EasyChair Preprint

№ 4123

Antixodant Activity of Black Soybean Extract (Glycine Soja) by the DPPH (1,1-Diphenyl-2-Pcrylhydrazyl) Method

Nurisyah Nurisyah, Elisabeth Natalia Barung and Alfrida Monica Salasa

September 1, 2020

Antixodant activity of black soybean extract (*Glycine soja*) by the DPPH (1,1-Diphenyl-2-Pcrylhydrazyl) method

Nurisyah[1,*], Elisabeth Natalia Barung[2] and Alfrida Monica Salasa[1]

[1]Health Polythecnic, Makassar, Indonesia
[2]Health Polythecnic, Manado, Indonesia

Email: nurisyah31@gmail.com

Abstract. Black soybeans (*Glycine soja*) is a potential plant as a source of antioxidants rich in flavonoids, isoflavones and anthocyanins. Because it was conducted research that aims to determine the antioxidant activity of black soybean (*Glycine soja*) extract. The extract was obtained by maceration using ethanol solvent 70%, methanol, methanol-water (9:1), and ethyl acetate. *Antioxidant activity test using scavenging 1,1-diphenyl-2-picrylhydrazil (DPPH) method, where the absorbance is measured on λ = 517 nm by spectrophotometer.* The results showed that black soybeans had antioxidant activity as showed on the IC_{50} value obtained. IC_{50} value of ethanol extract 70% was 2.99 mg/mL, methanol extract was 2.17 mg/mL, methanol-water extract (9:1) was 1.58 mg/mL, and ethyl acetate extract was 0.23 mg/mL. The results suggest that ethyl acetate extract has the best activity as an antioxidant, so effective as an alternative source of antioxidants that can be developed in the pharmaceutical field. Keywords: Antioxidants, black soybeans, DPPH

1. Introduction

Black soybeans are currently being targeted by nutrition and health researchers, because black soybeans contain phenolic, tannin, anthocyanin and isoflavones and antioxidant activity is higher than yellow soybeans. Black soy flavonoid content 6 times more than yellow soybeans. Soybeans generally contain isoflavones, as well as black soybeans [1].

Black soybeans have higher antioxidant content than yellow soybeans [2]. In addition, black soybeans contain antocyanin which is very potential to prevent the oxidation process that occurs early and cause degenerative diseases. In black soybeans, there are three kinds of anthocyanins: delphinidin-3-glucoside 0-3,71 mg / mL, cyanidin-3-glucoside 0.94 -15.98 mg / mL, and petunidine-3-glucoside 0-1.41 mg / mL. The total anthocyanin content in black soybeans is 1.58-20.18 mg/mL [3].

Antosianin of black soybean can inhibit LDL cholesterol oxidation, diligently consume black soybean processed products as much as 150 grams / day can lower cholesterol levels. In addition, to inhibiting LDL oxidation, the flavonoid of black soybean have function as an anticancer. Not only serves as an antioxidant, black soy is able to reduce the symptoms of menopause in women because of the content of soy isoflavones whose structures resemble estrogen. In addition, black soybeans can inhibit premature aging in women if taken regularly [4].

Processed black soy is not as interesting as yellow soybeans. In Indonesia, black soybean is generally used for sauce material because black soybeans make the quality of soy sauce to black chocolate [3].

Active compounds in plants was extracted by solvent extraction. The choice of solvent type should consider several factors such as selectivity, ability to extract, toxicity, ease of evaporation and solvent prices [5]. The solution used is adjusted to the desired polarity of the compound. According to the principle of like dissolves like, a solvent will tend to dissolve a compound having the same polarity. The polar solvent will dissolve the polar compound and vice versa. Flavonoids are polyphenol group compounds that are widely distributed in plants in the form of glycosides that bind to a sugar, therefore flavonoids are polar compounds. Polar solvents commonly used for the extraction of flavonoids are methanol, ethanol, water and ethyl acetate. Therefore, this study was conducted to determine which type of solvent is best to obtain the extract with the highest antioxidant activity, so it is expected that the use of black soybeans can be improved.

