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Comparison of Antioxidant and Anti-collagenase Activities Ethanol Extract of Black Soybeans with Daidzein Compounds

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Abstract

Aging is a natural process in human life; one of the triggers is free radicals. The use of antioxidants from natural ingredients is an effort to overcome premature aging and counteract the harmful effects caused by free radicals. Black soybean (Glycine soja), which easily obtained in Indonesia, contains a nutritional value that is very good for human health. The protein content is quite high (40.4 g / 100g) and rich in antioxidants. The results presented in this study revealed that black soybean extract (Glycine max (L.) Merr.) Had a higher antioxidant activity of FRAP compared to Daidzein compounds, black soybean extract had antiaging activity through better collagenase inhibition compared to Daidzein compounds. The largest FRAP activity at 50 µg / mL black soybean extract concentration was 146.35 µM Fe (II) / µg) and Daidzein 50 µg / mL concentration was 96.93 (µM Fe (II) / µg). Anticolagenase activity based on the IC50 value of black soybean extract was 84.73 µg / mL, and Daidzein was 98.18 µg / mL. From the above results, it can conclude that ethanol extract of black soybeans has excellent potential as an antioxidant that is useful as inhibiting collagenase.

Keyword: Antioxidant; Anti-collagenase; Black Soybean; Daidzein.

1. Introduction

Disease and health are essential problems that have to do with free radicals and antioxidants. There was a drastic change in the 20th century from the work of a Russian named Moses Gomberg, who made the first organic free radicals from triphenylmethane (a hydrocarbon compound used as a basis for various dyes).

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As a result of the research of [1]. concluded that the term free radical then interpreted as a relatively unstable molecule that has one or more unpaired electrons in its outer orbit. Because the unpaired electrons circle their orbits. In their molecules, they form a kind of magnetic effect that causes free radicals to bond to nearby molecules. Many free radicals are so unstable that their existence is only for a moment, during their concise lives, free radicals act like catalysts that bridge chemical reactions and change their shape in other molecules [2]. Oxidative stress refers to an imbalance between free radicals and their stabilizing antioxidant enzymes in the body. Reactive oxygen species (ROS) can be produced by healthy cellular metabolism and react with biomolecules such as proteins, lipids, and DNA to cause cell damage and are responsible for degenerative changes. Molecules that are reactive when paired with electrons contain nitrogen are called reactive specific nitrogen (RNS = Reactive nitrogen species). At low concentrations, free radicals play an essential role in physiological regulation and cellular signaling processes, but high levels can cause damaging changes in cells. In contrast to these antioxidants, they reduce oxidants by donating their electrons to stabilize free radicals and make them not reactive compounds to minimize the harmful effects produced by these radicals in cells. Human life intended to realize scientific knowledge and then try to distribute it throughout the world. Materialistic civilization emphasizes our bodies. Therefore our cells age faster and suffer the adverse changes that occur in the body [3-5] Intrinsic and extrinsic factors influence the aging process in the skin tissue. Intrinsic is a natural process that occurs over time. Biologic/genetic clock processes play a role in determining the amount of multiplication in each cell until the cell stops dividing and then dies [6-10]. while extrinsic factors include excessive sun exposure, pollution, smoking habits, and unbalanced nutrition [11]. Black soybean (Glycine soja), which easily obtained in Indonesia, contains a nutritional value that is very good for human health. The protein content is quite high (40.4 g / 100g) and rich in antioxidants such as anthocyanins, isoflavones (flavonoids), and other polyphenol groups. Isoflavones in black soybean have beneficial effects on patients with diabetes mellitus by increasing the production of serum insulin and protecting the organs of the pancreas and preventing damage from free radicals [12]. High anthocyanins in black soybeans and 1,1-diphenyl-2-picrylhydrazyl and O2 make black soybeans when boiled to form Liver-butyl hydroperoxide (t-BuOO) substances that are useful for active prevention against the generation of Thiobarbituric acid-reactive substances (TBARS)), where TBARS is known to be the cause of liver disorders [13]. Othe reaserch explained that anthocyanins pigments have higher antioxidants than tocopherol. Secondary metabolites and extracts of black soybeans have been widely investigated and found to have anti-collagenase, anti-elastase activity from Daidzein compounds, polyphenols such as flavonoids, tocopherols, phenolic acids, and tannins. These compounds provide the benefit of inhibiting Chc or a platform for synthesizing active molecules [14]. Based on the background of black soybeans, which have high nutritional and antioxidant content, the researchers wanted to examine the comparison of antioxidant and anti collagenase activities of ethanol extract of black soybeans with Daidzein compounds.

