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Research Articles

Antioxidant and Elastase Inhibitor from Black Soybean (*Glycine max* L.) and Its Compound (Daidzein)

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Abstract

Background: Skin aging occurs along with age. Genetic, nutrients, hormones, and sun exposure can affect directly and indirectly the structure of the skin. These things will induce an increase in free radicals which disrupts one of the most important enzymes in the extracellular matrix which is elastase enzyme. High elastase enzyme synthesis will cause skin wrinkle. Free radicals can be inhibited by the presence of antioxidants. Black soybean contains natural phytochemical compounds which act as antioxidants and anti aging. Black soybeans are rich in daidzein compound, which scavenging against free radicals and prevent premature aging.

Objective: This research evaluates the antioxidant and antiaging potential of Black Soybean (*Glycine max* L.) extract and its compound, daidzein.

Methods: Analysis of antioxidants from black soybean extract and daidzein were carried out using ABTS scavenging activity assay. The antiaging assay was carried out through inhibition of elastase enzyme.

Results: Black soybean extract had lower IC_{50} value of ABTS scavenging activity around 77.39±4.05 µg/ml better than daidzein with IC_{50} of 83.34±3.89 µg/ml. The results of elastase inhibition activity assay showed that daidzein compound has a lower IC_{50} value, 57.35±5.64 µg/ml compared to black soybean extract with IC_{50} value, 93.36±6.39 µg/ml.

Conclusion: Black soybean's extract had higher antioxidant. Daidzein had better elastase inhibition activity compared to black soybean extract.

Keywords: Black soybean; daidzein; antioxidant; anti-aging; anti-elastase
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INTRODUCTION

Aging process occurs in every human being with age. Aging is a process of reduced ability of tissue functions that bring effect for all organs of the body, both internal and external organs such as the skin.[1] Social progress and industrialization have gradually led to the development of sociocultural factors that have a direct and indirect influence on aging, including nutrients consumed, UV radiation, smoking and stress.[2] Other causes include genetic factors, free radicals, elastase enzymes, and reduced hormones.[3]

Free radicals are one of the aging factors because they can damage biological molecules in the body such as tissues and cells, causing oxidative stress and cell apoptosis.[3] Other aging factors, such as photoaging by exposure to UV light also affects the elasticity of the skin, which is degraded by the enzyme elastase in the extracellular matrix shown as wrinkles. Elastase enzyme which plays a role in degrading elastin, protein usually found with Extracellular Matrix. These enzymes have their respective roles in their involvement in the aging process.[4]

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In this decade, modern science has developed in identifying alternative solutions with very few side effects, one of which is natural herbal plants.[5] The use of natural ingredients has been carried out by the community for a long time because many contain compounds that are efficacious in medicine known as phytochemical compounds, namely groups of natural compounds that can be used to maintain health.[6]

Black soybeans have the highest isoflavone content, including genistein and daidzein. Isoflavones as active substances have a working mechanism by inhibiting free radicals. In addition, isoflavones are also found in other legume plants.[7,8] Antioxidant of other compounds possessed by black soybeans also play a role in the process of inhibiting premature aging.[9,10]

MATERIALS AND METHODS

Materials

Black soybean (Glycine max (L.) Merr.), ethanol 70%, ABTS (2,2'-39;-Azino-Bis-3-Ethylbenzothiazo line-6-Sulfonic Acid) (Sigma A1888 lot 061M5308V), Potassium persulfate (Merck 1.05091.0250 lot K443 103913), PBS 1x (Gibco 1740576), Dymethilsufoxide (DMSO) (Merck 1.02952.1000 lot K46505352), N-Sucanyl-Ala-Ala-Ala-P-nitroanilide, elastase substrate (Sigma 54760), Elastase from porcine pancrease (Sigma 45124), Tris (Pharmacia biotech 17-1321-01), Sodium chloride (Merck1064040500), Hydrochloride acid solution (Merck 1090631000), Daidzein (Chengdu Biopurify, BP0445), ddH_2O.

Instruments

Macerator, Evaporator, pH meter, Erlenmeyer tube, Multiskan Go Reader, Mikropipet (1-10 µl, 50- 200 µl, 100-1000 µl), Tips (1-10 µl, 50- 200 µl, 100-1000 µl), Falcon tube (15 ml, 50 ml), Tube Effendorf (1,5 ml), 96 well plate, Analytical Balance, Vortex.

