Antioxidant potential ethanolic extract of *Glycine max* (l.) Merr. Var. Detam and daidzein

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**Abstract.** Antioxidants in chemical terms are electron donors and antioxidants are biologically compounds that can overcome the negative effects of oxidants in the body such as damage to vital cells of the body. The balance between oxidants and antioxidants is very important. Antioxidant from natural sources are safer than synthetic antioxidants. Soybeans are functional food source that contains essential amino acids, vitamin e, saponins and are rich in antioxidants such as flavonoids, isoflavones and anthocyanins. The aim of this research is to investigate antioxidant potential of ethanolic extract of black soybean (EEBS) and its compound daidzein.

The phytochemical screening assay evaluated by modified Farnsworth methods and to find out phenolic and flavonoid content, total phenols and flavonoids were tested. DPPH (2,2-diphenyl 1-pichyllhydazy), ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) reducing activity, FRAP (ferric reducing antioxidant power) activity and H2O2 scavenging activity assay were used to measure antioxidant activity. The analysis of phytochemical of EEBS exhibit the presence of saponins, alkaloids, tannins, steroids and triterpenoids and terpenoids. Total phenol and flavonoid assay showed the presence of phenols and flavonoids. Four assay of antioxidant activity showed that EEBS at highest concentration exhibit higher activity (%), and from three assays EEBS has higher antioxidant activity (expressed as IC50) than daidzein. Overall, three of the four antioxidant tests performed can be concluded that EEBS has better antioxidants activity than daidzein. Further research is needed regarding black soybean as a promising antioxidant resource.

**Keywords :** antioxidant, oxidant, black soybean, daidzein, phytochemical

1. **Introduction**

Antioxidants in chemical terms are electron donors and antioxidants are biologically compounds that can overcome the negative effects of oxidants in the body such as damage to vital cells of the body. The balance between oxidants and antioxidants is very important because it is related to the work function of the body's immune system, especially to maintain the integrity and functioning of lipid membranes, cell proteins, and nucleic acids, and control signal transduction and gene expression in immune cells [1,2]. Free radicals are able to oxidize biomolecules, leading to mutagenic changes, tissue damage and cell death. They play a significant pathological role in atherosclerosis, cancer, arthritis, cirrhosis, emphysema and various other degenerative diseases [3-5].

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The production of antioxidants in the human body occurs naturally to compensate for the production of free radicals. The antioxidants function as a defence system against free radicals, but increased production of free radicals that are formed due to stress factors, UV radiation, air pollution and the environment results in inadequate defence systems, so that additional antioxidants from outside are needed [6,7].

Antioxidants exogen can be obtained in synthetic and natural forms. Synthetic antioxidants such as butylated hydroxy toluene (BHT), butylated hydroxyanisol (BHA) and tert-butylhydroquinone (TBHQ) can effectively inhibit oxidation. However, the use of synthetic antioxidants is limited by government rules because if its use exceeds the limit it can cause toxic effects in the body and carcinogenic effect, so it needs safe natural antioxidants. One promising potential source of natural antioxidants is plants because they contain flavonoids, tannins, carotenoids, terpenoids, vitamins C and E and polyphenols [8,3,4].

Soybeans are functional food source that contains essential amino acids, vitamin E, saponins and are rich in antioxidants such as flavonoids, isoflavones and anthocyanins. One important aspect of soybeans as a functional food source is that isoflavonoids are secondary metabolites produced by plants through the synthesis of 2hydroxyisoflavone synthase (IFS). This compound is not synthesized by microorganisms, therefore, this plant is the main source of isoflavonoids in nature. Soybeans must have a fairly high isoflavone content [9].

Isoflavones in soybean consist of malonil-glycosides, acetyl-glycosides, glycosides, and aglycones. Diophys of the four forms of isoflavonoids, the highest biological activity by isoflavonoids aglycones, especially genistein (5,7,4'-trihydroxy isoflavones), daidzein (7,4'-dihydroxy isoflavonoids) and glycitine (6-methoxy-7,4' dihydroxy isoflavonoids) [10].

The active compounds in soybeans that are responsible for biological activity were requested by daidzein and genistein. Several studies have shown that genistein, isoflavones in soybeans can prevent cancer [11], breast cancer [12]. Daidzein genistein compounds can prevent osteoporosis in postmenopausal women [13], antimetastatic prostate cancer in humans [14], anti-inflammatory [15].