2. Materials and Method

2.1 Material

Multiskan Go Reader (Thermo Fisher Scientific 1510), Incubator (ESCO), Micropipette (1-10 µL, 50- 200 µL, 100 1000 µL) (Eppendorf), Tips (1-10 µL, 50- 200 µL, 100-1000 µL) (NEPTUNE), 96 well plate (TPP 92096),

Falcon tube 15 mL (SPL 500, pH meter (OHAUS Starter300 portable), Beaker glass (IWAKI CTE33), Spatula, Incubator, Multiskan Go Reader (Thermo Fisher Scientific 1510), Mikropipet (1-10 µL, 50- 200 µL, 100-1000 µL) (Eppendorf), 96well-plate (TPP 92096), Falcon tube 15 mL (SPL 50015), Falcon tube 50 mL(SPL 50050), Analytical Balance (AXIS), Tube Effendorf 1.5 mL (SPL 60015-1), Vortex (WiseMix VM-10), Asetat buffer (pH 3,6) , 2,4,6-Tripyridyl-s-Triazine (TPTZ) (Sigma-Aldrich, 3682-35-7), Ferrous (III) Chlorida (FeCl3) (Sigma-Aldrich, 12322-2.5L). , Dimethyl sulfoxide (DMSO) (Merck, 1029522500) , Sodium Acetate (Merck 1062681000), Hydrochloride Acid (Merck 1090631000), Daidzein (Chengdu Biopurify BP0445), Falcon tube 50 ml (SPL 50050), Tube Effendorf 1,5 ml (SPL 60015-1), Vortex (WiseMix VM-10), N-[3-(2-Furyl)acryloyl]-leu-gly-Pro-Ala (FALGPA) (Sigma F5135), Collagenase from Clostridium histolyticum (Sigma C8051), Tricine (Sigma SA10377), Calcium Chloride (Merck 1023821000), Sodium Chloride (Merck 106406), Destilled water, Dymethilsufoxide (Merck 1.02931.1000), Hydrochloric acid solution (Merck 109057).

2.2 Preparation of ethanol extract of black soybean

Air-dried leaves of Black soybean (500g) were extracted with 70% ethanol (12L) three times (2h each) using a soxhlet under reflux. The ethanol extract was concentrated under vacuum to give a crude extract (100g).

3. Antioxidants activity test

3.1 Ferric reducing antioxidant power (FRAP) Activity

A total of 7.5 µL samples of various concentrations of Black Soybean extract and Daidzein (1000, 500, 250, 125, 62.5, 31.25 µg / mL and FRAP solution of 142.5 µL were added to the sample well, and DMSO added to the well blank and control well Microplate closed, then incubated 37 ° C for 6 minutes Measurement of absorbance with a microplate reader at a wavelength of 595 nm Standardization with Ferro sulfate (0.03) grams of FeSO4 in 100 mL d2 H2O [15].

