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Research Article

Antioxidant Activity of Water and Ethyl Acetate Fractions of Qust Al Hindi Root (*Saussurea lappa*) Using DPPH Inhibition Method

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ABSTRACT: Free radicals are molecules that do not have an electron pair in the outer shell, making them highly reactive. Oxidation due to free radicals can be prevented and inhibited by substances, namely antioxidants. Natural antioxidants are obtained from plants or fruits, one of which is Qust al hindi (*Saussurea lappa*). Qust al hindi is a plant native to India that is used as a traditional medicine. Qust al hindi contains various phytochemical compounds, one of which is flavonoid compounds that function as antioxidants. The purpose of this study was to analyze the antioxidant activity of water and ethyl acetate fractions of qust al hindi plant roots by *1,1-diphenyl-2picrylhydrazyl* (DPPH) silencing method using UV-Vis spectrophotometry based on the calculation of IC_{50} value using GraphPad Prism Application. The results of this study indicate that the water and ethyl acetate fractions of qust al hindi plant roots have antioxidant activity against DPPH free radicals with IC_{50} values of 203.5 µg/mL; 193.3 µg/mL, respectively, which are included in the very weak and weak antioxidant groups.

KEYWORDS: Antioxidants; DPPH; Fraction; Qust Al Hindi *(Saussurea lappa*)

1. INTRODUCTION

Qust al hindi (*Saussurea lappa*) is native to India, Pakistan and China, growing in the Himalayan region at an altitude of 2500-3500 meters. The plant is found in cold temperature regions and polar regions in Asia, Europe and North America (Kamalpreet, *et al*. 2019). In the traditional Indian system of medicine, qust al hindi is used either in combination with other drugs or as a single drug. The dried root has a slightly bitter taste and is gray to yellow in color. The volatile oil obtained from the root is mostly used in medicine. The root is mainly used for asthma, cough and also for the treatment of chronic skin diseases, rheumatism and cholera (Zahara *et a*l., 2014).

Qust al hindi is in high demand in the pharmaceutical industry, but in recent decades, it has become more popular in the world as an endangered species. As it is an endangered species, it has been listed in Appendix I of CITES. Trade in qust al hindi is effectively banned under the Foreign Trade Development Act of 1992. Qust al hindi was first listed in CITES Appendix II on July 1, 1975 and then in Appendix I in 1985 as a critically endangered plant (Wahab, A *et al*. 2015).

Free radicals are atoms or molecules that have unpaired electrons, are unstable and highly reactive, as a result free radicals will attract electrons from molecules in cells and cause damage to cells and tissues. The formation of free radicals in the body is a continuous and inevitable process in the human body. The immune system, metabolic processes, stress, nutritional factors, environmental influences, toxins and some drugs are responsible for the formation of free radicals. Free radicals can cause various diseases, such as diabetes mellitus, Parkinson's disease, emphysema, acute renal failure, aging and cancer. Therefore, the body needs antioxidants that can stabilize free radicals (Qazi & Khursid, 2018). Free radicals play an important role in tissue damage and pathological processes in living organisms (Velazquez, *et al*. 2003). Free radicals that enter the body can attack vulnerable compounds, such as lipids and proteins, and are implicated in the onset of various diseases (Amic, *et al*. 2003).

Chemically, antioxidant compounds are electron donor compounds (Fawwaz et al., 2020). Biologically, antioxidants are compounds that can counteract or reduce the negative effects of oxidants. Antioxidants work by donating one electron to compounds that are oxidants so that the activity of the oxidant compound can be inhibited. Antioxidant is a compound or chemical component that in certain levels or amounts is able to inhibit or slow down the damage caused by the oxidation process (Sayuti & Rina, 2015).

One of the plants that can be utilized as a source of antioxidants is qust al hindi root. Qust al hindi is a plant known mostly in prophetic medicine as well as in Ayurveda, Unani, and Siddha. Various active compounds isolated from the plant are reported to have medicinal properties, for example, the main components are sesquiterpene lactones such as costunolide and dehydrocostus lactone (Zahara *et a*l., 2014). In addition, these plants contain phytochemicals from various classes of chemicals, such as alkaloids, glycosides, coumarins, flavonoids, phenols, quinones, steroids, tannins, and terpenoids (Sukmawati *et al.*, 2022). The antioxidant activity of qust al hindi is due to the presence of chlorogenic acid compounds that function to prevent oxidation and free radicals (Singh, *et al*. 2017).

Based on the publication (Sukmawati *et al.*, 2022), ethanol extracts from the roots of qust al hindi have pharmacological effects as anti-inflammatory, antimicrobial, anticancer, anti-arthritis, immune and antioxidant responses, besides that the active compounds contained in the qust al hindi plant, namely tannin, have pharmacological effects as anti-bacterial, antifungal, antidiarrheal, hepatoprotective, anti-hepatotoxic, anti-viral, antioxidant, and immunostimulant.