2. Methods

2.1. Sampling and Processing of Sample

Black soybean samples (Glycine soja) are cleansed from epidermis attached to soybeans using warm water. After cleansing, black soybean samples are dried in a clean container and smoothed, and black soy samples (Glycine soja) are ready for extraction.

2.2. Sample Extraction

The soybean seed is smoothed, put into a maseration vessel (erlenmeyer 500 ml). Then 70% ethanol canvasser was added at a ratio of 1: 3 g / mL. The vessel was shaken using a shaker for 4 hours at 200 rpm. The result of maceration was filtered, the flotate obtained was concentrated with rotary vacum evaporator to obtain the viscous extract. The condensed extract is dried over a water bath of 70ºC. The same treatment was performed using methanol solvent, methanol-water (9:1), and ethyl acetate.

2.3. Test Antioxidant Activity with DPPH Method

Antioxidant activity (free antiradical) was calculated by the DPPH method in which the sample was reacted with DPPH solution. The test was carried out by 1.0 ml of the sample solution in ethanol solvent reacted with 4.0 ml 40% w / v DPPH solution in the ethanol solvent, then the mixture incubated at room temperature for 30 minutes in the dark (in vials enclosed in aluminum foil) measured uptake by UV - Vis spectrophotometer at a wavelength of 400 - 600 nm. The color change of the solution showed DPPH free radical capture activity and can be measured by the difference in absorbance produced in the sample compared with the controls / blanks (unsupported DPPH). Antiradical activity is expressed in terms of percent DPPH radical capture and calculated by equations (Yen and Chen, 1995).

$$\% \text{ inhibition} = \left(1 - \frac{\text{sample absorbance}}{blank\ absorbance}\right) \times 100\%$$

A 0% value means no free antiradical activity or antioxidant, while a 100% value means total absorption. Determination of IC_{50} value is done by measuring the antioxidant activity of a series of concentration of sample solution, then made the equation of regression line of concentration VS% inhibition. From the regression line equation can be seen the value of IC_{50} by entering the value of 50 as the value of Y axis, so it can be obtained how much the value of X that will present IC_{50} scale.

3. Results and Discussion

Extraction of active compounds from a plant tissue with different types of solvents at different polarity levels aims to obtain optimal results, both the amount of extract and active compounds contained in the sample. Extraction may use solvent and mixed solvents, commonly used mixtures of mixtures of water and ethanol and mixtures of water and methanol [6]. A number of irreplaceable hydroxyl groups or a sugar causes the flavonoid to be polar so dissolve in a polar solvent such as methanol. The effect of glycosylation (sugar bound to flavonoids) causes the flavonoids to be less reactive, thus more easily soluble in polar solvents such as water [5,7].

Ethanol solvent 70%, methanol and methanol: water (9: 1) is a polar solvent that can attract polar compounds such as flavonoids-O-glycosides [7]. Flavonoids-O-glycosides contain sugar molecules. This sugar molecule also contains a hydroxyl group. The hydroxyl group is polar, so it will easily dissolve also with high polarity. Phenolic components can be extracted from plant material using polar solvents such as water, methanol, ethanol or semi-polar solvents such as ethyl acetate [8].

Black soybeans analyzed antioxidant activity is black soy varieties Mallika small seed. Black soybean samples were extracted by maceration using ethanol solvent 70%, methanol, methanol-water (9: 1), and ethyl acetate. The concentration line of the tested sample solution is adjusted to the regression line equation which is expected to give the value of IC_{50} (50% antioxidant activity) which is in the concentration series of the prepared solution. Data of % inhibition determination can be seen in **Table 1.**

Table 1. Data of measurement result of antioxidant activity of each extract.

