The Levels of Quercetin and Antioxidant Activity of Patikan Kebo Leaves Extract

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Abstract: Patikan kebo is a wild plant that is commonly found in tropical and subtropical areas on soil that is not so moist. Patikan kebo contains a number of active compounds such as flavonoids, terpenoids, and several other active compounds such as alkaloids and polyphenols. This study aims to determine the concentration of quercetin and antioxidant activity in patikan kebo leaf extract. Samples were taken from the Swadaya Alam Jaya Herbal Research Center for Medicinal Plants, and bioactive compounds were extracted from the leaves of the patikan kebo plant through the mesarasi method with ethanol. Quercetin levels were analyzed using the High-Performance Liquid Chromatography (HPLC) method, and antioxidant activity was carried out using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed that the patikan kebo leaf extract showed that the content of quercetin compounds in 100 g of the patikan kebo leaf extract was 110.73 mg and the IC_{50} antioxidant activity results were 80,151 % and 79,962 %, respectively.

1 INTRODUCTION

Indonesia is a tropical island and is famous for its natural wealth with various types of nutritious plants that have properties (Widjaja et al., 2014). Globally, there are 40,000 types of plants are known as traditional medicines, 30,000 of which are in Indonesia, of which 7000 types of plants have been used as raw materials for herbal medicines. One of them is the patikan kebo plant (Jumiarti and Komalasari, 2017).

Patikan kebo is a wild plant that is easily found on the surface of the soil that is not so moist, and contains a number of active compounds such as flavonoids, terpenoids, and a number of other active compounds such as alkaloids and polyphenols (Asha et al., 2014). The important bioactive components of patikan kebo are flavonoids, which function as antioxidants. Several studies have shown that antioxidant compounds can play a role in fighting oxidative damage through their reactions with free radicals in a way that correlates with oxidative stress defense so that antioxidant compounds can play a role in fighting oxidative damage (Uppala and Reddy, 2014). One of the flavonoid groups found in the patikan kebo plant is quercetin (Huang et al., 2012) (Karim, Jura, & Sabang, 2015). Quercetin may protect against the environmental causes of free radicals (David et al., 2016). The benefits of antioxidants and quercetin for health are the basis for exploring plants or foods that contain these compounds. One of them is the patikan kebo plant.

The bioactive components in patikan kebo leaves can be increased by extraction because this method can attract and separate several compounds using certain solvents (Leba, 2017). The extraction methods that are widely used for solid samples into solvents are soxtellation, percolation, and maceration. The maceration method is a simple extraction method that is most commonly used for the extraction of patikan kebo. This method can also avoid the risk of damage to compounds in plants (Tetti M, 2014).

Quercetin compounds and antioxidant activity can be dissolved in polar solvents such as ethanol, methanol, ethyl acetate, butanol, potreleum, and others (Tran, 2020). So far, there have been no studies analyzing compounds in patikan kebo leaves taken from local soil in Gianyar, Bali, which have the potential to contain quercetin and antioxidant activity. This research is about the content of these compounds, which can be used as empirical evidence that patikan kebo leaf extract can be used for health. Based on the

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above background, this study aims to determine the concentration of quercetin and antioxidant activity in patikan kebo leaf extract.

2 MATERIALS AND METHODS

2.1 Methods

This study used patikan kebo leaves, which were taken homogeneously in the local area by the Jaya Herbal Self-Help Research Center for Medicinal Plants, Gianyar Regency, Bali, Indonesia. The extraction of patikan kebo leaves was carried out at the Center for Food and Nutrition Studies, Gadjah Mada University (UGM), and the evaluation of quercetin levels and antioxidant activity was carried out at the Chemical Laboratory of the University of Muhammadiyah Malang (UMM).

Instruments and Materials

The tools used in this study were digital scales, test tube glass, UV-Vis spectrophotometer, Whatman no. 1 filter paper, shaker and rotating evaporator, mortar hammer, measuring pipette, suction cup, measuring flask, glass funnel, erlenmeyer, beaker, test tube, cuvette. The main ingredients used in the manufacture of patikan kebo leaves extract are patikan kebo leaf simplisa and 96% ethanol. Methanol p.a, DPPH 0.2 mM (Dissolve as much as 0.078 g in methanol p.a to 1000ml volume), distilled water, H3Po4 solution, and 25% acetonitrile.

