

Chromatogram Profile of *Vigna radiata* and *Phaseolus vulgaris* Related to Chemical Hydrolysis

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ABSTRACT: Mungbean (*Vigna radiata*) and red bean (*Phaseolus vulgaris*) is a type of food containing isoflavone in the form of glycoside (genistin, daidzin, glycitin). Isoflavones are one type of phytoestrogen which have similar chemical structure with estradiol. Therefore, it can be used not only to inhibit but also to prevent many symptoms related to estrogen deficiency. Human body cannot adsorb isoflavone in the glycoside form. In order to make it absorbable, isoflavone should be hydrolyzed to defuse its glycoside chain to be aglycon (genistein, daidzein, glycitein). The aim of this study was to determine the influence of hydrolysis on chromatogram profile of mungbean and red bean. Hydrolysis was conducted by chemical method using hydrochloride acid (HCl). The chromatogram profile was determined by High Performance Liquid Chromatography method (HPLC) using C₁₈ reverse phase column, the mobile phase was methanol : water (7:3), the sample injected automatically as much as 10 ml, the flow rate of 1 ml/min with a temperature of 28°C, at a wavelength of 254 nm. This study showed that chemical hydrolysis successes to remove the glycoside chain in both mungbean and red bean extract.

KEYWORDS: *Vigna radiata*; *Phaseolus vulgaris*; isoflavone, glycoside; High Performance Liquid Chromatography.

1. INTRODUCTION

Generally, the legumes or *leguminosae* contain isoflavone compound (Zubik and Meydani, 2003). The basic chemical structure of isoflavones is almost the same as flavonoids, which consists of two benzene rings (A and B) and attached to the heterocyclic ring C pyran, but the orientation of the B ring is different. In flavones, the B ring bound by carbon number 2 rings middle C, while isoflavones bound by carbon number 3 (Schmidl and Labuza, 2000). Isoflavones are compounds that have a molecular similarity to estrogen (Uesugi *et al.*, 2002), which has been used to treat symptoms of clinical in women postmenopausal.

Isoflavones contained in nuts or dairy products consists in two chemical forms namely the aglycon and glycon. To obtain the aglycone form can be done by enzymatic process using probiotic microorganisms, like *lactobacillus* and *bifidobacterium* has β -glucosidase enzyme that plays an important role in endogenous hydrolyze isoflavone during fermentation (Donkor and Shah, 2007). Hydrolysis through a chemical reaction can also be done by strong acid such as hydrochloric acid or acetic acid (Zhang *et al.*, 2007).

Hydrolysis by chemical reaction can remove glycoside chain in soybean although the level of aglycone extract lower than enzymatic process (Fawwaz and Baits, 2016). This study was conducted to determine the influence of hydrolysis on chromatogram profile of mungbean and red bean by High Performance Liquid Chromatography (HPLC) method.

2. EXPERIMENTAL SECTION

2.1. Chemicals and standard solution

Standard genistein G6649 that contains 5 mg was purchased from Sigma Aldrich Chemie GmbH, with purity $\geq 98\%$ over the analysis using HPLC. Pro analysis grade of acetone, hydrochloric acid (HCl), and methanol was purchased from Merck (Darmstadt, Germany). Distilled water was obtained through a Millipore-Q50 Ultrapure water system (Sartorius). Mungbean and red bean was purchased from commercial market in Makassar City. The stock solution ($c = 200 \mu\text{g/mL}$) was prepared by dissolving 1 mg of genistein standard with 5 mL of methanol: water (8: 2). Pipette 1 ml of the stock solution and add 5 ml of methanol: water (8: 2) to obtain a concentration of 40 ppm.

2.2. Sample preparation

The samples (250 g) was added 500 ml of 70% ethanol in a ratio of 1:2 (g/ml), the mixture obtained is then heated at a temperature of 90°C, stirring constantly for 2 hours. Solute separated from the mixture using a Whatman filter paper. The half of filtrate is then evaporated with a rotary evaporator to obtain a thick extract, and the other filtrate was added 37% hydrochloric acid until the mixture reaches a pH of 3. The mixture is then heated at 90°C, stirring constantly, for 2 hours. This mixture is then added to distilled water in a ratio of 1:1 (ml/ml) and stirred continuously at room temperature. The precipitate formed is separated using a vacuum filter, the results are stored at 4°C (Zhang *et al.*, 2007).

2.3. HPLC analysis

Analysis of the genistein standard and samples (300 $\mu\text{g/ml}$) were conducted using a HPLC system equipped with C₁₈ reverse phased column, then automatically injected into the tool as much as 10 ml, and ultraviolet detector wavelength

of 254 nm was applied. The mobile phase used was methanol: water (7:3), a flow rate of 1 ml/min, and analysis was performed at 28°C.