Sample	Concentration (mg/L)	average absorbance	% inhibition
Blanko	-	0.83029	-
Ethanol extract 70%	1	0.67103	19.18
	1.5	0.60256	27.43
	2	0.53826	35.17
	2.5	0.47356	42.96
	3	0.41986	49.43
	3.5	0.34934	57.93
Methanol extract	1	0.61117	26.39
	1.5	0.52666	36.57
	2	0.44471	46.44
	2.5	0.35845	56.83
	3	0.28073	66.19
Methanol-water extract (9: 1)	0.5	0.66031	20.47
	1	0.54414	34.46
	1.5	0.41369	50.18
	2	0.32437	60.93
	2.5	0.22421	72.99
Ethyl acetate extract	0.05	0.74182	10.66
	0.1	0.65053	21.65
	0.15	0.58516	29.52
	0.2	0.52528	36.74

In this study, the antioxidant potential of a radical trap is determined using DPPH, a stable radical in aqueous solution or methanol and capable of receiving an electron or a hydrogen radical to become a stable diamagnetic molecule [9,10]. DPPH in this test is captured by antioxidants that release hydrogen, thus forming a reduced DPPH-H. The color changes from violet to yellow and follows a decrease in absorption at 517 nm wavelength. The decrease in absorption is then the antioxidant activity of radical catcher can be known.

Table 1 shows that all extracts have antioxidant activity as indicated by the decrease in DPPH uptake. The decrease in DPPH uptake is then calculated as% inhibition (% of free radical binding of DPPH). The concentration of treatment at ethanol extract 70% and methanol extract ranged from 1 to 3.5 mg/mL, this was done so that the value of 50% inhibition (= IC_{50}) was in the range of treatment concentration. This shows that the smaller the concentration of treatment will give the value of IC_{50} is getting smaller as well.

From the data Table 1 above made a curve of the relationship of concentration to% inhibition so that obtained the equation of regression line of each sample extract. The curves are presented in Figure 1 - 4 below:

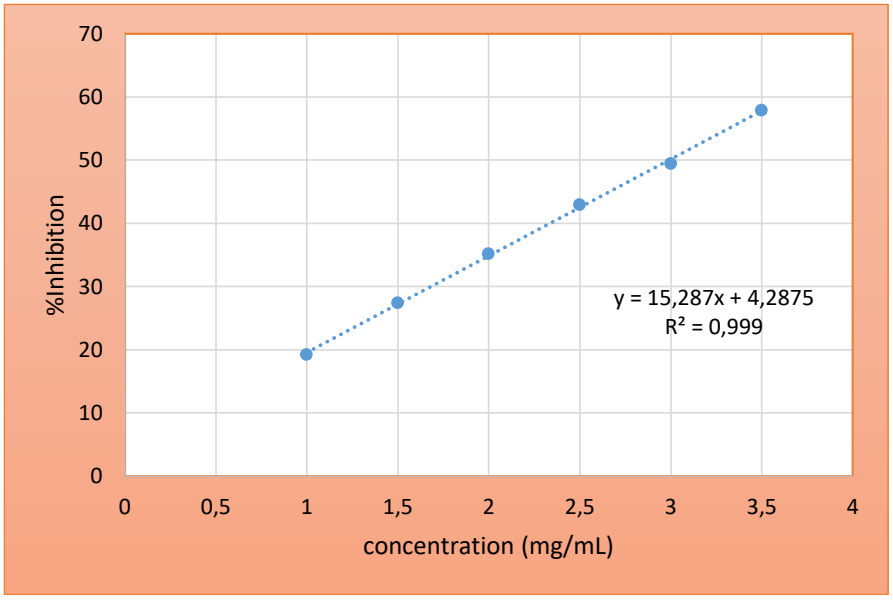

Figure 1. Regression curve of ethanol extract 70%.

