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Quantitative Test Of Quercetin Content Of Rome Beauty Apple Flour Extract Using The High-Performance Liquid Chromatography Method

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Abstract

Introduction: Apple is a fruit containing antioxidant phytochemicals, one of which is quercetin, a flavonoid compound. Apples are one of the natural ingredients that have a beneficial effect on health. Rome beauty apples (*Malus sylvestris Mill.*) are natural antioxidants that are safer for consumption compared to synthetic antioxidants, which have the potential to cause harm. Oxidative stress occurs due to an imbalance in the amount of antioxidants in the body, causing cell damage. High-Performance Liquid Chromatography (HPLC) was conducted to determine the antioxidant content, especially quercetin, in Rome Beauty apple extract.
Objective: to find out how to quantitatively test the quercetin flavonoids from Rome Beauty apple extract (Malus sylvestris Mill.) using HPLC to determine the levels of quercetin flavonoids in Rome Beauty apple extract (*Malus sylvestris Mill.*).
Method: The method of sample preparation was carried out using a maceration extraction technique with 70% ethanol solvent. Determination of the quercetin content in Rome Beauty Apple Extract was carried out using the High-Performance Liquid Chromatography (HPLC) method and a UV spectrophotometer detector.
Results: The results of the quantitative tests that have been carried out conclude that Rome Beauty apple extract (*Malus sylvestris Mill.*) has a quercetin content of 557.999 ppm and a quercetin content of 0.557%.
Conclusion: Rome Beauty apples (*Malus sylvestris Mill.*) positively contain the flavonoid compound quercetin

Keywords: Rome Beauty Apple, HPLC, Quercetin

Introduction

Fruit is a natural ingredient source containing various antioxidant compounds (Jideani *et al.*, 2021). Natural antioxidants are generally safer to consume than synthetic ones, which have potentially detrimental effects such as pro-oxidative properties (Liu, 2022). Apple is a fruit with abundant antioxidants (Asma *et al.*, 2023). Apples have beneficial effects against oxidative stress and related diseases (Oyenihi *et al.*, 2022).

Oxidative stress is a condition where there is an imbalance between free radicals and the amount of antioxidants in the body, causing cell damage (Oyenihi *et al.*, 2022). Oxidative stress can cause cancer, diabetes mellitus, neurodegenerative disease, cardiovascular disease, rheumatoid arthritis, kidney disease, and eye disease (Pisoschi *et al.*, 2021). Malang is the largest producer of apples in Indonesia, which have the potential to be a source of natural antioxidants. *Apples* are plants that originate in West Asia. This plant lives in areas with subtropical climates with cold temperatures and air conditions. Batu City is an expansion city of Malang Regency that produces many varieties of apples, one of which is the Rome Beauty apple, which has the potential to have properties as a natural antioxidant (Dohitra *et al.*, 2015). *Rome Beauty apples* are a natural ingredient that has beneficial effects on health. Rome Beauty apples contain catechin, epicatechin, phloridzin, allergic acid, chlorogenic acid, and quercetin, which act as antioxidants. One of the flavonoid compounds in quercetin, which has the potential as an antioxidant, has a working mechanism by reducing free radicals (Rusita *et al.*, 2019; Zaddana *et al.*, 2020). Quercetin is a flavonoid reported to exhibit various biological activities and is used for medical applications (Tronins *et al.*, 2023).

The potential pharmacological activity of flavonoids, especially quercetin compounds, is the basis for using High-Performance Liquid Chromatography (HPLC) to determine the quercetin content of Rome Beauty apple extract, as in previous research (Mizzi *et al.*, 2020). The purpose of this study was to find out how to quantitatively test the flavonoid quercetin from Rome Beauty apple extract (*Malus sylvestris Mill.*) using HPLC to determine the levels of the flavonoid quercetin found in Rome Beauty apple extract (*Malus sylvestris Mill.*).