Formula of inhibition of FRPA :

% Reducing Activity = [(A absorbance of control – Absorbance of extract)/ absorbance of control x 100]

3.2 Anticolagenase activity

Inhibition of collagenase enzyme activity was measured based on the method described by Sigma Aldrich and Wittenauer and his colleagues (2015) with a slight modification (Utami and his colleagues 2018). A mixture of solutions consisting of 30 µL samples (0.78 - 50 µg / mL), 10 µL Collagenase enzyme from Clostridium histolyticum (0.1 mg / mL, Sigma C8051) and 60 µL buffer tricine (50 mM Tricine, 10 mM calcium chloride, 400 mM calcium chloride Sodium chloride, pH 7.5) incubated at 37 ° C for 20 minutes. Besides that, a control containing only 10 µL of enzyme and 90 µL of phosphate buffer prepared as well as a blank containing 10 µL of enzyme, 80 µL of phosphate buffer and 30 µL of a sample. Next, the solution mixture added as much as 20 µL of FALGPA substrate (1 mM, Sigma F5135) except blank. Absorbance was measured using a wavelength of 335 nm.

Formula of inhibition of Anticolagenase activity :

% Reducing Activity = [(A absorbance of control – Absorbance of sample)/ absorbance of sample x 100]

3.3 Statistical analysis

Initial sample weight data and black soybean sample extraction data obtained from the research results by using the SPSS program. FRAP activity test (FRAP method antioxidant test that will obtain the FeSO4 standard) and analysis of FRAP antioxidant activity data from black soybean extract and Daidzein with One Way test. Then the effect of various concentrations of black soybean and Daidzein extract on FRAP activity will be obtained, after which the Post Hoc Test of Tukey HSD conducted. Analysis of collagenase inhibitory activity data from black soybean extract and Daidzein was carried out by Linear Regression Analysis Test. The results of the effects of various concentrations of black soybean and Daidzein extract on collagenase inhibition to determine the value of Inhibition Concentration 50 (IC50).

4. Result and discussion

4.1 Analysis of antioxidant activities test by using FRAP method

The antioxidant activity in the ethanol extract of soybean and daidzein compounds was analyzed by the FRAP method. Data from the analysis of antioxidant activity were analyzed by the Post Hoc Test Turkey HSD test, as shown in the table 2 below.

Table 1: Results of analysis of post hoc test of tukey HSD test on antioxidant activity FRAP method on ethanol extract of black soybean

Sample	Final concentration	FRAP activity (µMFe (II)/µg)			Avergae	SD	RSD
		1	2	3			
Black soybean extract	50.00	146.65	145.80	146.60	146.35	0.48	0.3
	25.00	100.80	100.50	100.45	100.58	0.19	0.2
	12.50	95.60	91.20	87.20	91.33	4.20	4.6
	6.25	79.30	76.70	85.55	80.52	4.55	5.6
	3.13	66.30	63.80	64.30	64.80	1.32	2.0
	1.56	23.05	22.60	26.15	23.93	1.93	8.1

Table 2: Results of analysis of post hoc test of tukey HSD test on antioxidant activity FRAP method on ethanol extract of Daidzein

Sampel	Final concentration	Reduction activity (µM Fe(II)/µg)			Average	SD	RSD
		1	2	3			
Daidzein	50.00	99.65	98.55	92.60	96.93	3.79	3.9
	25.00	86.85	74.45	91.70	84.33	8.90	10.5
	12.50	72.45	72.65	64.85	69.98	4.45	6.4
	6.25	49.50	63.90	58.80	57.40	7.30	12.7
	3.13	41.60	35.50	49.45	42.18	6.99	16.6
	1.56	15.90	14.95	11.00	13.95	2.60	18.6

Table 3: Results of analysis of post hoc test of tukey HSD test on antioxidant activity FRAP method on ethanol extract of extract ethanol black soybean and daidzein

Final concentration (ug/ml)	Average of FRAP activity (µM Fe(II)/µg) by sample	
	Black soybean extract	*Daidzein*
50	146.35 ± 0.48^{f}	96.93 ± 3.79^{e}
25	100.58 ± 0.19^{e}	$84.33 \pm 8.90^{d,e}$
12.5	91.33 ± 4.20^{d}	$69.98 \pm 4.45^{c,d}$
6.25	80.52 ± 4.55^{c}	$57.40 \pm 7.30^{b,c}$
3.13	64.80 ± 1.32^{b}	42.18 ± 6.99^{b}
1.56	23.93 ± 1.93^{a}	13.95 ± 10.54^{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).