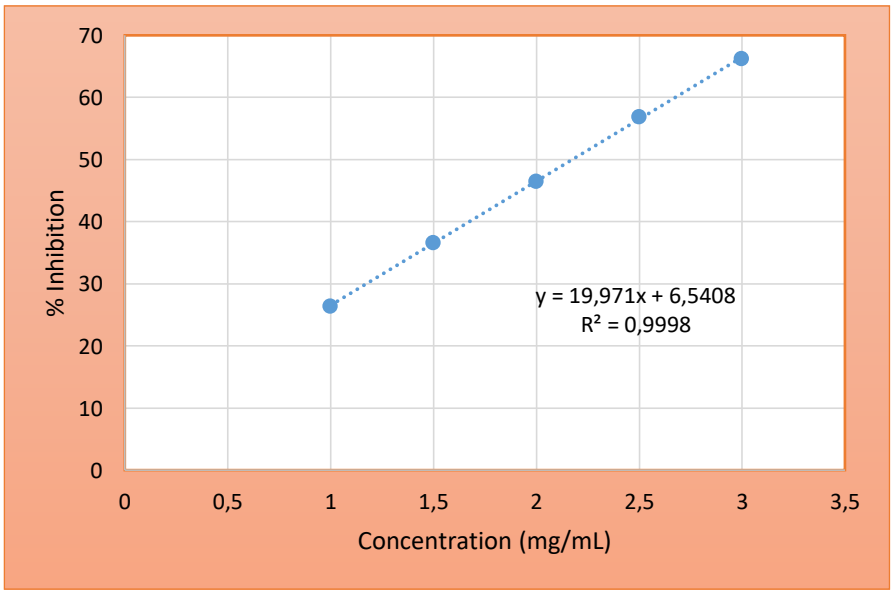

Figure 2. Regression curve of methanol extract

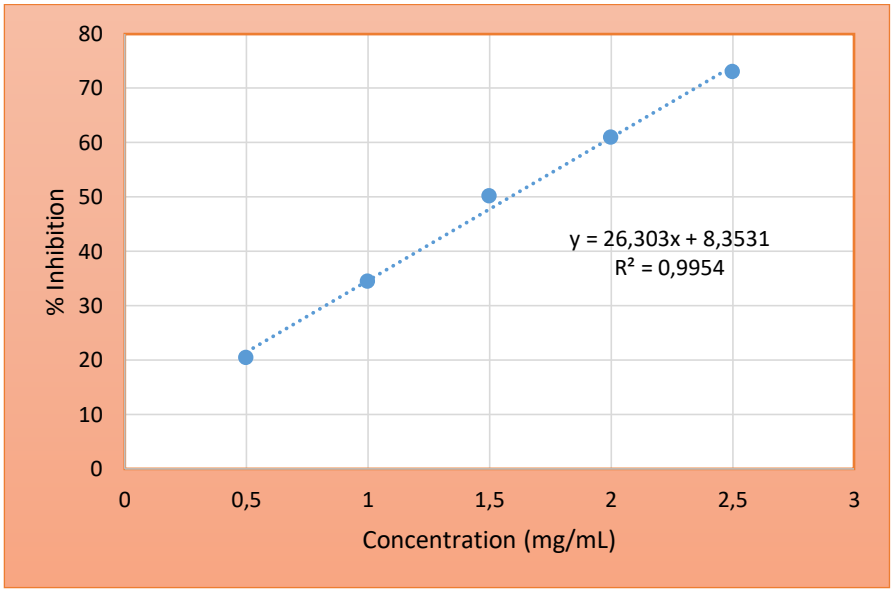

Figure 3. The regression curve of methanol-water extract (9:1).