Preparation of black soybean extract

Black soybeans (*Glycine max* (L.) Merr.) were dried using incandescent light to speed up the drying process. After that, Glycine max (L.) Merr. were ground and extracted by maceration technique using 70% ethanol for 3 days at room temperature. The filtrate obtained was evaporated by the solvent with a rotary evaporator at 50°C, so that a thick extract was obtained.[11]

ABTS assay

The sampel solution was made from 2 µl of Glycine max (L.) Merr. extract and daidzein with various concentrations (1000; 500; 250; 125; 62.5; 31.25 µg/ml), added into the well that containing 198 µl ABTS. The control solution was made from 200 µL ABTS. The blank only contains 200 µL of DMSO. Microplate was closed, then incubated 37°C for 6 minutes. Measurement of absorbance with a microplate reader at a wavelength of 745 nm.[12,13,14]

% scavenging activity = [(control-sample) / control] x 100

Elastase inhibition assay

The solution consists of 10 µl samples (concentration of 0.78-50 µg/ml), 5 µl Elastase from porcine pancrease (0.01 mg / ml, Sigma 45124) and 125 µl tris buffer (100 mM, pH 8) were incubated at 25°C for 15 minutes. In addition, it was also prepared for control that contains 5 µl enzyme and 135 µl tris buffers. Blank contains 130 µl tris buffer and 10 µl samples. Next, mixture of 10 µl of the SucAla-3-pNA substrate was added and re-incubation at 25°C for 15 minutes. The absorbance is measured using a wavelength of 410 nm. [12,13,14,15]

% scavenging activity = [(control-sample) / control] x 100

Table 1. ABTS reduction activity

Concentration (µg/ml)	Averange ABTS reduction activity (%)	
	Glycine max (L.) Merr.	Daidzein
50	33.03 ± 2.00[d]	28.68 ± 1.19[d]
25	17.05 ± 0.98[c]	17.27 ± 2.02[c]
12.5	14.76 ± 0.98[c]	9.86 ± 0.83[b]
6.25	7.36 ± 0.95[b]	2.76 ± 0.08[a]
3.13	4.48 ± 0.64[b]	0.81 ± 0.05[a]
1.56	1.29 ± 0.18[a]	0.60 ± 0.05[a]

Data were presented as mean ± standard deviation. Different small letters in the same coloumn are significant at P < 0.05 (Tukey HSD Post Hoc Test).

Table 2. IC_{50} value of ABTS reduction

Sample	Equation	R_2	IC_{50} (µg/ml)	IC_{50} Averange
Glycine max L. (1st repetition)	Y = 0.6429x + 2.7548	0.95	73.49	77.39 ± 4.05
Glycine max L. (2nd repetition)	Y = 0.5718x + 3.3567	0.97	81.57	
Glycine max L. (3rd repetition)	Y = 0.6104x + 2.9278	0.95	77.12	
Glycine max L. (averange)	Y = 0.6084x + 3.0131	0.96	77.23	
Daidzein (1st repetition)	Y = 0.6174x + 0.2597	0.96	80.56	83.34 ± 3.89
Daidzein (2nd repetition)	Y = 0.6112x – 0.1743	0.99	82.09	
Daidzein (3rd repetition)	Y = 0.5627x + 0.5199	0.97	87.93	
Daidzein (averange)	Y = 0.5971x + 0.2017	0,98	83.40	

RESULTS

ABTS reduction antioxidant activity

In antioxidant testing of ABTS (2,2'-39;-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acids), ABTS solution was prepared first and the absorbance measurement of black soybean extract and daidzein samples. The antioxidant activity test used the ABTS reduction method with concentrations of 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.25 µg/ml, 3.13 µg/ml and 1.56 µg/ml.

Table 1 show the decrease that occurred was significantly different ($p < 0.05$). Figure 1 show the antioxidant activity of Glycine max (L.) Merr. and daidzein increased in proportion to the concentration. Highest antioxidant activity at concentration 50 µg/ml with value of 33.03±2.00 % (Glycine max (L.) Merr.) and 28.68±1.19 % (daidzein). Daidzein has lower antioxidant activity compared to Glycine max (L.) Merr. at various concentrations.

Table 2 shows the value of IC_{50} Glycine max (L.) Merr. and daidzein at 77.39±4.05 µg/ml and 83.34±3.89 µg/ml.

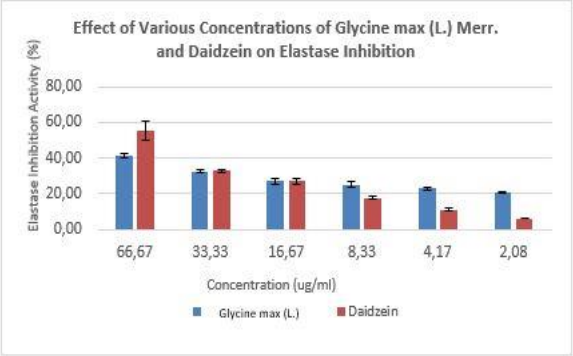

Figure 2. Elastase inhibition activity in various concentrations

Elastase inhibition activity

Each black soybean extract and daidzein concentration in elastase inhibitor activity were 66.67 µg/ml; 33.33 µg/ml; 16.67 µg/ml; 8,33 µg/ml; 4,17 µg/ml; 2.08 µg/ml.