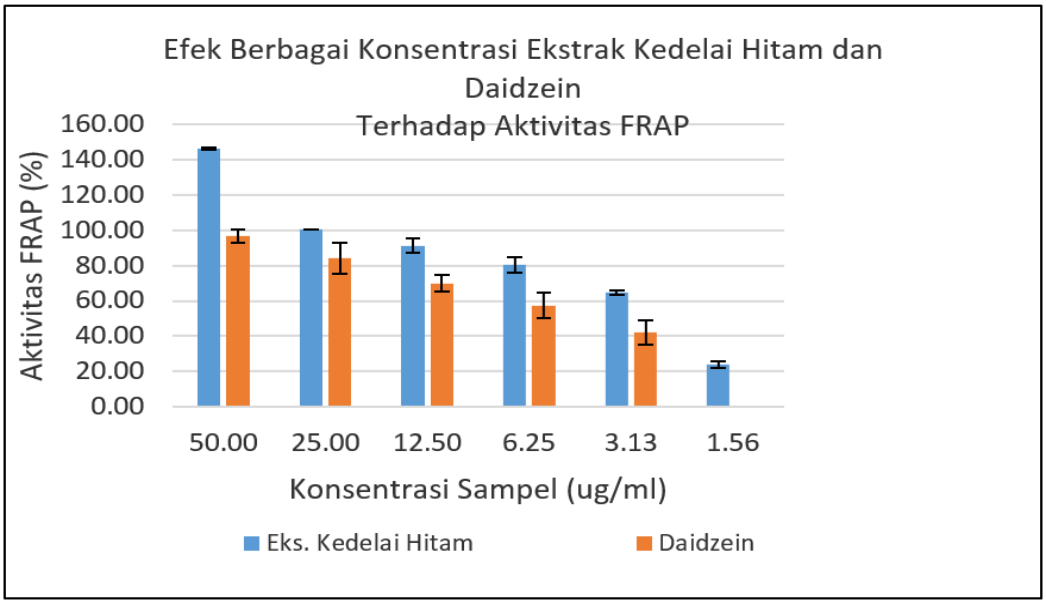

Figure 1: Results of analysis of post hoc test of tukey HSD test on antioxidant activity FRAP method on ethanol extract of extract ethanol black soybean and daidzein

4.2 Analysis of Anti-collagenase activity

The anti-collagenase activity in the ethanol extract of soybean and daidzein compounds was analyzed by the inhibition of collagenase method. Data from the analysis of anti-collagenase activity were analyzed by the Post Hoc Test Turkey HSD test, as shown in the table 4 below.

Table 4: Results of analysis of post hoc test of tukey HSD test on anti-collagenase activity on ethanol extract of black soybean

Sample	Final concentration	Inhibitory activity (%)			Average	SD	RSD
		1	2	3			
Black soybean extract	250.00	79.95	76.3	76.51	76.46	0.50	0.65
	125.00	58.34	59.78	60.50	59.54	1.10	1.85
	62.50	48.44	48.97	49.20	48.87	0.39	0.80
	31.25	42.33	43.62	42.86	42.94	0.65	1.51
	15.63	34.86	37.06	36.07	35.99	1.10	3.05
	7.81	31.33	33.41	33.68	33.40	0.28	0.85

Table 5: Results of analysis of post hoc test of tukey HSD test on anti-collagenase activity on Daidzein

Sample	Final concentration	Inhibitory activity (%)			Average	SD	RSD
		1	2	3			
Daidzein	250.00	89.83	76.3	76.51	88.16	0.50	0.65
	125.00	63.05	59.78	60.50	98.2	1.10	1.85
	62.50	49.88	48.97	49.20	98.2	0.39	0.80
	31.25	29.77	43.62	42.86	98.3	0.65	1.51
	15.63	25.64	37.06	36.07		1.10	3.05
	7.81	19.11	33.41	33.68	2.5	0.28	0.85