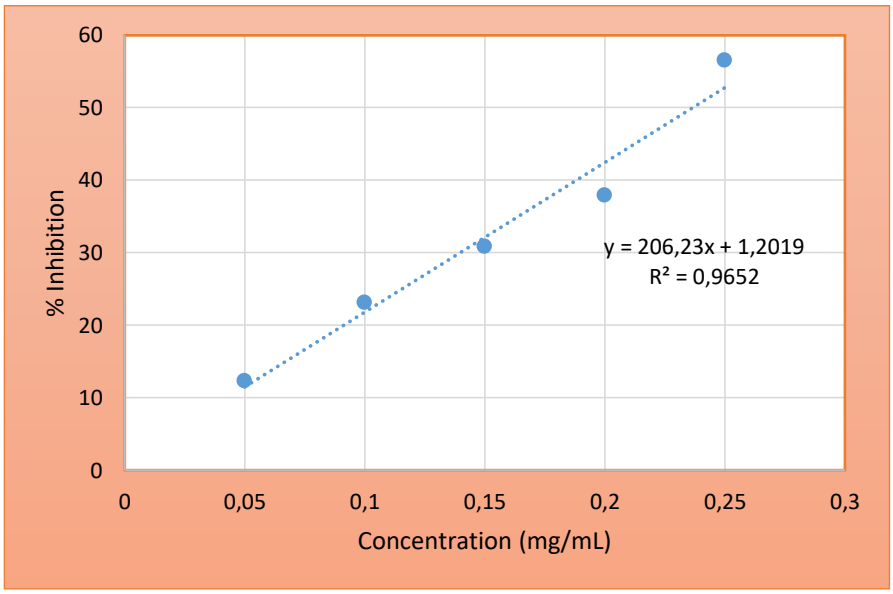

Figure 4. The regression curve of ethyl acetate extract.

The calculation results of IC_{50} value of each sample can be seen in **Table 2** this below :

Table 2. The antioxidant activity of each sample

Sample	The equation of the regression line and the linearity value (R)	IC_{50} value
Ethanol extract 70%	y = 15.287x + 4.2875 R = 0.999	2.99 mg/mL
Methanol extract	y = 19.971x + 6.5408 R = 0.9998	2.17 mg/mL

Methanol-water extract (9: 1)	y = 26.303x + 8.3531 R = 0.9954	1.58 mg/mL
Ethyl acetate extract	y = 0.2062x + 1,2019 R = 0.9652	0.23 mg/mL

Antioxidant activity was measured by looking at DPPH damping capability by the extract, also known as percent of inhibition. The parameter used to show the antioxidant activity is inhibition concentration (IC_{50}). Determination of IC_{50} of each extract aims to obtain the number of doses of extract that can absorb free radicals by 50%. The average value of IC_{50} extract can be seen in **Table 2**, and based on **Figure 5** above shows that the solvent of ethyl acetate solvent gives the result of high antioxidant activity because IC_{50} value is relatively lower compared to other solvent.

Based on IC_{50} values, it was found that black soybean seed extract with ethyl acetate solvent had stronger antioxidant activity compared with soybean extract with ethanol solvent 70%, methanol, and methanol-water (9: 1). This is because the smaller the value of IC_{50} the higher the antioxidant activity of a material. An antioxidant compound is very strong when IC_{50} value is less than 0.050 mg / mL, strong antioxidant if IC_{50} value is between 0.050 - 0.1 mg / mL, moderate antioxidant if IC_{50} value ranges from 0.1-0.15 mg / mL and antioxidants weak if IC_{50} ranges from 0.15-0.2 mg / mL and if the above IC_{50} value of 0.2 mg / mL is included into very weak antioxidants [11].

Extract of black soybean seed with ethyl acetate solvent has the highest antioxidant activity (lowest IC_{50} value) compared with soybean extract with other solvent. This suggests that the bioactive compound which acts as a free radical inhibitor of black soybean extract can be extracted well if using ethyl acetate solvent. Thus it can be said that ethyl acetate extract has the best activity as an antioxidant than other extracts, so effective as an alternative source of antioxidants that can be developed in the pharmaceutical field.

The ethyl acetate solvent is a solvent used to extract compounds with intermediate polarities such as flavonoids in the form of O-glycosides and tannins, whereas triterpenoids are compounds composed of long chains of C30 hydrocarbons which are nonpolar, but triterpenoid compounds mostly contain an -OH group, the presence of substituents of the hydroxyl groups attached to the hydrocarbon chain causes their properties to be semi-polar in that they can be extracted in an ethyl acetate solvent. In this study we found that the activity of antioxide extract of ethyl acetate which is still very weak category (IC_{50}> 0.2 mg / mL). This is because the compound contained in ethyl acetate extract which acts as antioxidant like flavonoid is not pure so that its antioxidant power is low.