The types of soybeans that are mostly studied are black and yellow soybeans [16,17]. Previous study stated that black soybean has a better ability to prevent oxidation-related diseases compared to yellow soybeans [17]. Black soybeans have a higher antioxidant content than yellow soybeans [18]. The use of black soybeans in Indonesia is still limited compared to other kind of soybeans even though black soybeans have a lot of potential to be developed [16]. The potential for black soybean as a therapeutic agent is very promising, therefore it is necessary for further research on black soybeans especially as antioxidants because of its rich compounds.

2. Material and Method

2.1. Preparation of EEBS (Ethanol Extract of Black Soybean)

Fresh Black Soybeans obtained from Unit Pengelolaan Benih Sumber (UPBS) Balai Penelitian Tanaman Aneka Kacang dan Umbi, Malang, East Java. The plants were identified by herbarium staff, Department of Biology, School of Life Science and Technology, Bandung, West Java, Indonesia. The dried soy bean then ground, mashed into powder. The powder of Glycine max (L.) Merr. var. Detam (250g) were extracted with distilled ethanol 70% by a maceration methods. Ethanol filtrate was filtered, and wastes were remacerated until colourless filtrate in triplicates. The pasta form is obtained by condensation using 50°C evaprapor (Zhengzhou Well-known, RE-201D). The yield of EEBS was 2.63 g stored at 20°C [3,19-23,24]. EEBS was used for next experiment. Daidzein (Chengdu Biapurify Phytochemical Ltd, BP0445) were used as standard compound.

2.2. Phytochemical Assay

2.2.1. Flavonoids. 10 mg EEBS was dissolved in 2 N HCl [Merck 1003171000] in the test tube. Added enough Mg [Merck EM105815, USA], then heated for 5-10 minutes, cooled and filtered and add 1 ml of amyl alcohol. The sample contains flavonoid compounds if it produces red/orange [20,21,24].
2.2.2. **Saponins.** 10 mg EEBS was dissolved using ddH$_2$O in the test tube, boiled in a bath for 5 minutes then filtered and shaken vigorously and added 1 N. HCl. The sample contained saponin compounds if the foam remained stable and still after dropping HCl 1 N [19-24].

2.2.3. **Phenols.** Dissolved 10 mg EEBS in 5 ml ddH$_2$O, added 1% FeCl$_3$ [Merck 103943] solution as much as 500 µl. Sample contains phenol compounds if it produces green/red/purple/blue/black [19-24].

2.2.4. **Tannins.** 10 mg EEBS was dissolved in 2 ml 2 N HCl [Merck 1003171000] in a test tube, then heated in a waterbath for 30 minutes, wait until it's cold then add 500 µl amyl alcohol [Merck 10979, USA]. The sample contains tannin compounds if the aml alcohol layer is orange/red [19-24].

2.2.5. **Alkaloids.** 10 mg EEBS dissolved in 5 ml ddH$_2$O was evaporated in the waterbath. The resulting residue dissolved with 5 ml 2N HCl [Merck 1003171000] in a test tube, then heated in a waterbath for 30 minutes, wait until it's cold then add 3 drops of HCN 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 sediment formed indicate the presence of alkaloids [19-24].

2.2.6. **Steroids/Triterpenoids.** 10 mg EEBS put in a drop plate, add glacial acetic acid until submerged, let stand for 10-15 minutes then adding one drop of H$_2$SO$_4$ [Merck 109073, USA] concentrated. If it produces blue green, the sample contains steroid class compounds, whereas if it produces purple/red/orange, the sample contains a triterpenoid compound [19-24].

2.2.7. **Terpenoids.** 10 mg of EEBS put into a drop plate, added vanillin sufficiently, then drip H$_2$SO$_4$ [Merck 109073, USA] concentrated one drop and homogenized. The sample contains terpenoid compounds if it produces purple [19-24].

2.3. **Total Phenol Assay**
Briefly 15 µL standard solution in 6 concentration level (50; 25; 12.5; 6.25; 3.125; 1.5625 µg/mL) of gallic acid [Sigma 398225] and sample (EEBS) in concentration of 50 µg/mL were prepared for total phenol assay. Standard and sampled was mixed with 75 µL of Folin-Ciocalteu’s reagent 2.0 M [Merck 1.090.010.500] and 60 µL of Na$_2$CO$_3$ 7.5 % [Merck A897992745] in microplate. The reaction then incubated at 50°C for 10 min. The absorbance was measured in 760 nm of wavelength using Multiskan Go Reader [Thermo Fisher Scientific 1510]. The linear regression equation (y = ax + b) was created based on the standard (gallic acid) absorbance value. The analysis of phenol content of sample was performed based on the each of standard linear regression equation [19-25].