Extraction of the Patikan Kebo

The patikan kebo leaves that have become simplisa are soaked in a 96% ethanol solution with a ratio of 1 g simplisa to 10 ml ethanol for ± 48 hours while stirring regularly using a shaker at 150 rpm at 600 C. After going through the soaking process, the patikan kebo leaves are filtered The filtrate was taken using Whatman filter paper number 1. Then the filtrate was inserted into a rotatory evaporator to make it more concentrated and obtain patikan kebo leaves extract.

Evaluation of the Total Quercetin

Analysis of quercetin levels was carried out using the HPLC method on a Shimadzu UFLC system, equipped with a LC 20AT quaternary gradient pump using an SPD-M20A PDA diodearray detector (DAD). The data were obtained on a liquid chromatography solution administrator data system

Evaluation of the Antioxidant Activity

For the determination of antioxidant activity, first sample preparation, i.e. sample taken 0.1 ml dissolved in methanol pa to 100 ml in a measuring flask, to obtain a sample solution of 100 ppm, then homogenized and allowed to stand for about 30 minutes, then filtered and if necessary centrifuged at 3000 rpm for 10 minutes then supernatant was taken. Next, 1.5 ml of the sample solution was added with 3 ml of 0.2 mM DPPH solution, then the mixture was shaken until homogeneous and allowed to stand for 30 minutes, then the absorbance was measured at 516 nm. The procedure was carried out on a blank of 1.5 ml of methanol pa. The antioxidant activity of the sample could be determined by calculating the percentage of DPPH absorption inhibition with the formula:

$$\text{Antioxidant Activity (\%)} = \left(1 - \frac{s}{b}\right) x\ 100\%$$

Information :
b = Absorbance Blank
s = Absorbance Test Sample

3 RESULTS AND DISCUSSION

This study aims to determine the concentration of quercetin and antioxidant activity in patikan kebo leaf extract. Samples were taken from the Jaya Herbal Bali Self-Help Research Center for Medicinal Plants and extracted using the maceration method with ethanol as a solvent to obtain the samples used in this study. The advantage of using the maceration method is that there is no heating process, so it is unlikely that the active substances in simplisa are damaged or decomposed (Istiqomah, 2013). The solvent used in this study was ethanol. Extraction solvents such as ethanol, methanol, dichloromethane, ethyl acetate, water, and hexane are widely used to extract antioxidants and other compounds, but ethanol solvents have a high polarity when compared to other types of organic solvents. Ethanol has a low boiling point. Ethanol is also non-toxic and dangerous, so it tends to be safe (Bakhouche et al., 2015; Hoon et al., 2015).

The patikan kebo leaf extract contains a number of phenolic and flavonoid compounds (Asha et al., 2014), one of which is quercetin. The Patikan kebo leaves extract contains a number of phenolic and flavonoid compounds (Asha et al., 2014), one of which is quercetin. Quercetin is a compound classified as a flavanol, which is the main polyphenol flavonoid found in various plants (Anand et al., 2016). These compounds have anticancer, antiviral, and anti-

inflammatory properties, as well as the ability to treat metabolic disorders, cardiovascular disease, arthritis, allergies, and inflammation (Batihah et al., 2020). Quercetin can also control postprandial blood sugar levels by activating α-glucosidase enzyme inhibitors (Jadhav, 2012). In this study, the levels of quercetin were identified using the HPLC method, which is a method of separating a mixture of substances using a mobile and stationary phase, the separation technique in HPLC occurs because of differences in absorption power, solubility, partitioning, molecular size, ion size, and vapor pressure on the components carried by the mobile phase through the stationary phase (Aulia et al., 2016).

Table 1: Results of analysis of quercetin levels using HPLC.

