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THE ANTIOXIDANT COMPOUNDS IN METHANOL EXTRACT OF Glycine max L. Merr DETAM I AND II VARIETIES by KINETIC MACERATION METHOD

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ABSTRACT

The study aims to identify the active compound of antioxidants in the methanol extract of black soybean (Glycine max L. Merr) Detam I and II varieties. The extract results from maceration kinetics was analyzed with GC-MS. Soybeans have been crushed then carried defatting using n-hexane solvent, the pulp obtained from the defatting then extracted using methanol 90%. Qualitative analysis was performed using TLC and GC-MS Shimadzu QP-2010 Model SE with a total time of analysis 20 and 80 minutes. The presence of compounds flavonols 3',4',5,7-OH, 3-O araglucoside and hexadecanoic acid, methyl ester; pentadecanoic acid, 14-methyl, -methyl ester; 9,12-Octadecadienoic acid (Z, Z) - methyl ester and methyl 10-trans, 12-cis-octadecadienoate. TLC results condensed methanol extract of black soybean (Glycine max L. Merr) Detam II varieties also contain flavonoids, but the GC-MS's profile showed no detectable flavonoids but there are several antioxidant compounds i.e Octanoic acid; Methyl 10-trans, 12-cis-octadecadienoate; Hexadecanoic acid; and 9,12-Octadecadienoic acid (Z, Z).

KEY WORDS:

Detam I and II varieties of Glycine max L. Merr, Antioxidants, TLC and GC-MS

INTRODUCTION

The most diseases are initiated by the presence of excessive oxidation reactions in the body. The oxidation reaction is needed by aerobic organisms due to energize the process metabolism and respiration, but on certain conditions of its existence may be implicated in various diseases and degenerative conditions, such as aging, arthritis, cancer, and others[1]. The body naturally has a defense system against free radicals, which endogenous intracellular antioxidant consisting of enzymes synthesized by the body such as superoxide dismutase (SOD), catalase and glutathione peroxidase. Antioxidants in the body must be a sufficient

number to eliminate and neutralize the effects of free radicals . If an increase in free radicals in the body is required exogenous antioxidants (derived from food consumed) in an amount more[2].

The antioxidant compounds are contained in many vegetables and fruits, especially nuts and grains[3]. Soybean is one of the food -producing natural antioxidants. Extract soybean (Glycine max) Anjasmoro varieties is proven effective in lowering levels of lead in the blood of mice with lead intoxication, equivalent to the effect of vitamin C supplementation[4]. The The Detam II varieties of Glycine max was proven to reduce levels of lead in the blood of mice, but did not reduce levels of malondialdehyde and significant organ damage[5]. The content of soybeans that have antioxidant properties, among others, α-linolenic acid, isoflavones, lecithin, lectins, linoleic acid, peptide, phytosterols, proteins, and saponins[6].

Among the compounds that are antioxidants, isoflavones become an object of research, and intensively evaluated potentially hipocholesterolemia, antioxidant, and estrogenic in blood vessels[7]. The isoflavones in soybean consists of genistein (4',5'7 - tryhydroxyisoflavone) and daidzein (4', 7-dihydroxyisoflavone), as well as the derivative β-glycoside, genistin and daidzin[2]. Isoflavones are phytoestrogens that are found in soy beans and some studies have shown that isoflavones are the best phytoestrogens compared with other phytoestrogens[8]. One of the two main isoflavones that have estrogen effect as an antioxidant and like molecule is genistein[6].

The each varieties of Soybean consists has a Specific Ingredients[9]. The Agency research beans and tubers of Indonesia (2010) states that black soybean Detam I varieties is an Indonesian native varieties has a protein content higher than other soybean varieties .

To determine the existence of genistein and other antioxidant compounds found in soybean Glycine max Detam I varieties, it is necessary to research using Gas Chromatography Mass Spectrometry qualitatively from soy beans are extracted by

maceration method kinetic in methanol 80 % . Kinetic maceration is a technique of extracting organic compounds are easy and simple, in addition to doing the stirring will enhance the solid contact with the solvent and accelerate the process by increasing the diffusion of the components to be extracted[10]. Thus, obtained methanol extract of soybean suspected to contain genistein and other antioxidant compounds .