2.4. **Total Flavonoids Assay**
Briefly 15 µL standard solution in 6 concentration level (50; 25; 12.5; 6.25; 3.125; 1.5625 µg/mL) of quercetin [Sigma Q4951-10G] and sample (extract from black soybean) in concentration of 50 µg/mL were prepared for total flavonoid assay. Standard and sampled was mixed with 75 µL AlCl$_3$ 2% [Merck 449598] into the well plate containing the sample (well sample) and add 150 µl sample solvent (DMSO [Merck1029522500]) to well blank. The absorbance was measured in 415 nm of wavelength using Multiskan Go Reader. The linear regression equation (y = ax + b) was created based on the standard (quercetin) absorbance value. The analysis of flavonoids content of sample was performed based on the each of standard linear regression equation [26].

2.5. **DPPH scavenging activity assay**
Method from Widowati et al. was used for the DPPH assay [20-22]. The method is based on the formation of non-radical DPPH-H results from the addition of hydrogen from an antioxidant characterized by a reduction in alcoholic DPPH solution [19-24]. Briefly 50 µl various concentration of samples (EEBS, daidzein), was introduced in 96-well microplate followed by addition of 200 µl of
0.077 mmol/L DPPH [Sigma Aldrich D9123, USA] into the well. The mixture incubated at room temperature for 30 min in the dark. After that, absorbance was measured using a microplate at 517 nm. The scavenging activity (%) calculated as below:

\[
\text{Scavenging Activity (\%)} = \frac{(A_c - A_s)}{A_c} \times 100
\]

\(A_c\) = negative control absorbance (without samples)
\(A_s\) = samples absorbance

The scavenging activity (%) was the continued to be calculated as median inhibitory concentration (IC_{50}) [19-23,27,28].

2.6. ABTS-reducing activity assay

The antioxidant capacity of EEBS and daidzein was evaluated by ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) [Sigma A1888-2G, USA] diammonium salt-free radical assay. Two microlitres of various level samples (EEBS, daidzein) were added to the sample well, then ABTS*+ solution (198 μl) was added to each well (96 well-plate), incubated for 6 min at 30 °C. Absorbance was measured at 745 nm wavelengths using microplate spectrophotometer [19-24].

2.7. FRAP assay

FRAP assay was performed using previous modified method [19-24]. Briefly 7.5 μl of various level samples (EEBS, daidzein) was added with 142.5 μl FRAP reagent into each well in a 96 well-microplate and then incubated at 37 °C for 30 min. Absorbance was measured at 593 nm wavelength using microplate spectrophotometer.

2.8. \(H_2O_2\) scavenging activity

The mixture of 12μL ferrous ammonium sulphate 1mM [Sigma 7783859], 60μL of various level samples (EEBS, daidzein), 3 μL of \(H_2O_2\) 5 mM Merck 1.08597.1000 was incubated at dark room temperature for 5 min. After incubation, 75 μL of 1,10-phenanthroline 1 mM [Sigma 131377] was added to the well plate containing samples (well samples) and blank, the plate was incubated for 10 min in a dark place at room temperature. The absorbance of scavenging activity was measured at 510 nm wavelength [27,29]. The formula used to measured \(H_2O_2\) scavenging activity:

\[
\text{\(H_2O_2\) scavenging activity (\%)} = \frac{(Abs \text{ control} - Abs \text{ sample})}{Abs \text{ control}} \times 100
\]

Abs sample= Sample absorbance
Abs control= Control absorbance

3. Results and Discussions

Soybean is one of the most important plants for human and animal consumption, and the most important organic component of soybean seeds is protein (about 40%) and oil (about 20%). With the exception of isoflavonoids, few studies have been done on other phenolic classes present in soybean [30]. Black soybeans contain essential amino acids, vitamin E, saponins and are rich in antioxidants, for example with flavonoids, isoflavones and anthocyanins. However, black soybeans have a 4-fold tannin content compared to yellow soybeans. Tannins is one of the anti-nutrients because of its ability to bind proteins so that proteins are difficult to digest by the protease enzyme. From the results of phytochemical screening that has been done, it is known that black soybeans contain saponins, tannins, steroids/triterpenoids, terpenoids and alkaloids. Total phenol and flavonoids show the presence of phenols and flavonoids [9,31].