Sampel ID	RT min	Result (µg/g)
patikan kebo leaf extract	6,739	110726,51549

Based on the results, it can be seen that the quercetin compound was seen at a retention time of 6,739 minutes. From the results of this study, it can be seen that the patikan kebo leaf extract contains quercetin at a concentration of 110.73 mg/gr. The ethanol solvent used is able to attract quercetin compounds, because kuers is a flavonoid group compound and can be dissolved in ethanol solvents (Sukmawati et al., 2019). Factors that affect the production of secondary metabolites are divided into two factors, namely extrinsic and intrinsic. Extrinsic in the form of climatic conditions and the soil where it grows, while the intrinsic factors are in the form of the genes of the plant concerned, as well as climatic conditions, which include temperature, rainfall, lighting, and soil elevation, while soil conditions include the content of nutrients, minerals, moisture, pH, rocks, sand, and mud (Liu et al., 2017).

Previous studies reported that having tested quercetin levels in patikan kebo leaf extract with various solvents showed results of 276.6 mg/gr, 189.2 mg/gr, 164.1 mg/gr, 124.3 mg/gr, and 84,8 mg/gr (Tran, 2020). The difference in quercetin value results depends on differences in plant species, solvents and concentrations, as well as the method used at the time of extraction (Hardinsyah et al., 2019).

The antioxidant activity test of patikan kebo leaves extract was identified using the DPPH method, because the test is simple, fast, and easy and only requires a small sample (Shalby et al., 2013). The working principle of DPPH (2,2-Diphenyl-1-picrylhydrazyl) is that antioxidant compounds will donate hydrogen atoms to DPPH when the DPPH solution reacts with antioxidant compounds. Then, measured by UVVis at a wavelength of 516nm, there was a color change (from dark purple to yellow or pale yellow) when the extract sample showed the ability to absorb DPPH free radical activity (Kedare et al., 2011). The results of the research on antioxidant activity in patikan kebo leaves extract can be seen in the following table.

Table 2: Antioxidant Activity of Patikan Kebo Leaf Extract (%) concentration 100 ppm.

ppm concentration	ul	Abs	%antioxidant activity
patikan kebo leaf extract	1	0.105	80,151
	2	0.106	79,962

Based on table 2, patikan kebo leaf extract has antioxidant activity of 80.151% and 79.962%, respectively. The ability of antioxidants as free radical scavengers is associated with the ability of these antioxidants as proton donors. The number of hydrogen protons that can be donated is influenced by the number and position of the aromatic hydroxyl or hydroxyl groups of the phenolic component. The more aromatic hydroxyl groups, the more effective the chain reaction inhibition in the oxidation process is by donating hydrogen atoms or acting as free radical acceptors (Septiana & Asnani, 2013).

Research conducted by Karim, K et al., (2015) reported the optimum results of antioxidant activity in patikan kebo leaf extract of 99.21%. Antioxidants are said to be very strong if they have an IC_{50} value of less than 50 ppm, strong categories range from 50-100 ppm, moderate antioxidants are 100-150 ppm, and weak antioxidants are in the range of 150-200 ppm. al., 2017). The smaller the IC_{50} value of a compound, the more effective the compound is as an antioxidant (Putri et al., 2013). The difference in antioxidant activity values obtained from each type of extract may be due to differences in the content and number of active compounds contained in the extract (Purwanto et al., 2017).

The level of polarity of the solvent also greatly affects the antioxidant activity. In addition, the decrease in temperature during storage can also affect the levels of phytochemicals or antioxidant activity (Li et al., 2012). Plants that have high antioxidant bioactive compounds can help the body prevent damage by radical compounds or decrease body resistance (Mensaah et al., 2014).

The results of the analysis of phytochemical compounds in patikan kebo leaf extract proved to contain quercetin and strong antioxidant activity. Therefore, to continue the potential pharmacological use of patikan kebo leaf extract, it is necessary to conduct appropriate preclinical research.

4 CONCLUSIONS

Based on the results of the research, it was concluded that the patikan kebo leaf extract contained a quercetin compound of 110.73 mg/gr. The antioxidant activity obtained from patikan kebo leaves extract has a strong antioxidant activity category.

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