MATERIALS AND METHODS

Materials Research : Detam I varieties of Glycine max soybean seed, n-hexane, 80 % methanol, n-hexane, silica gel GF254, chloroform ($CHCl_3$), ethyl acetate, 5 mg of Genistein (Sigma) ≥98 % synthetic powder G6649 and aquadem

Instrument Research: Maceration kinetic (Stirring the motor IKA Labortechnik RW 20 N), rotary evaporator (BUCHI Rotavapor R - 114), waterbath electric (BUCHI Waterbath B - 480), BUCHI Vacuum Controller V - 850, BUCHI Vacuum Pump V700, sieve mesh 20, analytical scales (Ohaus PA 214 , AND GR - 202), Water Bath Memmert, TLC (Camag), Camag UV lamp, GC - MS Shimadzu QP Model - 2010 SE, injection syringes, 0.2 µm of nylon membrane filter (Whatman)

The manufacture of Soybean Methanol Extracts

The soybean seed Detam I varieties was mashed in a blender and sieved to 20 mesh to form a fine powder. 500 grams of Soy bean powder were macerated kinetic with 1 L n-hexane and then were remacerated the same way as much as 2 times. The pulp was reextracted with 1 L 80% methanol for 1 hour , filtered and the filtrate was collected in a container, and then macerated with 80 % methanol again for 3 times. The extract of 80 % methanol was concentrated using a rotary evaporator and electric water bath until thick extract obtained.

Identification of Flavonoids Extract Soybean Methanol with TLC

The methanol extract of soy content of flavonoids was identified using TLC with silica gel GF254 with stationary phase and a mobile phase of chloroform : ethyl acetate (6 : 4). The results elution was observed at 365 nm UV light will give a blue fluorescence and steamed ammonia will stain yellow color quickly faded .

Analysis of Genistein and other antioxidant compounds in Glycine max Detam I Varieties with GC-MS

61.3 mg the methanol extract of soybean extract were dissolved in 10 ml methanol in measuring flask and then the filtering was done with a syringe injection and a 0.2 μm membrane filter (Whatman). The extract was analyzed the content of compounds qualitatively using GC - MS Shimadzu QP 2010 SE in the Research Laboratory of the University of Surabaya . The column was used was Restek semipolar (Crossbond® 5 % diphenyl / 95 % dimethyl polysiloxane) 60 m lenght x 0.25 mm ID x 0.25 μm dF , with helium carrier gas , injection volume of 1.00 mL and the ionization energy of the instrument 70 eV . Observed on two conditions , as follows :

The first condition :

Injector temperature : 230 ° C

Column temperature : 150 ° C for 2 minutes, then increased to 240 ° C for 10 minutes

Antarfase temperature : 250 ° C

Flow rate : 1.21 ml / min

Total analysis time : 20 minutes

The second condition :

Injector temperature : 230 ° C

Column temperature : 60 ° C for 10 minutes, then enhanced with a temperature rise of 4 ° C per minute to 220 ° C for 10 minutes , then increased with a rise of 1 ° C per minute until the final temperature of 240 ° C

Interfase temperature : 250 ° C

Flow rate : 0.8 ml / min

Total analysis time : 80 minutes

The chromatogram of the methanol extract of soy compared with standard chromatograms genistein (Sigma) ≥98 % synthetic powder G6649 , which is made by 0.3 mg genistein was dissolved in 3 ml methanol using a micropipette, and then filtered with a membrane filter and observed in both conditions

THE RESULTS AND DISCUSSION

The results of organoleptic of methanol extracts of Glycine max seed Detam I varieties, which include color, shape and smell can be seen in Table 1.