Table 6: Results of analysis of post hoc test of tukey HSD test on aanto-collagenase activity on ethanol extract of extract ethanol black soybean and daidzein

Final concentration (ug/ml)	Average of FRAP activity (µM Fe(II)/µg) by sample	
	Black soybean extract	*Daidzein*
66,67	76.46 ± 0.50^{f}	88.64 ± 1.04^{f}
33,33	59.54 ± 1.10^{e}	61.77 ± 1.13^{e}
16,67	48.87 ± 0.39^{d}	48.15 ± 1.56^{d}
8,33	42.94 ± 0.65^{c}	30.38 ± 1.43^{c}
4,17	35.99 ± 1.10^{b}	24.74 ± 0.78^{b}
2,08	33.40 ± 0.28^{a}	19.26 ± 0.20^{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 7: IC50 of anti-collagenase from black soybean extract and daidzein

Sample	Equatiom	R2	IC50 (µg/mL)	IC50 (µg/mL)
Black soybean (repeation 1)	Y = 0.1722x + 34.707	0,97	88.81	84.75 ± 3.54
Black soybean (repeation 2)	Y = 0.1718x + 35.865	0,97	82.28	
Black soybean (repeation 3)	Y = 0.1728x + 35.629	0,97	83.17	
Black soybean (Average)	Y = 0.1723x + 35.401	0,97	84.73	
Daidzein (repeation 1)	Y = 0.2839x + 22.921	0,96	98.18	98.21 ± 49,94
Daidzein (repeation 2)	Y = 0.2784x + 22.119	0,95	95.38	
Daidzein (repeation 3)	Y = 0.2753x + 22.72	0,97	100.15	
Daidzein (Average)	Y = 0.2792x + 22.587	0,96	99.09	

According of the result of this reaserch was in correlation with another reaserch. Isoflavones in black soybean have beneficial effects on patients with diabetes mellitus by increasing the production of serum insulin and protecting the organs of the pancreas and prevent damage from free radicals.[16]. High anthocyanins in black soybeans and 1,1-diphenyl-2-picrylhydrazyl and O2 make black soybeans when boiled to form Liver-butyl hydroperoxide (t-BuOO) substances that are useful for active prevention of the generation of Thiobarbituric acid-reactive substances (TBARS)), where TBARS is known to cause liver disorders [17]. Secondary metabolites and extracts of black soybeans have been widely investigated and found to have anti-collagenase, anti-elastase activity from Daidzein compounds, polyphenols such as flavonoids, tocopherols, phenolic acids, and tannins. These compounds provide the benefit of inhibiting Chc or a platform for synthesizing active molecules [18]. Daidzein (4, 7-dihydroxyisoflavone) naturally occurring isoflavone phytoestrogens belonging to non-steroidal estrogen and mainly derived from legumes such as soybeans and green beans. It is also a primary bioactive ingredient in traditional Chinese medicine, which is often used in the treatment of fever, acute dysentery, diarrhea, diabetes, heart dysfunction, liver injury and his colleagues [19]. The chemical structure of daidzein is similar to mammalian estrogens and provides a two-way function by replacing/interfering with estrogen and estrogen-receptor (ER) complexes. Therefore, daidzein provides a protective effect against several diseases related to estrogen regulation, such as breast cancer, osteoporosis, diabetes, cardiovascular disease [20]. The pharmacological effect of daidzein which is a phytoestrogen which can be a valid strategy in the prevention and treatment of various diseases, such as malignancy in the breast through ER modulation mechanism and anti-angiogenesis [21], treatment of tumor disease through the role of daidzein in the regulation of tumor cell invasion caused by TNF-α through the kappa B (NF-BB) and AP-1 signaling pathways, followed by a reduction in uPA secretion from malignant cells, thus inhibiting migration and invasion of cancer cells [22]; besides, daidzein displays Anti-proliferative effects on cancer through termination of the cell cycle in the G1 and G2 / M phases and the induction of apoptosis [23]. Daidzein is also very useful for the treatment of cardiovascular diseases such as coronary heart disease, atherosclerosis, hypertension and heart disease due to disorders of the

