Determination of quercetin in *Hibiscus sabdariffa* L. calyces by High-Performance Liquid Chromatography (HPLC)

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Abstract

Quercetin is a flavonoid and common present in vegetables and fruits, usually found in rutin glucosides. Its antioxidant effect is implied to be helpful for human health. Quercetin content in *Hibiscus sabdariffa* L. calyces was determined and validated by high-performance liquid chromatographic (HPLC) method. *Hibiscus sabdariffa* L. calyces extract was made by using mixture of hydrochloride acid 2.8 M solution and methanol (60:40) solvent that heated at 60, 70, and 80°C for 30 minutes. The chromatographic separation was performed on Shim-pack CLC-ODS (M) C18 column, detection at 372 nm and 1 mL min⁻¹ of flow rate. Optimum mobile phase for separation quercetin from other constituent in *Hibiscus sabdariffa* L. Calyx was a mixture of acetonitril and water (97:3). The result showed that limit of detection and limit of quantitation of quercetin were 22.8 µg/mL and 76.16 µg/mL, respectively. Recovery and coefficient of variance were 102.96% and 2.80%, respectively. The extract was heated at 80°C gave the highest content of quercetin that is 0.89±0.03%.

Keywords: Quercetin, *Hibiscus sabdariffa* L., high-performance liquid chromatography

Introduction

Phytochemicals are biologically active, non-nutrient plant chemicals found in many commonly consumed fruits and vegetables (Schreiner & Huyskens-Keil, 2006). Flavonoids are the most commonly found phytochemicals, and typically these chemicals help protect the plant against UV light, fungal parasites, herbivores, pathogens and oxidative cell injury (Cook & Samman, 1996). When consumed regularly by humans, flavonoids have been linked to a reduction in the incidence of diseases such as cancer and heart disease (Beecher, 2003; Cook & Samman, 1996; Lako, Wattanapenpaiboon, Wahlqvist, & Treenery, 2006; Liu, 2004). There is currently great interest in flavonoid research due to the possibility of improved public health through diet, where preventative health care can be promoted through the consumption of fruit and vegetables. Flavonols are a class of flavonoid commonly found in many fruits and vegetables, and content varies widely, depending on variety and environmental factors, such as growing conditions, growing climate, storage and cooking conditions (Lachman et al., 2003; Yang, Meyers, van der Heide, & Liu, 2004). Studies have shown that quercetin, exhibits anti-cancer, anti-inflammatory, antiviral activity, and may also prevent cardiovascular disease in humans (Gil, Ferreres, & Tomas-Barberan, 1999).

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one; 3',3',5,7-pentahydroxy flavone) as shown in Figure 1. is a polyphenolic product of the phenylpropanoid biosynthetic pathway in plants and therefore an integral part of the mammalian diet both as an aglycone and as b-glycosidic conjugates.

Quercetin occurs naturally in plants as conjugated glycosides, with the most common glycosides being quercetin-3,4-0-diglucoside, quercetin-4-0-monoglucoside and quercetin 3-monoglucoside. Quercetin has been detected in many fruits and vegetables in varied concentrations. Thus, problem to quantization of quercetin many plants is hydrolises of many glycosides to become quercetin. The acid catalyzed hydrolys of many simple glycosides have been studied rather carefully (Nuutila, Kammiovirta, & Oksman-Caldentey, 2002; Morrice, Wood, & Duthie, 2000). There is no precise information about the optimum temperature of hydrolys of the more complex flavonol glycosides.

*Hibiscus sabdariffa*, commonly named as “roselle” are rich flavonoid sources, especially flavonol. The leaves and flowers are used as a tonic tea for digestive and kidney functions. The calyces and seeds are diuretic, laxative and tonic (Qi, Chin, Malekian, Berhane, & Gager, 2005).

The aim of this study was to determine of quercetin in *Hibiscus sabdariffa* L. calyces by high-performance liquid chromatography (HPLC) with varied temperature of acid catalyzed hydrolys.
 Materials and Methods

Material

Hibiscus sabdariffa L. calyces from Manoko, Lembang, Indonesia, quercetin standard (Sigma), HPLC grade methanol and acetonitril, double distilled water, and 2.8 M hydrochloride acid solution. Standard stock solution of 1000 µg/mL quercetin were prepared in methanol.