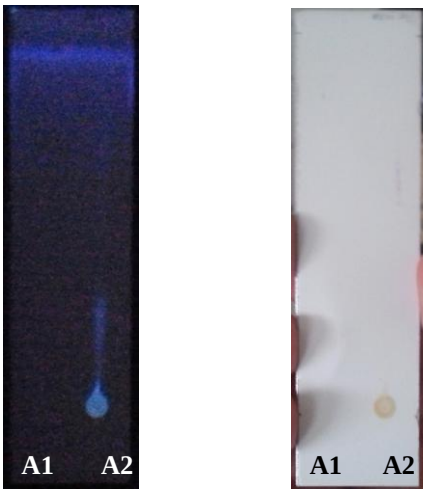
Table 1. The organoleptic test of Methanol Extracts of Glycine max Seed Detam I Varieties

No	Characteristic	Observation
1	Shape	Hard l aglomerate (very thick)
2	Color	blackish brown
3	Smell	rather stimulate

Identification of Flavonoids By TLC

Flavonoid identification results (Table 2) was observed using a 365 nm UV light produces dark blue fluorescence (Figure 1a) and steamed with ammonia produces yellow color quickly faded (Figure 1b). Stains yellow and blue fluorescence at a wavelength of 365 nm indicates that the extract of Glycine max varieties Detam I contains flavonoids qualitatively by thin

layer chromatography. While the standard does not appear Genistein blue fluorescence at 365 nm UV light and shows a yellow stain that quickly faded after steamed with ammonia.



(a) (b)

Fig 1. Identification of Flavonoids in Extract Seeds of Glycine max Detam I varieties with (a) UV ray 365 nm produces blue fluorescence and (b) Reagent ammonia vapor
A1 : spot of Genistein (Sigma)
A2 : spot of methanol extract of Glycine max Detam I varieties

Analysis of Genistein and Other Antioxidant Compounds with GC-MS Method at the time of analysis of a total of 20 minutes there are 4 antioxidant compounds were detected, such as hexadecanoic acid, methyl ester and pentadecanoic acid, 14-methyl, -methyl ester at a retention time of 14.070 minutes. As well as 9,12-Octadecadienoic acid (Z, Z) - methyl ester and Methyl 10-trans, 12-cis-octadecadienoate at a retention time of 17.035 minutes. In observation with the first condition is detected Flavonols compound 3',4',5,7-OH, 3-O Araglucoside indicating that the extract contains flavonoids. In the total analysis time of 80 minutes there are 3 of the same antioxidant compounds were detected in a total of 20 minutes of analysis, namely hexadecanoic acid, methyl ester and pentadecanoic acid, 14-methyl, -methyl ester at a retention time of 56.010 minutes. and 9, 12-Octadecadienoic acid (Z, Z) -, methyl ester at a retention time of 63.705 minutes. From these two conditions are not detected presence of genistein in soy methanol extract. This is due to limited research on

semipolar column GC-MS used have a maximum temperature limit of 250°C, while in the genistein previous studies can be observed at 300°C.

Table 2. Compound Composer Standard of Genistein on 20 Minutes

Retention time (min)	MR	Compounds corresponding database from GC-MS Shimadzu QP 2010 SE	Formula
3,720	162	Ethanesulfonyl chloride*	$C_2H_4Cl_2O_2S$
	103	Butanenitrile	C_4H_6ClN
	108	Carbonochloridic acid	$C_3H_5ClO_2$
	186	Ethane, 1,2-bis (2-Chloroethoxy)	$C_6H_{12}Cl_2O_2$
	152	1-Chloro ethyl carbonate	$C_5H_9ClO_3$
14,365	562	Tetracontane	$C_{40}H_{82}$
	618	Tetracontane	$C_{44}H_{90}$
	366	Hexacosane	$C_{26}H_{54}$
	408	Celidoniol, deoxy	$C_{29}H_{60}$
	296	Heneicosane*	$C_{21}H_{44}$