Some other foodstuffs that have been analyzed, known to be the first row of soybeans, contain isoflavones and their derivatives. Isoflavones and derivatives are compounds known to function as antioxidants, antitumors, antiosteroklerosis [12,13]. This isoflavone compound is generally a complex or conjugate compound with a sugar compound through a glucoside bond. This type of isoflavone compound is mainly genistin, daidzin, and glycitin. This form of compound has a small physiological activity. During processing, either through the fermentation process or non-fermentation process, isoflavone compounds can undergo a transformation, especially through the process of hydrolysis so that it can be obtained free isoflavone compound called aglikon higher activity. The aglycone compounds are genistein, glycinein, and daidzein. Thus to increase the antioxidant activity of black soybean seeds, it should be done first hydrolysis process or fermentation process.

4. Conclusion

Based on the results of research that has been done, it is concluded:

1. All extracts have antioxidant activity with IC_{50} value each is ethanol extract 70% of 2.99 mg/mL; methanol extract of 2.17 mg/mL; methanol-water extract (9:1) of 1.58 mg/mL; and ethyl acetate extract of 0.23 mg/mL.
2. Ethyl acetate extract has the strongest antioxidant activity.

Acknowledgment
The authors would like to thank the Head of Pharmacy Department and the Director of Poltekkes of Kemenkes Makassar who have provided financial support to this research.

References
[1] Chang S K C and Xu B J 2007 Comparative Analyses of Phenolic Composition, Antioxidant Capacity, and Color of Cool Season Legumes and Other Selected Food Legumes. *J. Food Sci* **72**(2) : S167
[2] Kwon S H, Ahn I S, Kim S O, Kong C S, Chung H Y, Do M S and Park K Y 2007 Anti-obesity and hypolipidemic effects of black soybean anthocyanins, *J. Med. Food*, **10**(3), 552-556.
[3] Adie dan Krisnawati 2012 *Kedelai Hitam : Varietas, Kandungan Gizi Dan Prospek Bahan Baku Industri*. Badan Litbang Pertanian, Balai Penelitian Tanaman Kacang-kacangan dan Umbian-umbian
[4] Takahashi R, Ohmori R, Kiyose C, Momiyama Y, Ohsuzu F and Kondo K 2005 Antioxidant activities of black and yellow soybeans against low density lipoprotein oxidation. *J. Agric. Food Chem.* **53**(11): 4578-82.
[5] Harborne J B 1996 *Metode Fitokimia*, translated by Kosasih Padmawinata and Iwan Soediro, Edisi II, (Bandung: ITB Press) p.14; 21-22; 69;72.
[6] Agoes, 2007?
[7] Markham 1988 *Cara Identifikasi Flavonoid*, translated by Kosasih Padmawinata, (Bandung: Penerbit ITB) p. 1-20.
[8] Ramle S F M, Kawamura F, Sulaiman O and Hashim R 2008 Study on Antioxidant Activities, Total Phenolic Compound, and Antifungal Properties of Some Malaysian Timbers from Selected Hardwoods Species *International Conference of Environmental Research and Technology*, 472-475.
[9] Oke et al., 2002??
[10] Hou et al., 2002??
[11] Molyneux P 2004 The Use of The Stable Free Radical Diphenylpicryl-hydrazyl (DPPH) for Estimating Antioxidant Activity *Songklanakarin J. Sci.Technol*. **26**(2): 211-21
[12] Dixon R A and Steele C L 1999 Flavonoids and isoflavonoids–a gold mine for metabolic engineering *Trends Plant Sci* **4**(10): 394-400.
[13] Yuan D, Pan Y, Chen Y, Uno T, Zhang S and Kano Y, 2008, An improved method for basic hydrolysis of isoflavone malonylglucosides and quality evaluation of Chinese soy materials. *Chem. Pharm. Bull.* **56**(1): 1-6.