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Methods

Design

This research is a descriptive observational study. This study focuses on the quercetin content of Rome Beauty apples (*Malus sylvestris Mill*.). This research was carried out in different laboratories; namely, the manufacture of Rome Beauty apple Simplicia was carried out at the UPT of the Materia Medika laboratory in Batu City Malang, and the manufacture of apple extract and analysis of the quercetin content was carried out in the Food Engineering laboratory of Soegijapranata Catholic University Semarang.

Simplicity-making Process

The study began with preparing the dry extract (simplicial), namely Rome Beauty apples washed thoroughly in running water and then sliced thinly. After that, it was dried in an oven at 50 ^{o}C for three days. Then, dry Simplicia is obtained, crushed using a blender, and sieved to get uniform Simplicia particles.

Rome Beauty Apple Extract Process

The extract was prepared by maceration using 70% methanol. Simplisia powder weighed as much as 50 grams, dissolved in 250 mL of 70% methanol in a glass vessel, and stirred until homogeneous. The maceration process was carried out for five days. On the fifth day, filtering and remaceration were carried out by adding 250 mL of 70% methanol for two days with the same treatment. On the second day of repatriation, screening is carried out. The results of the filtering (filtrate) of maceration and maceration are mixed and evaporated using a vacuum rotary evaporator at a temperature of 500C until a thick extract is obtained. Pour it into a porcelain cup, heat it with an electric stove and with the help of a fan while continuing to stir, and keep the temperature controlled with a thermometer. This process evaporates ethanol to obtain a thick extract with a concentration of 100%. This maceration method is very advantageous because it is cheap and easy to do and does not damage compounds that cannot withstand heating. It is adjusted to the physical and chemical properties of the class of compounds in Rome Beauty apples (*Malus sylvestris Mill.*), namely the form of flavonoid compounds that are not heat resistant and easily oxidized at high temperatures (Setiani *et al*., 2017).

Quercetin Standard Solution Process

The quercetin standard solution dissolves the standard with methanol and sonicating for 15 minutes, then filters with a 0.22 µm filter membrane. The results of making a standard quercetin solution are soluble and ready for analysis in a High-Performance Liquid Chromatography (HPLC) system.

Standard and Sample Preparation

The first standard preparation performed was 10.0 mg of quercetin standard. Then, the standard was dissolved in a 10 mL measuring flask with methanol solvent up to the mark (Quercetin content became 1 mg/mL or 1000 µg/mL). Then 1000 µg/mL of mother liquor was taken, and as much as 1 mL was dissolved in a 10 mL measuring flask with methanol solvent up to the mark (Quercetin level became 100 µg/mL). A standard curve was made from 100 µg/mL mother liquor and the solution with a 0.22 µL membrane filter. For sample preparation, weigh 25 mg of the sample, dissolve it in methanol up to 10 mL, and filter the sample solution using a 0.22 µm membrane filter. Next, inject 20 µL of sample filtration into the HPLC system. Repeat replication at least five times (Husnia & Budiarti, 2021)

Results and Discussions

The Rome Beauty apple is used in this research, as in **Figure 1.** The Rome Beauty apple is a type of plant in the dicotyledon class. Rome Beauty apples are round, slightly oval, and have a slight indentation at the top. Its skin is flimsy, with a slightly rough surface and green to shiny red. The flesh of the Rome Beauty apple is yellowish-white, and there are seeds in the flesh. Rome Beauty apple seeds are extended with a brown pointed tip (Dohitra *et al*., 2015; Husaini *et al*., 2017). Rome Beauty apples are taken from the city of Batu, and Simplicia powder is carried out at the Batu Herbal Materia Medica Laboratory UPT, which is one of the UPTs of the East Java Province health service and is a research centre for herbal medicinal plants in East Java Province as in **Figure 2.**

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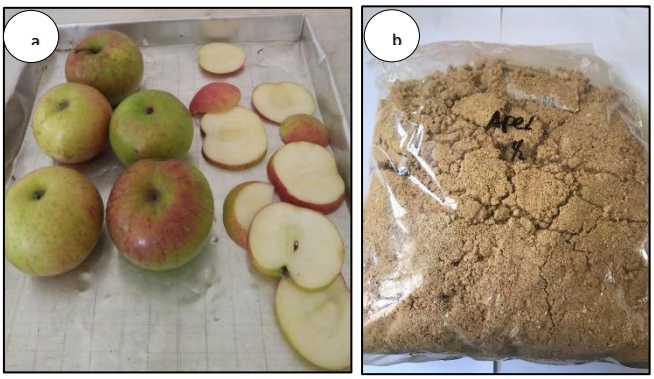

Figure a. Rome Beauty Apples (*Malus sylvestris* Mill.). **Figure b .** Rome Beauty Apple Simplicia Powder (Malus sylvestris Mill.)