2.4. Data analysis

The qualitative parameter is used retention time by comparing the retention time of chromatogram of the sample solution with reference standard solution of genistein in the same HPLC conditions (Fawwaz and Baits, 2016). A calibration standard curve for genistein was obtained by running on HPLC and then plotting area against concentrations. The best fit of the line curve was calculated by equation of line. Linearity was evaluated through the correlation coefficient (R^2). The correlation coefficient, intercept and slope of calibration curve were calculated. The best fit of data was determined by linear regression using the following equation: $Y = bx + a$, where:

- Y = Absorbance
- b = Slope
- x = Concentration
- a = Intercept

3. RESULTS AND DISCUSSION

The mungbean (*Vigna radiata* (L.)), a leguminous seed, is a commodity nuts are very well known to the public. It has been used as a food source, and it is commonly sold in food markets, particularly in Asian Countries. The main nutritional components of mungbean include carbohydrate and high-quality protein; and they are highly nutritious. Lee et al. (2000) explained that extracts of mungbean can inhibit aldehyde oxidation that has an important role as primary factor in aging and degenerative diseases, like heart disease, cataracts, cognitive dysfunction, and cancer (Blake and Winyard, 1995; Halliwell and Gutteridge, 1998; Pietta, 2000). The red beans (*Phaseolus vulgaris* L.). According to the Central Bureau of Statistics (2011), the production of red beans in Indonesia is quite high, reaching 116,397 tons in 2010 (Pangastuti, 2013).

Table 1. Weight of mungbean (*Vigna radiata*) extract

	Sample (g)	Volume (ml)	Extract (g)	Extract (%)
After hydrolyzing	250	500	0.56	0.22
Before hydrolyzing	250	500	15.06	6.02

Table 2. Weight red bean (*Phaseolus vulgaris*) extract

	Sample (g)	Volume (ml)	Extract (g)	Extract (%)
After hydrolyzing	200	500	0.02	0.51
Before hydrolyzing	200	500	15.32	7.66

The main secondary metabolites present in some *leguminosae* including mungbean and red bean are isoflavones. This compound has many benefits, particularly as hormone replacement therapy of estrogen. In mungbean and red bean the form of isoflavone is glycosides and aglycone (Marcelia, 2015). Genistein is an isoflavone aglycon that is contained in many nuts or other dairy products. Fermentation will release the sugar molecule from isoflavones, so that it will produce isoflavone aglycon (genistein) (Rowland, 2003). The chemical process of hydrolysis process using strong acid can also break the sugar chain in isoflavones glycosides (Fawwaz and Baits, 2016).

In this study obtained percent of mungbean extract before and after hydrolyzing is different, where the percentage of extract before hydrolyzing near to 15 times higher than after hydrolyzing. The condition same as red bean extract, although the different only seven times higher. The clear data can be seen in the Table 1 and 2. Both extracts did not contain genistein, either before or after hydrolysis. From the results of qualitative and quantitative analysis on both extracts using standard genistein as a comparison show that there is no peak in the same retention time with the standard. Nevertheless, there is a peak shift that indicated a change in the compound from the hydrolysis process. The newly formed compound is an isoflavone aglycon compound other than genistein, like daidzein or glycitein. The data is provided in the Figure 1-5.