3.1. Phytochemical Assay of EEBS

Phytochemical screening of EEBS aimed to evaluate qualitatively presence of flavonoids, saponins, phenols, tannins, steroids/triterpenoids, terpenoids and alkaloids. The result of EEBS phytochemical
screening showed in Table 1. Phytochemical screening shows that only saponins, tannins, steroids/triterpenoids and alkaloids were detected.

Table 1. The result of qualitative phytochemical screening of EEBS.

| Phytochemical content       | EEBS |
|-----------------------------|------|
| Flavonoids                  | -    |
| Saponins                    | +    |
| Phenols                     | -    |
| Tannins                     | +    |
| Steroids/Triterpenoids      | +/-  |
| Terpenoids                  | +    |
| Alkaloids                   | +    |

+ : detected; - : Not detected

3.2. Total Phenol and Flavonoids Assay

Total phenol bioactivity was to find out phenol content contained in EEBS. Total phenol of EEBS based on gallic acid equivalent (GAE) was performed using linear regression equation (y = 0.0374 x + 0.1452 with $R^2 = 0.99$) [19-23]. The total phenol obtained from EEBS was 5.60±0.18 µg GAE/mg EEBS. Total flavonoid assay is to find out flavonoid content contained in EEBS. Total flavonoids of EEBS based on quercetin equivalent (QE) was performed using linear regression equation (y = 0.0174 x + 0.0368 with $R^2 = 0.99$) [26,27]. This study shows that EEBS has total flavonoids content is 2.03±0.12 µg QE/mg EEBS.

In the phytochemical screening assay, phenol and flavonoids were not detected in EEBS, according to the results of total phenol (5.60±0.18 GAE µg/mg EEBS) and flavonoid (2.03±0.12 QE µg/mg EEBS) which showed low results when compared to previous studies which reported total phenol from black soybean (80% ethanol) was 17.75 ± 0.39 (GAE mg/g extract) and total flavonoids 5.73 ± 0.26 catechin equivalent (CE mg/g extract), so that they are not detected qualitatively by phytochemical assay [32].

Other ingredients in black soybeans are essential amino acids, vitamin E, saponins and are rich in antioxidants such as flavonoids, isoflavones and anthocyanins. The several foods that have been analyzed, it is known that soybeans rank first, containing isoflavones and their derivatives. Isoflavones and their derivatives are compounds known to function as antioxidants, antitumor, antiinflammatory [33,34]. Isoflavones are flavonoid antioxidants that have aglycone, namely genistein (5,7,4′-trihydroxy isoflavones), daidzein (7,4′-dihydroxy isoflavones) and glycitein (6-methoxy-7,4′ dihydroxy isoflavones)[10] that exert antioxidant action [3,35]. Total isoflavone content in soybean seed was around 558.2 µg/g - 1716.9 µg/g [36]. When compared with other types of soybeans, black soybeans contain than yellow soybeans. The total polyphenols in yellow soybeans are 0.02 mg/g, while black soybeans have a total polyphenol content of 0.56 mg/g [9].

3.3. Antioxidant Assay

3.3.1. DPPH scavenging activity. The antioxidant capacity of EEBS and daidzein was evaluated using DPPH free radical scavenging activity. This method is based on the formation of non-radical DPPH-H results from the addition of hydrogen from antioxidants which is characterized by a reduction in the DPPH alcohol solution [37,20-23]. Antioxidant molecules extinguished by DPPH free radicals are marked in purple from the change in DPPH sample to colourless [19-23,24]. The inhibitory concentration (IC$_{50}$) of EEBS and daidzein to scavenge DPPH can be seen in Table 4.
Table 2. The IC$_{50}$ value of DPPH scavenging activity of EEBS and daidzein.

| Samples     | Equation          | R$^2$ | IC$_{50}$ μg/mL | IC$_{50}$ (μM) |
|-------------|-------------------|-------|-----------------|---------------|
| EEBS        | y = 0.2463x + 22.069 | 0.99  | 113.40          | -             |
| Daidzein    | y = 0.2052x + 27.376 | 0.99  | 110.25          | 433.66        |

*Linear equations, coefficient of regression (R$^2$) and IC$_{50}$ of each sample were calculated. IC$_{50}$ of EEBS was presented in μg/mL, while daidzein were presented in μM and μg/mL.

As shown in Table 2, the IC$_{50}$ value of daidzein (110.25 μg/mL) was lower than EEBS (113.40 μg/mL). These results indicate low scavenging activity of EEBS compared to daidzein. Nevertheless, at the highest concentration (200 μg/mL) EEBS has higher scavenging activity (70.18 ± 0.8%) than daidzein (67.34 ± 1.16%) (shown in Figure 1).

