deviation. Different small letters in the same column are significant at $P<.05$ (Tukey HSD Post Hoc test).
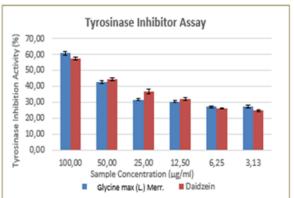


**Figure 2.** Various concentrations in tyrosinase inhibition activity

**Table 5. The IC$_{50}$ Value of Tyrosinase Inhibitor Activity**

| Sample | | Equation | R$_2$ | IC$_{50}$ (μg/ml) | Average IC$_{50}$ (μg/ml) |
|---|---|---|---|---|---|
| Glycine max | 1 | Y = 0.3676x + 24.629 | 0.99 | 69.02 | |
| | 2 | Y = 0.3374x + 25.489 | 0.99 | 72.65 | 70.71 ± 1.83 |
| | 3 | Y = 0.3483x + 25.454 | 0.99 | 70.47 | |
| | Mean | Y = 0.3511x + 25.191 | 0.99 | 70.66 | |
| Daidzein | 1 | Y = 0.3385x + 25.966 | 0.97 | 71.00 | |
| | 2 | Y = 0.3131x + 26.236 | 0.97 | 75.90 | 72.65 ± 2.81 |
| | 3 | Y = 0.3327x + 26.360 | 0.97 | 71.06 | |
| | Mean | Y = 0.3281x + 26.187 | 0.97 | 72.58 | |

Based on Tabel 4 and Figure 2, the tyrosinase inhibition activity of Glycine max (L.) Merr. was highest at a concentration of 100 μg/ml of 60.77±1.12 % It was different at a concentration of 50 μg/ml, 25 μg/ml, and 12.5 μg/ml where tyrosinase inhibition activity by *Glycine max* (L.) Merr). decreased with a percentage of 42.66±0.66 %, 31.68±0.57 %, and 30.57±0.80 %. *Daidzein* compound inhibition activity was higher at the same concentration of 44.36±0.97 %, 36.94±1.43 % and 32.02±0.72 % and higher than the daidzein compound at the same concentration of 57.47±1.16 %.

Table 5 shows the *Glycine max* (L.) Merr). and *daidzein* compound showed that both could inhibit the tyrosinase enzyme with IC$_{50}$ at 70.71±1.83 μg/ml and 72.65±2.81 μg/ml.

## DISCUSSION

The phytochemical screening is carried out as a preliminary stage which aims to determine the class of bioactive compounds in a plant studied. The selection of the solvent and extraction method is the most important factor in conducting a phytochemical screening assay. The phytochemical screening is done qualitatively using a color reagent and see the color changes that occur. Black soybean seeds (*Glycine max* (L.) Merr.) is known as

nuts with nutritional value and is rich in protein. Not identified in our research, daidzein is one of the highest bioactive compounds in black soybean seeds and acts as an antioxidant. [14] Therefore, *daidzein* was used as a comparison.

DPPH method is a simple method used to determine the antioxidant activity of plant material by capturing hydrogen atoms from the component of the extract of the plant. DPPH is sensitive to light, oxygen, and pH. However, it is stable in a radical form so that it may be quite an accurate measurement of antioxidant activity. [15]

The lower the concentration shows the smaller the percentage of antioxidant activity of Glycine max (L.) Merr). and *daidzein*. Antioxidant activity with DPPH scavenging on daidzein compound was higher than *Glycine max* (L.) Merr). at concentration of 200 μg/ml, 100 μg/ml, and 50 μg/ml.

*Glycine max* (L.) Merr). and *daidzein* with DPPH scavenging activity obtained $IC_{50}$ values averaging 116.52±2.50 μg/ml and 109.34±2.80 μg/ml. The result was different from the previous study conducted by Fidrianny et al., the IC50 result by *Glycine max* (L.) Merr. was 3.13 μg/ml. [16] Kladna et al. reported the antioxidant test for several phytoestrogen compounds, one of which is daidzein, showed an IC50 result of 2.81±0.03 mM through the DPPH scavenging method. [17] Both of these can be caused by different procedures in the extracted method, the solvent used and the

different wavelengths in the DPPH method Tyrosinase or polyphenol oxidase is an oxidoreductase that participates in the biosynthesis of melanin, the main pigment found in hair, eyes, and skin. The tyrosinase enzyme reaction with the L-DOPA substrate produces an orange color. Inhibition activity of tyrosinase enzyme is characterized by a reduction in the orange color formed or the result of a clearer colored reaction. This also marks the presence of antioxidant activity in the reaction. [13]

*Glycine max* (L.) Merr). and *daidzein* compound showed that both could inhibit the tyrosinase enzyme with IC50 at 70.71±1.83 μg/ml and 72.65±2.81 μg/ml. The tyrosinase enzyme inhibition study showed anti-tyrosinase activity produced by *Glycine max* (L.) Merr). and *daidzein* compound, according to Lai et.al (2012) using black soybean sprouts extract which has a strong activity of 98% in inhibiting the tyrosinase enzyme and can be used as a lightening agent in skin cosmetic product. [18] Another study by Sitanggang et al. also showed a positive correlation in inhibiting >50% of the tyrosinase enzyme activity by yellow soybean. [19]

The effectiveness of a compound in biological or biochemical functions that can inhibit the oxidation process by 50% (IC50) is classified in several groups including <50 μg/ml (very strong); 50-100 μg/ml (strong); 101-150 μg/ml (medium); >150 μg/ml (weak).[20] In this study, the IC50 value in each test is grouped in Table 6.

**Table 6. The IC50 Value of *Glycine max* (L.) Merr.) and *Daidzein***

| Sample | Assay | $IC_{50}$ (µg/ml) | Activity |
|---|---|---|---|
| Glycine max (L.) Merr. | DPPH assay | 116.52 ± 2.50 | Medium |
| Daidzein | | 109.34 ± 2.80 | Medium |
| Glycine max (L.) Merr. | Tyrosinase inhibition assay | 70.71 ± 1.83 | Strong |
| Daidzein | | 72.65 ± 2.81 | Strong |

## CONCLUSIONS

Bioactive compounds that presence in black soybean (*Glycine max* (L.) Merr.) are saponin, tannin, triterpenoid, terpenoid, an alkaloid. *Glycine max* (L.) Merr.) and *daidzein*, both have antioxidant and tyrosinase inhibition activity as anti-aging. From the result, it shows that *Glycine max* (L.) Merr.) has tyrosinase inhibitor activity better than *daidzein*. Therefore *Glycine max* (L.) Merr.) can be developed into anti-aging ingredients in the product.

## ACKNOWLEDGEMENTS

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