Table 3. Elastase inhibition activity

Concentration (µg/ml)	Averange elastase activity (%)	
	Glycine max (L.) Merr.	Daidzein
66.67	41.48 ± 1.48^{e}	55.72 ± 5.40^{d}
33.33	32.48 ± 0.76^{d}	32.79 ± 1.06^{c}
16.67	27.13 ± 1.47^{c}	27.08 ± 1.57^{c}
8.33	25.10 ± 1.75^{b,c}	17.82 ± 0.94^{b}
4.17	22.95 ± 1.03^{a,b}	11.25 ± 1.02^{a,b}
2.08	20.97 ± 0.49^{a}	6.29 ± 0.18^{a}

Data were presented as mean ± standard deviation. Different small letters in the same column are significant at P < 0.05 (Tukey HSD Post Hoc Test)

Table 3 and Figure 2 provide an overview of the results of increased antioxidant activity at each concentration with a peak concentration of 66.67 µg/ml, value of 41.48±1.48 % in Glycine max (L.) Merr. and 55.72±5.40 % on daidzein. There was a very thin difference between the two samples on 66.67 µg/ml concentration, which is 27.13±1.47 % (Glycine max (L.) Merr.) and 27.08±1.57 % (daidzein).

IC_{50} value of elastase inhibition activity can be seen in table 4 below, where the table shows IC_{50} values in Glycine max (L.) Merr. at 93.36±6.39 µg/ml and daidzein at 57.35±5.64 µg/ml.

DISCUSSION

Black soybean is a native plant of Asia which is very well grown in tropical regions such as Indonesia.[16] This plant has high content of the aglycone isoflavone compound daidzein. In addition there are also other compounds such as genistein. These active compounds act as antioxidants. Antioxidants are molecules that are able to stabilize or free radicals before attacking cells and can inhibit or delay the oxidation of a substrate.[17]

ABTS (2,2'-39;-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acids) produced by reacting strong oxidizing agents (potassium permanganate/potassium persulfate) with ABTS salt. ABTS antioxidant test measures the relative ability of antioxidant based on the loss of blue color due to the antioxidant donates hydrogen absorption spectra measured with a long wave. The wavelength that used in this experiment is 745 nm.[12,14]

ABTS reduction activity from daidzein has IC_{50} of 83.34±3.89 µg/ml greater than black soybean extract which has an IC_{50} value of 77.39±4.05 µg/ml, this because daidzein is a pure compound that is very active. This result is different from the previous study by Fidrianny *et al.*, which shows the IC_{50} results with the ABTS method around 0.54 µg/ml because of the differences in solvent and reagent used in the ABTS assay.[18] In other study by Lee et al. for 56 black soybeans landraces gave the same result as the current study with IC_{50} values averaging 53.5-127.7 µg/ml.[19]

The method used is to measure the levels of SucAla3 (N-Succinyl-Ala-Ala-Ala). The resulting yellow change is an indicator of the reaction. The higher ability of the sample to inhibit elastase activity, the less Sucala3 is formed (yellow was slightly/clear).[12,13,14,20]

IC_{50} values of black soybean extract and daidzein were 93.36 ± 6.39 µg/ml and 57.35±5.64 µg/ml. Budaraga et al. reported strong activity around 50-100 µg/ml, the stronger the activity with the smaller the value.[21] Blacksoybean inhibits the hydrolysis of elastin or fibrillin (fibers found in the extracellular matrix). The secretion and elastase activity of dermal fibroblasts is affected by UV exposure during photoaging, which triggers remodeling of the structure of elastin fibers and leads to the formation of wrinkles. The existence of NF-kB transcription activity is induced by UV radiation and greatly contributes to the photoaging process. [22]

CONCLUSION

Black soybean extract (*Glycin max* L.) has better antioxidant activity through ABTS reduction than daidzein compound from black soybean extract (*Glycin max* L.) extract, while daidzein has antiaging activity through elastase inhibition which is better than black

Table 4. IC_{50} value of elastase inhibition activity

Sample	Equation	R2	IC_{50} (µg/ml)	IC_{50} Averange
Glycine max L. (1st repetition)	Y = 0.3136x + 22.073	0.97	89.05	93.36 ± 6.39
Glycine max L. (2nd repetition)	Y = 0.3134x + 21.692	0.98	90.33	
Glycine max L. (3rd repetition)	Y = 0.2845x + 21.352	0.98	100.70	
Glycine max L. (averange)	Y = 0.3038x + 21.706	0.99	93.13	
Daidzein (1st repetition)	Y = 0.6998x + 10.561	0.96	56.36	57.35 ± 5.64
Daidzein (2nd repetition)	Y = 0.6267x + 10.245	0.95	63.42	
Daidzein (3rd repetition)	Y = 0.8011x + 8.1299	0.97	52.27	
Daidzein (averange)	Y = 0.7092x + 9.6454	0.96	56.90	

soybean. This conclude that black soybean is better for skin treatment.

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