![Figure 1](image_url)

**Figure 1.** Effect various concentration of EEBS and daidzein toward DPPH scavenging activity. EEBS and daidzein were diluted in DMSO to reach the final concentration of 6.26; 12.5; 25; 50; 100; 200 (μg/mL).

Research conducted by Takahata et al. (2001) showed that DPPH radical scavenging activity of black soybeans was the strongest among the three types of soybeans associated with seed peels, which also included reddish brown, chocolate, and black beans, and were highly dependent on the content of phenolic compounds [38].

According to Table 4, the most active extract in the DPPH scavenging activity of the two tested compounds was daidzein extract which was shown by a lower IC$_{50}$ value compared to EEBS. Daidzein has higher antioxidant activity through DPPH scavenging activity and IC$_{50}$ value compared to EEBS. This is because daidzein is a polyphenolic compound that has a broad spectrum of physiological and pharmacological functions and is known to act as an antioxidant in vivo and in vitro [39].

3.3.2. ABTS-reducing activity assay. The ABTS reduction activity assay measures the relative ability of antioxidants to scavenge the ABTS generated. The reaction of a strong oxidizing agent (potassium permanganate / potassium persulfate) with ABTS salt will produce ABTS. Reduction of the blue-green ABTS radical solution by antioxidants that donate hydrogen is measured by 745 nm long wave absorption spectrum [19-23,24,28]. ABTS-reducing activity of EEBS and daidzein based on IC$_{50}$ values can be seen in Table 3.
Table 3. The IC$_{50}$ value of ABTS reducing activity of EEBS and daidzein.

| Samples  | Equation               | $R^2$ | IC$_{50}$ μg/mL | IC$_{50}$ (μM) |
|----------|------------------------|-------|-----------------|----------------|
| EEBS     | $y = 0.5008x + 12.441$ | 0.99  | 75.00           | -              |
| Daidzein | $y = 0.4736x + 8.9021$ | 0.99  | 86.78           | 341.34         |

*Linear equations, coefficient of regression ($R^2$) and IC$_{50}$ of each sample were calculated. IC$_{50}$ of EEBS was presented in μg/mL, while daidzein were presented in μM and μg/mL.

EEBS has higher ABTS-reducing activity as indicated by lower IC$_{50}$ (75.00 μg/mL) compared to daidzein (86.78 μg/mL). Along with reducing activity at the highest concentration (50 μg/mL) EEBS have higher activity (36.97 ± 0.09%) than daidzein (32.30 ± 1.87%) shown in Figure 2. The results show that EEBS has high antioxidant activity compared to standard compounds.

![Figure 2](image.png)

**Figure 2.** Effect various concentration of EEBS and daidzein toward ABTS-reducing activity. EEBS and Daidzein were diluted in DMSO to reach the final concentration of 0.78; 1.56; 3.13; 6.25; 12.5; 25; 50 (µg/mL).

IC$_{50}$ EEBS value in ABTS reducing activity assay is lower than daidzein, this shows that EEBS has higher antioxidant potential than daidzein. Some studies reported that black soybeans have the potential to possess antioxidants.

3.3.3. FRAP activity. FRAP activity can be used to determine the EEBS antioxidant capacity and daidzein. FRAP activity of the EEBS and daidzein can be seen in Figure 3. The method of FRAP activity based on the reduction Ferroin analog, complex tripyridyltriazine Fe$^{3+}$ of Fe (TPTZ)$^{+}$ into Fe$^{2+}$ complex dark blue coloured Fe (TPTZ) $^{2+}$ by antioxidants in acidic medium [5,19-23,28]. In this study, FRAP activity of EEBS and daidzein at the highest concentration (50 μg/mL) was 148.89 ± 4.16 and 98.36 ± 4.57 μM Fe (II)/μg respectively. These results indicate that EEBS has higher FRAP activity than daidzein [21,23].
Figure 3. Effect various concentration of EEBS and daidzein toward FRAP activity.
EEBS and daidzein were diluted in DMSO to reach the final concentration of 0.8; 1.6; 3.1; 6.3; 12.5; 25; 50 (µg/mL).

The content of flavonoids in EEBS, especially isoflavones and their derivatives (daidzein and genistein), has an important role as an antioxidant in black soybeans. As one of the flavonoid groups, isoflavone (daidzein is one of them) bioactive compounds containing phenolic groups have been reported to have ability as an antioxidant and prevent the occurrence of free radical damage through two mechanisms, namely donating hydrogen ions, and acting as a direct free radical scavenger [40].