<Chromatogram>

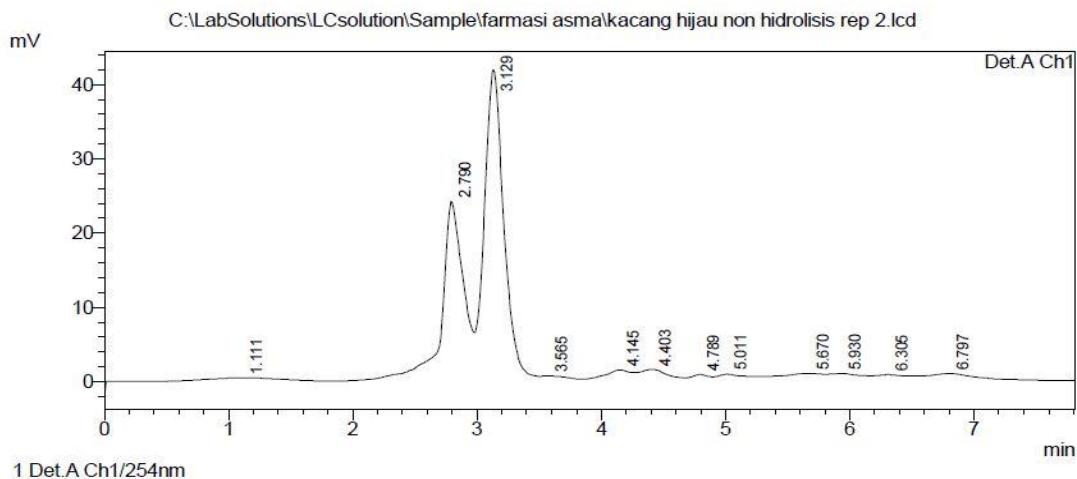


Figure 1. Chromatograms before hydrolyzed of mungbean (*Vigna radiata*) extract

<Chromatogram>

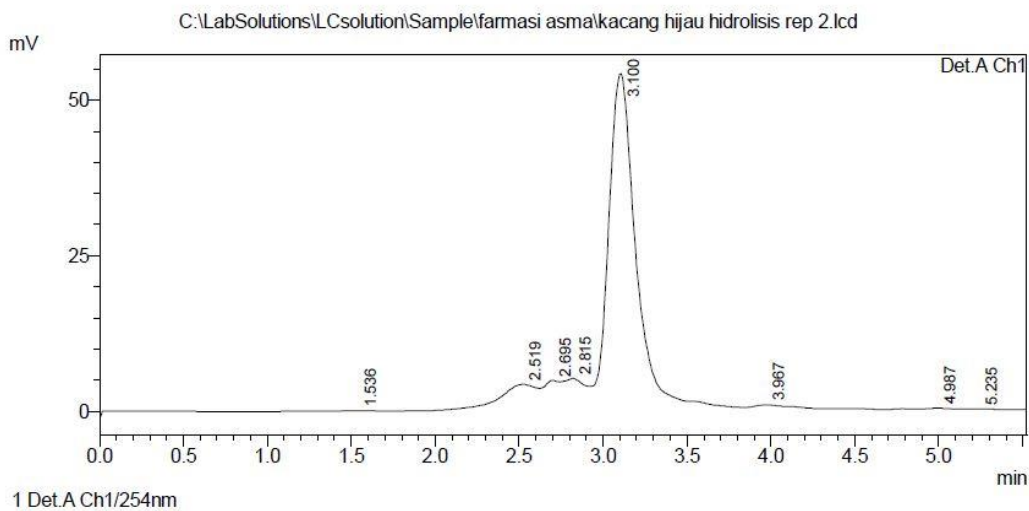
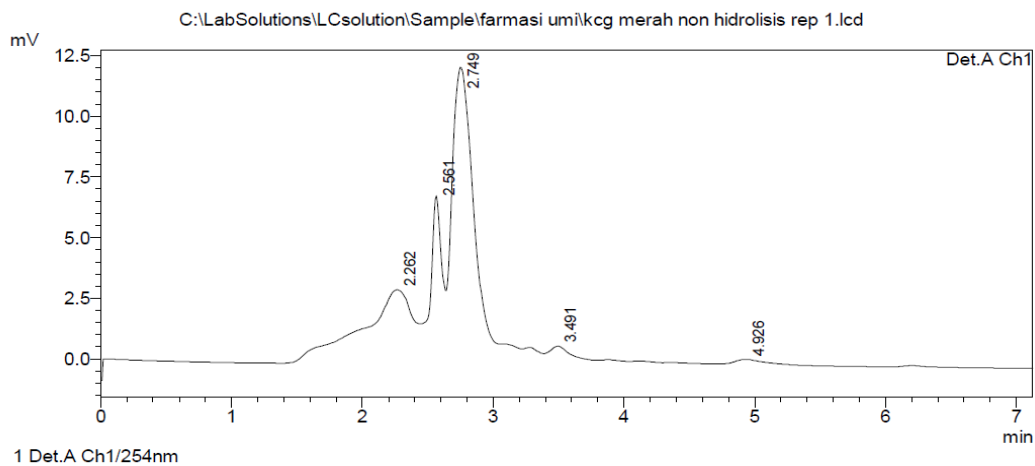


Figure 2. Chromatograms after hydrolyzed of mungbean (*Vigna radiata*) extract

<Chromatogram>



<Results>

Figure 3. Chromatograms before hydrolyzed of red bean (*Phaseolus vulgaris*) extract