Apparatus and software

The Shimadzu LC-10AT HPLC system with SPD-10A UV detector connected to computer loaded Shimadzu Class-VP software. The chromatographic separation was performed on Shim-pack CLC-ODS (M) C18 column, detection at 372 nm and 1 mL min⁻¹ of flow rate.

Method

Preparation sample

Two gram of Hibiscus sabdariffa calyces were hydolysed in 100 mL mixture of 2.8 M hydrochloride acid solution and methanol (60:40) solvents that heated at 60, 70, and 80°C for 30 minutes. The extract was allowed to cool and was made up to 100 ml and sonicated. The extract was filtered through a 0.45-mm filter for organic solvents, prior to injection.

Determination of linearity, LOD, and LOQ

Standard series of quercetin in the linear concentration range of 5.0.0-400.0 µg/mL were obtained from the above standard stock solutions. Optimum mobile phase for separation quercetin from other constituent in Hibiscus sabdariffa L. Calyx was a mixture of acetonitril and water (97:3). The linearity of quercetin was carried out by make calibration curve of standard series solution. The peaks area in the linear concentration range of 5.0-400.0 µg/mL were performed. Calibration equation of quercetin was y = 74522.69x – 76162.04 and coefficients correlation (r) = 0.9989. LOD and LOQ were 22.85 µg/mL and 76.16 µg/mL, respectively.

The determination of accuracy and precision analytical method was carried out by standard addition method with three different concentrations of standard solution quercetin that was added. Parameter for accuracy is percentage recovery. Percentage recovery of quercetin that heated at 80°C were 102.96%. Coefficient of variation (CV) is parameter for precision of analytical method. Coefficient of variation quercetin that heated at 80°C were 2.80 %. The result showed that method gave good accuracy and precision. All results of determination accuracy and precision were presented in Table 1.

Results and Discussion

Determination of wavelength and mobile phase for analysis quersetin has been carried out in former research. Quersetin maximum wavelength was obtained at 372 nm in methanol solvent. Whereas optimum mobile phase was mixture of acetonitrile:water (97:3). This composition can separate quersetin from other components perfectly. Retention time (tr) of quercetin was 3.27 menit.

Chromatograms of quercetin are presented in Figure 2.

The extract was made by hydrolises of fresh Hibiscus sabdariffa calyces in mixture of 2.8 M hydrochloride acid solution and methanol (60:40) solvents that heated at 60, 70, and 80°C for 30 minutes. The aim of this procedure was to hydrolyses glicosides to become quercetin, perfectly.

Linierity has shown significant correlation between peaks area chromatogram and concentrations. Linearity are expressed with correlation coefficient (r) where acceptable value is r = 0.98 for bulk. The linearity of quercetin was carried out by make calibration curve of standard series solution. The peaks area of quercetin was 74522.69x – 76162.04 and coefficients correlation (r) = 0.9989. LOD and LOQ were 22.85 µg/mL and 76.16 µg/mL, respectively.

The result showed that method gave good accuracy and precision. All results of determination accuracy and precision were presented in Table 1.

Hibiscus sabdariffa L. calyces that heated at 80°C have highest level of quersetin. At 80°C is optimum temperature for hydrolysis glicosides flavonoid becomes quercetin at Hibiscus sabdariffa L. calyces concentrations, that are 80 µg/mL, 100 µg/mL and 120 µg/mL from standard stock solution and added with methanol. Each solution were filtered through a 0.45-mm filter and 20 µL filtrat were injected into HPLC. Procedure is was replicated 2 times.
Conclusions

The chromatographic separation was performed on Shim-pack CLC-ODS (M) C18 column, detection at 372 nm and 1 mL min$^{-1}$ of flow rate. Optimal mobile phase for separation quercetin from other constituent in *Hibiscus sabdariffa* L. Calyx was a mixture of acetonitril and water (97:3). The result showed that limit of detection and limit of quantitation of quercetin were 22.8 µg/mL and 76.16 µg/mL, respectively. Recovery and coefficient of variance were 102.96% and 2.80%, respectively. The extract was heated at 80°C gave the highest content of quercetin that is 0.89±0.03%.

References