Compounds detected using database WILEY8.LIB

* : The compound was detected database NIST08s.LIB

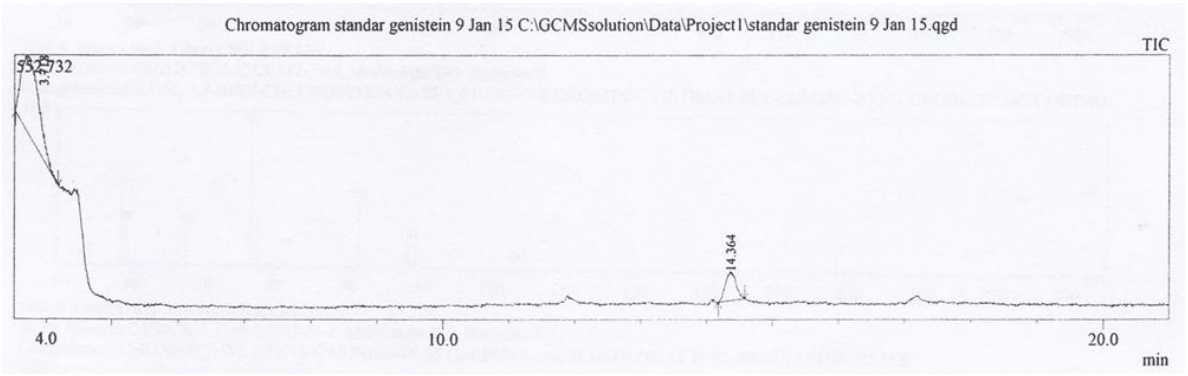


Figure 2. The spectra of Gas Chromatography of Genistein on 20 Minutes

Table 3. The constituent compounds Methanol Extracts of Glycine max Detam I varieties on 20 Minutes

Retention time (min)	MR	Compounds corresponding database from GC-MS Shimadzu QP 2010 SE	Formula
10,015	194	Alpha-D-Glucopyranoside, methyl*	$C_7H_{14}O_6$
	194	Alpha-D-Galactopyranoside, methyl	$C_7H_{14}O_6$
	194	Alpha-methyl-D-Manopyranoside*	$C_7H_{14}O_6$
10,115	194	Alpha-D-Galactopyranoside, methyl	$C_7H_{14}O_6$
	178	Alpha-L-Galactopyranoside, methyl 6-deoxy	$C_7H_{14}O_5$
	194	Alpha-D-Glucopyranoside, methyl	$C_7H_{14}O_6$

10,180	496	Ergosta-2,24-dien-26-oic-acid, 27-(acetyloxy)-5,6-epoxy-22-hydroxy-1-oxo-,delta-lactone	$C_{30}H_{40}O_6$
	155	4-Aminocyclohexanone, N-acetyl*	$C_8H_{13}NO_2$
	225	N-Cyclododecylacetamide*	$C_{14}H_{27}NO$
10,230	129	Butyraldehyde, semicarbazone	$C_5H_{11}N_3O$
	172	2-Isopropyl-4,6-dimethyl-1,3,2-Oxathiaborinane*	$C_8H_{17}BOS$
	598	Flavonol 3',4',5,7-OH, 3-O Araglucoside	$C_{26}H_{30}O_{16}$
10,470	194	Alpha-D-Galactopyranoside, methyl	$C_7H_{14}O_6$
	194	Alpha-Methyl-D-Mannopyranoside*	$C_7H_{14}O_6$
	194	Alpha-D-Glucopyranoside, methyl*	$C_7H_{14}O_6$
11,670	159	Indole-3-Acetaldehyde*	$C_{10}H_9NO$
	159	2-Hydroxylepidine*	$C_{10}H_9NO$
	370	N-Dinitrophenyl-1-Tryptophane*	$C_{17}H_{14}N_4O_6$
11,840	194	Mome Inositol	$C_7H_{14}O_6$
	194	3-O-Methyl-d-Glucose*	$C_7H_{14}O_6$
	194	4-O-Methylmannose*	$C_7H_{14}O_6$
14,070	270	**Hexadecanoic acid, methyl ester**	$C_{17}H_{34}O_2$
	270	**Pentadecanoic acid, 14-methyl, -methyl ester**	$C_{17}H_{34}O_2$
	354	9-Octadecenoic acid, 12-(Acetyloxy)-methyl ester [R-(Z)]	$C_{21}H_{38}O_4$
17,035	294	**9,12-Octadecadienoic acid (Z, Z)- methyl ester**	$C_{19}H_{34}O_2$
	294	**Methyl 10-trans, 12-cis-octadecadienoate***	$C_{19}H_{34}O_2$
	294	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$