<Chromatogram>

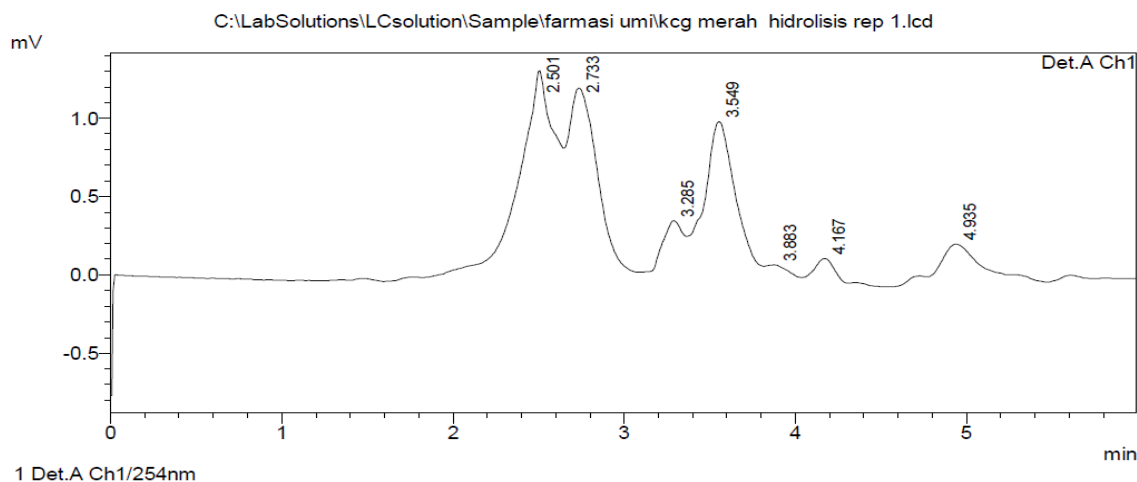


Figure 4. Chromatograms after hydrolyzed of red bean (*Phaseolus vulgaris*) extract

<Chromatogram>

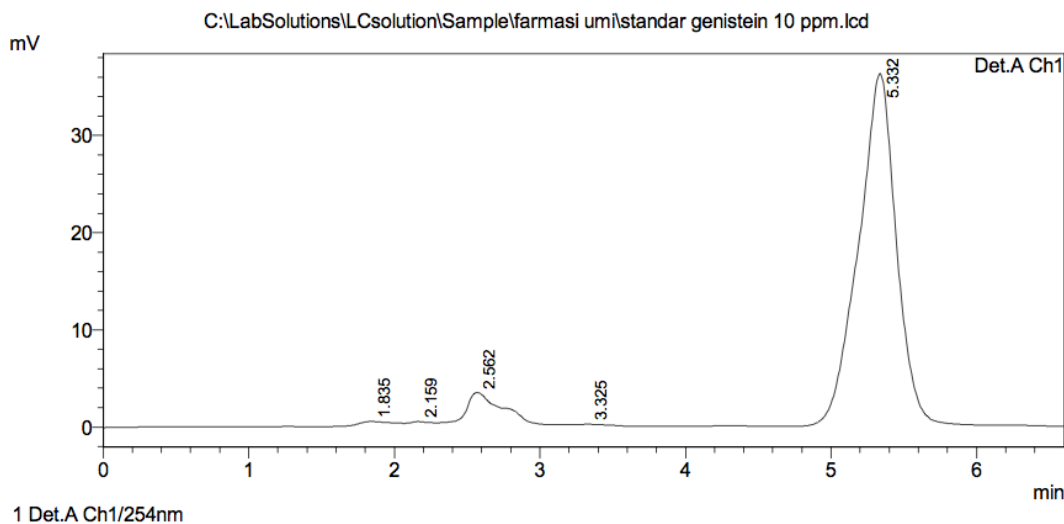


Figure 5. Chromatogram of genistein standard

This proves that the hydrolysis process carried out by chemical method is successful, although it cannot be determined what type of isoflavone aglycon is contained. There are several types of isoflavone aglycon in bean, isoflavones consist of 4 forms, namely aglycon, glycosides, malonyl glycosides, and acetyl glycosides, each of which has 3 types of isomers. The aglycone forms consist of genistein, daidzein, and glycitein. The glycoside form consists of genistin, daidzin, and glycitin. Malonyl glycosides consist of 6''-O-malonylgenistin, 6''-O-malonyldaidzine, and 6''-O-malonylglycitine. Acetyl glycosides consist of 6''-O-acetylgenine, 6''-O-acetyldaidzine and 6''-O-acetylglycitein (Anderson, 2002). The most common types of isoflavones found in soy protein and soy products are genistein and daidzein (Friedman *et al.*, 2001).

4. CONCLUSION

In this study we can concluded that there were chromatograms shift in both samples for both conditions, hydrolyzed and without hydrolyzed. This showed that chemical hydrolysis process occurred, however, the result of glycoside hydrolysis was not genistein. So, it is believed that there are other isoflavones aglycone than genistein like daidzein or glycitein.

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