estrogen hormone in menopausal women, through the mechanism of regulating blood lipid metabolism, attenuation of endothelial dysfunction, reduction in blood pressure and increased antioxidant effectiveness [24]. Daidzein is also useful in the prevention and treatment of osteoporosis as an anti-diabetic, anti-aging activity [25], antioxidant function, anti-inflammatory, neuroprotective activity [26].

5. Conclusions

Black Soybean Extract (Glycine max (L.) Merr.) Has a higher antioxidant activity of FRAP compared to Daidzein compounds. Black Soybean Extract has antiaging activity through better collagenase inhibition compared to Daidzein compounds. The greatest FRAP activity at 50 µg / ml black soybean extract concentration was 146.35 µM Fe (II) / µg) and Daidzein 50 µg / ml concentration was 96.93 (µM Fe (II) / µg). Anticolagenase activity based on IC50 Black Soybean Extract values of 84.73 µg / ml and Daidzein at 98.18 µg / ml.

References

[1]. Anderson,J.W. 2002. Meta-analysis of the Effect of Soy Proteins Intake of Serum Lipid. J.Med. 333: 276-282.

[2]. Arct J, Pytkowska K. Flavonoids of biologically active cosmeceuticals. Clin Dermatol. 2008(26): 347–357.

[3]. Bao, C., Namgung, H., Lee, J., Park, H.C., Ko, J., Moon, H., Ko, H.W., Lee, H.J. (2014). Daidzein suppresses tumor necrosis factor-α induced migration and invasion by inhibiting hedgehog/Gli1 signaling in human breast cancer cells. J. Agric. Food. Chem. 30:3759-3767.

[4]. Benaiges,A.,P.Marcet.,R.Armengol.,C.Betes.,E.Girones. 1998. Study of the refirming effect of a plant complex. Int J Cosmet Sci. 1998(20): 223–233.

[5]. Charkoudian, N. (2003). Skin blood flow in adult human thermo-regulation: How it works, when it works, when it does not, and why. Mayo Clinic Proceedings, 78(5), 603-612

[6]. Choi, E.J., Kim, G.H. (2013). Antiproliferative activity of daidzein and genistein may be related to ERα/c-erbB-2 expression in human breast cancer cells. Mol. Med. Rep. 7: 781-784.

[7]. Chu, D. H. (2008). Overview of biology, development, and structure of skin. In K. Wolff, L. A. Goldsmith, S. I. Katz, B. A. Gilchrest, A. S. Paller, & D. J. Leffell (Eds.), Fitzpatrick's dermatology in general medicine (7th ed., pp. 57-73). New York: McGraw-Hill.

[8]. Cotsarelis, G., Sun, T. T., & Lavker, R. M. (1990). Label-retaining cells reside in the bulge of the pilosebaceous unit: Implications for follicular stem cells, hair cycle, and skin carcinogenesis. Cell, 61(7), 1329-1337.

[9]. Coward L, Barnes NC, Serchell KD, Barnes S. 1993. Genistein, Daidzein, and their betha-glycosidase conjugates: antitumor isoflavone in soybean foods from American and asian diets. J Agric Food Chem 41:1961–1967.

[10]. Dajanta, K., Janpum, P. & Leksing, W. 2013. Antioxidant Capacities, Total Phenolics and Flavonoids in Black and Yellow Soybeans Fermented by Bacillus subtilis: A Comparative Study of Thai Fermented Soybeans (thuanao). International Food Research Journal. Vol. 20 (6): 3125-3132.