Compounds detected using database WILEY8.LIB
* : The compound was detected database NIST08s.LIB
Compounds that blocked the thick : compounds that have antioxidant effects

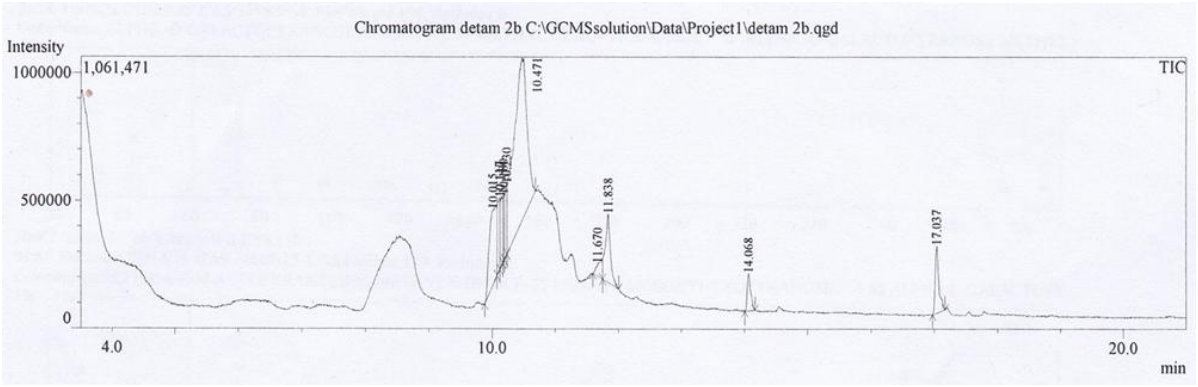


Fig 3. Gas chromatography spectra of Extract Glycine max varieties Detam I on 20 Minutes

Table 4. Summary of Compound Composer of Genistein on 80 Minutes

Retention time (min)	**MR**	**Compounds corresponding database from GC-MS Shimadzu QP 2010 SE**	**Formula**
5,520	104	Trimethyl borate	$C_3H_9BO_3$

Compounds detected using database WILEY8.LIB

* : The compound was detected database NIST08s.LIB

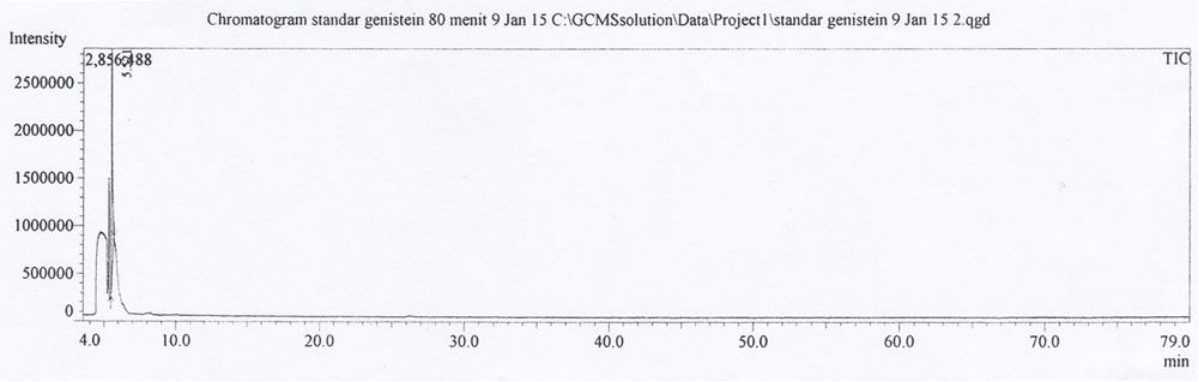


Fig 4. Gas chromatography spectra of Standard Genistein on 80 Minutes

Table 5. Compound Composerof Methanol Extracts Glycine max Detam I varieties on 20 Minutes