Simplicia powder weighed as much as 50 grams, dissolved in 250 mL of 70% methanol in a glass vessel, and stirred until homogeneous. The maceration process was carried out for 5. On the fifth day, filtering and remaceration were carried out by adding 250 mL of 70% methanol for two days with the same treatment. On the second day of remaceration, screening is carried out. The results of the maceration and maceration filtration (filtrate) are mixed and evaporated using a vacuum rotary evaporator at 500C until a thick extract is obtained.

The maceration method is very advantageous because it is cheap and easy to do and does not destroy heat-resistant compounds. It is adjusted to the physical and chemical properties of the class of compounds in the Rome Beauty apple (*Malus sylvestris* Mill.), namely the class of flavonoid compounds. Flavonoid compounds are a class of compounds that are not heat resistant and easily oxidized at high temperatures, whereas quercetin is a compound of the flavonol group of flavonoids (Badaring *et al.*, 2020; Asworo and Widwiastuti, 2023).

The maceration results are evaporated using a rotary vacuum evaporator until a thick extract is obtained. The analysis of quercetin in Rome Beauty apple extract by High-Performance Liquid Chromatography (HPLC) begins with determining the maximum wavelength. The maximum wavelength is when maximum light absorption occurs in the analyzed compound. Wavelength determination is made using a UV spectrophotometer between 250-800 nm wavelengths.

Table 1. Quantitative Test Results for the Content of Quercetin in Rome Beauty Apple Flour

Sample	Simplisia of Weight (g)	Quercetin (ppm)	Quercetin (%)
Apple Flour	100	557,999	0,557%

Based on the results of quantitative analysis using High-Performance Liquid Chromatography (HPLC) carried out on a sample of Rome Beauty (*Malus sylvestris* Mill.) extract, the sample was positive for containing the compound quercetin, which can be seen from the results of **table 1**. The analysis results obtained were 557,999 ppm and a quercetin content of 0.557%. High-performance liquid chromatography (HPLC) is the most widely used analytical tool for pharmaceutical analysis. The study of Miftahuljanna (2019) explained the results of the quercetin content analysis test in the form of 100-gram powder by HPLC using 96% ethanol, the ethanol extract of miana leaves Plectranthus scutellarioides (L.) R.Br.) contained an average level of quercetin, namely 3.122 mg/g and quercetin content in per cent amounting to 0.312%. In research by Husnia and Budiarti (2021), the high-performance liquid chromatography method results can be applied to determine the quercetin content in the ethanol extract of leunca fruit at 0.00086% using the isocratic technique. Study Hutagalung (2023) Quantitative test results using the UV-Vis spectrophotometer method obtained total phenolic compound levels of 335.77 mg GAE/mL based on the identification of secondary metabolites using high-performance liquid chromatography-mass spectrometry (UPLC-QTOF-MS/MS) obtained that kecarum cocktail arrack contains 24 secondary metabolite compounds, a class of phenolic compounds consisting of flavonoids and phenolic compounds.

Conclusions

Quantitative evaluation of quercetin in Roma Beauty Apples (Malus sylvestris Mill.) using High-Performance Liquid Chromatography (HPLC) equipped with a UV spectrophotometer detector showed the presence of the flavonoid component quercetin. Has a concentration of 557,999 ppm and 0.557% quercetin. Quercetin, as an antioxidant, has the

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potential to be claimed as a functional food that functions as an anti-free radical. Suggestions: For further research, it is necessary to test quercetin levels using other techniques as a comparison.

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