[11]. Danby, F. W. (2005). Why we have sebaceous glands. Journal of the American Academy of

Dermatology, 52(6), 1071-1072.

[12]. Daniel, R. C., & Scher, R. K. (1997). Nail changes secondary to systemic drugs and ingestants. In R. K. Scher, & R. C. Daniel (Eds.), Nails: Therapy, diagnosis, surgery (2nd ed., pp. 251-258). Philadelphia: Saunders.

[13]. Danilenko, D. M., Ring, B. D., & Pierce, G. F. (1996). Growth factors and cytokines in hair follicle development and cycling: Recent insights from animal models and the potentials for clinical therapy. Molecular Medicine Today, 2(11), 460-467.

[14]. Dubey, R.K., Imthurn, B., Zacharia, L.C., Jackson, E.K. (2004). Hormone replacement therapy and cardiovascular disease what went wrong and where do we go from here? Hypertension. 44: 789-795.

[15]. Ernita E. 1995. Senyawa-senyawa Isoflavon dari Limbah Tahu [skripsi]. Bogor: Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor.

[16]. Fairley, J. A., Scott, G. A., Jensen, K. D., Goldsmith, L. A., & Diaz, L. A. (1991). Characterization of keratocalmin, a calmodulin-binding protein from human epidermis. Journal of Clinical Investigation, 88(1), 315-322.

[17]. Farnsworth, N. R. 1966. Biological and Phytochemical Screening of Plants. J Pharm Sci ; 55(3): 243–264.

[18]. Fawwaz,M.,D.S.Muliadi.,A.Muflihunna. 2018. Kedelai hitam (Glycine soja) Terhidrolisis Sebagai Sumber Flavonoid Total. Jurnal Fitofarmaka Indonesia-Research Gate. 4(1): 194-198.

[19]. Fisher,G.,S.Kang.,J.Varani.,Z.Bata-Csorgo.,Y.Wan.,S.Datta.,J.J.Voorhees. 2002. Mechanisms of Photoaging and Chronological Skin Aging. Arch Dermatol. 138: 1462-1470.

[20]. Fisher,A.E.O.,T.A.Hague.,C.L.Clarke.,D.P.Naughton. 2004. Catalytic superoxide scavenging by metal complexes of the calcium chelator EGTA and contrast agent EHPG. Biochem Biophys Res Commun. 2004(323): 163–167.

[21]. Flaxman, B. A., Sosis, A. C., & Van Scott, E. G. (1973). Changes in melanosome distribution in Caucasoid skin following topical application of nitrogen mustard. Journal of Investigative Dermatology, 60(5), 321-326.

[22]. Franke AA, Custer LJ, Cerna CM, Narala KK. 1994. Quantitation of phytoestrogens in legumes by HPLC. J Agric Food Chem 42:1905–1913.

[23]. Friedman M, Brandon DL. 2001. Nutritional and helth benefits of soy protein. J Agric Food Chem 49:1069–1086.

[24]. Gayraud, B., Hopfner, B., Jassim, A., Aumailley, M., & Bruckner-Tuderman, L. (1997). Characterization of a 50-kDa component of epithelial basement membranes using GDA-J/F3 monoclonal antibody. Journal of Biological Chemistry, 272(14), 9531-9538.

[25]. Genovesse MI, Davila J, Lajolo FM. 2006. Isoflavones in processed soybean product from Ecuador. Braz Arch Biol Tech 49(5):853–859Haake, A. R., & Hollbrook, K. (1999). The structure and development of skin. In I. Freedberg, A. Eisen, K. Wolff, K. Austen, L. Goldsmith, S. Katz, et al. (Eds.), Fitzpatrick's dermatology in general medicine (5th ed., pp. 70-111). New York: McGraw-Hill.

[26]. Jenkins, D. J., C. W Kendall, L. S. Augustin et al. 2002. Glycemic index: overview of implications in health and disease. Am. J. Clin. Nutr. 76:266S-273S.