Retention time (min)	MR	Compounds corresponding database from GC-MS Shimadzu QP 2010 SE	Formula
4,320	44	Nitrogen oxide	N_2O
	44	Carbon dioxide	CO_2
4,610	82	1-Chlorofluoroethane	C_2H_4ClF
4,670	64	1,1-Difluoroethylene-2,2-D2	$C_2D_4F_2$
	92	2-Mercaptoethanoic acid	$C_2H_4O_2S$
5,260	64	1,1-Difluoroethylene-2,2-D2	$C_2D_4F_2$
	82	1-Chlorofluoroethane	C_2H_4ClF
5,465	104	Boric acid	$C_3H_9BO_3$
6,170	60	Acetic acid	$C_2H_4O_2$
8,525	102	Acetyl oxide, anhydride	$C_4H_6O_3$
	72	Acethylformaldehyde	$C_3H_4O_2$
46,925	194	Alpha-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
	194	Beta-D-Glucopyranoside, methyl	$C_7H_{14}O_6$
56,010	270	**Hexadecanoic acid, methyl ester**	$C_{17}H_{34}O_2$
	270	**Pentadecanoic acid, 14-methyl, -methyl ester**	$C_{17}H_{34}O_2$
63,705	294	**9, 12-Octadecadienoic acid (Z, Z)-, methyl ester**	$C_{19}H_{34}O_2$
	294	8,11-Octadecadienoic acid, methy ester	$C_{19}H_{34}O_2$

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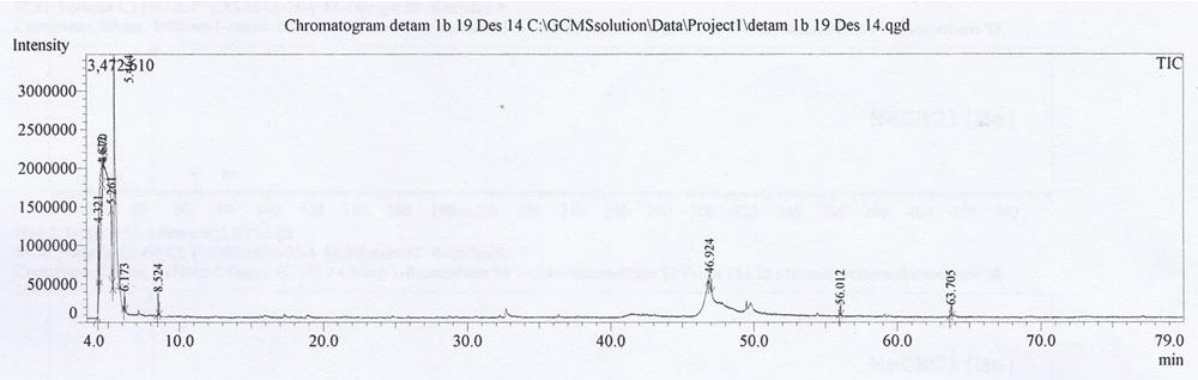

Figure 5. The spectra GC of Extract Glycine max Detam I varieties on 80 Minutes

This research is reinforced by several studies that get isoflavone content in soyabean [11,12], and soyabean may enhance the body immunity through the formation of immunoglobulin in diabetic rat with infected K.pneumoniae [13]

CONCLUSIONS

The extract of Glycine max Detam I varieties contain flavonoids with the observation of TLC and GC. The presence of compounds Flavonols 3 ' , 4' , 5,7 - OH , 3 - O Araglucoside and 4 of antioxidant compounds , such as hexadecanoic acid , methyl ester ; pentadecanoic acid , 14 - methyl , -methyl ester ; 9,12 - Octadecadienoic acid (Z , Z) - methyl ester and methyl 10 - trans , 12 - cis – octadecadienoate, but not detected any genistein in soy extracts .

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