3.3.4. \( \text{H}_2\text{O}_2 \) scavenging activity. The reaction of ferrous ammonium sulphate and phenanthroline could form \( \text{Fe}^{2+}\)-tri-phenanthroline complex with the color of orange, but if \( \text{H}_2\text{O}_2 \) exists in that reaction, \( \text{Fe}^{2+}\)-tri-phenanthroline complex would not be formed, thus scavenger of \( \text{H}_2\text{O}_2 \) might not form \( \text{Fe}^{2+}\)-tri-phenanthroline complex [29]. The \( \text{H}_2\text{O}_2 \) scavenging activity of EEBS and daidzein exhibited in Table 6. The present data showed that EEBS has higher \( \text{H}_2\text{O}_2 \) scavenging activity with an IC\(_{50}\) value 284.61 µg/mL, than daidzein with an IC\(_{50}\) value 363.60µg/mL. EEBS has higher antioxidant activity compared to daidzein.

Table 4. The IC\(_{50}\) value of \( \text{H}_2\text{O}_2 \) scavenging activity of EEBS and Daidzein.

| Samples   | Equation       | \( R^2 \) | IC\(_{50}\) µg/mL | IC\(_{50}\) (µM) |
|-----------|----------------|-----------|-------------------|-----------------|
| EEBS      | \( y = 0.0946x + 23.076 \) | 0.99      | 284.61            | -               |
| Daidzein  | \( y = 0.1284x + 3.3139 \) | 0.99      | 363.60            | 1,430.201       |

*Linear equations, coefficient of regression (\( R^2 \)) and IC\(_{50}\) of each sample were calculated. IC\(_{50}\) of EEBS was presented in µg/mL, while Daidzein were presented in µM and µg/mL.

In line with reducing activity (shown in Figure 4) at the highest concentration (300 µg/mL) EEBS has a higher activity (50.87 ± 1.4%) than daidzein (41.56 ± 0.40%).
Meta 5,7-dihydroxyl structure on ring A shows the ability of isoflavones to act as hydrogen ion donors so that more stable compounds are formed and peroxyl radicals are less reactive, while 4'-hydroxyl groups on ring B Isoflavone compounds act as ROS compound scavenger. The hydroxyl group configuration in ring B flavonoid compounds has been reported to act as a compound scavenger of ROS. It was further stated that the hydroxyl group at ring B can donate hydrogen ions by donating an electron to hydroxyl and peroxyl radicals; stabilize these two radicals, and form flavonoid radicals that are relatively more stable [40].

Daidzein included in the isoflavones group has a hydroxyl group responsible as a positive group for its antioxidant properties. The amount of hydroxyl groups possessed by daidzein can be a factor that can cause free radical scavenging activities. The more hydroxyl groups possessed by active compounds, the more free radicals can be scavenged [41]. Different from DPPH, the results of the ABTS reducing activity assay, FRAP activity and H$_2$O$_2$ scavenging the activity show that EEBS has a better antioxidant activity than daidzein. As previously explained, black soybeans contain essential amino acids, vitamin E, saponins and are rich in antioxidants, isoflavones and anthocyanins [33,34], also contain saponins, tannins, steroids/triterpenoids and alkaloids detected in the phytochemical test. The synergistic reaction of various compounds contained in EEBS causes EEBS to have a higher antioxidant activity than just one compound, daidzein.

The IC$_{50}$ value is defined as the concentration of the test compound which can reduce free radicals by as much as 50%. The smaller the IC$_{50}$ value the higher the free radical reduction activity. According to the standards that classified antioxidant capacity based on IC$_{50}$ values such as very strong (IC$_{50}$ value less than 50 ppm), strong (IC$_{50}$ value between 50-100 ppm), moderate (IC$_{50}$ between 100-150 ppm), and weak (IC$_{50}$ value between 151-200 ppm or more) (1 ppm equal to 1 µg/mL) [20]. DPPH showed the results of moderate antioxidant activity, ABTS showed the results of strong antioxidant activity and H$_2$O$_2$ showed low/weak antioxidant activity. The three tests (ABTS, FRAP and H$_2$O$_2$) showed better EEBS antioxidant activity than daidzein.

4. Conclusion
Overall, EEBS has better antioxidant activity capability than daidzein as evidenced by three assay results, ABTS reducing activity, FRAP activity and H$_2$O$_2$ scavenging activity.
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