Based on the above background, the authors are interested in conducting laboratory research on the antioxidant activity of water and ethyl acetate fractions of qust al hindi using the *1,1-diphenyl-2picrylhydrazyl* (DPPH) inhibition method.

2. EXPERIMENTAL SECTION

2.1. Population and Samples

This research was conducted in vitro laboratory experiment using DPPH free radical suppression method in February 2023 to May 2023 at the Pharmaceutical Chemistry Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia. The research population used in this study was the qust al-hindi plant. The samples used in this study were qust al-hindi roots made in the form of water and ethyl acetate fractions.

2.2. Materials and Tools

The materials used in this study are aquadest, DPPH (sigma aldrich®), ethyl acetate, quercetin (emsure®), ethanol 96%, ethanol extract of qust al hindi root. The tools used in this research are beaker glasses (pyrex®) 100 mL; 250 mL, measuring cup (pyrex®) 50 mL, l volumetric flask (pyrex®) 50 mL; 10 mL micropipette 1000 µL (dragonlab®), water bath (Memmert®), UV-VIS spectrophotometer *thermoScientific Tipe Genesys 10s Uv-Vis*, test tube (pyrex®), Vacuum Erlenmeyer (pyrex®), analytical balance *Kern ABJ-NM/ABS-N,* Separator Funnel (pyrex®) 50 mL.

The DPPH solution was prepared by accurately weighed as much as 5.0 mg of DPPH dissolved with 96% ethanol up to 5 mL then homogenized, then pipetted 1.75 mL then sufficed with 96% ethanol up to 50 mL. Homogenized and placed in a brown bottle.

The preparation of quercetin. A total of 5 mg was weighed, then dissolved in 5 mL of 96% ethanol, obtained a concentration of 1000 ppm. Quercetin 1000 ppm standard solution was pipetted 1 mL and enough to 10 mL with 96% ethanol for 100 ppm, from the solution was made with concentrations of 2, 4, 6, 8, and 10 ppm.

Sample solution was made with a concentration of 1000 ppm as a parent solution. The water and ethyl acetate fractions of qust al-hindi were weighed as much as 10 mg, put into a 10 ml volumetric flask and added 96% ethanol and shaken until homogeneous, then 96% ethanol was added to the limit mark. After that, it was diluted into 5 concentration series, namely 100, 150, 200, 250 and 300 ppm. The balnk solution was prepared by pipetted as much as 1 mL of 35 ppm DPPH then added 2 mL of 96% ethanol and then homogenized. After that, 1 mL of 96% ethanol was added.

2.3. Sample Preparation

The material used was 2 kilograms of qust al hindi root. Qust al hindi root was cleaned using running water to separate the dirt attached to the sample. Then the sample was dried without direct sunlight, then the dried sample was cut into small pieces and pulverized using a blender and then the sample was dried.

2.3.1. Preparation of Ethanol Extract of Qust Al Hindi Root

The obtained qust al hindi root simplisia powder was then extracted by the soxhlet method using 96% ethanol. Weighed as much as 50 grams of qust al hindi root simplisia powder and then put it into a cellulose thimble. Enter 300 mL of 96% ethanol into the soxhlet. The extract obtained was filtered using whattman filter paper. The filtrate obtained was then evaporated using a rotary vacuum evaporator at 60°C for 30 minutes to obtain a thick extract.

2.3.2. Preparation of Water and Ethyl Acetate Fractions of Qust Al Hindi Root

Ethanol extract of qust al hindi root as much as 3 mg was added with distilled water as much as 30 mL into a separatory funnel, then added ethyl acetate as much as 30 mL. Shaken until dissolved and allowed to stand until separated. After separating the ethyl acetate phase will be above and the water phase is below. The ethyl acetate phase was collected in a porcelain cup and then the water phase was shaken again by adding 30 mL of ethyl acetate until a clear solution was obtained. The remaining water phase is the water fraction. The results of the fraction were evaporated using a waterbath.

2.4. Quantitative Analysis

2.4.1. Determination of Maximum Wavelength of DPPH (ƛ max)

DPPH 35 ppm solution as much as 3 mL was put into a 5 mL volumetric flask after determining the maximum wavelength of DPPH solution at a wavelength of 400-800 nm using UV-Vis spectrophotometry. A wavelength of 515 nm was obtained.

2.4.2. Determination of Antioxidant Activity of Quercetin Comparator Standard

Solutions were made using quercetin as standard raw material (100 ppm) with concentration series of 2, 4, 6, 8, and 10 ppm. Each concentration series was pipetted as much as 1 mL into the vial after which, 3 mL of 35 ppm DPPH standard solution was added. Each mixture of the solution was incubated for 30 minutes in a dark place. Then the absorbance was measured at a maximum wavelength of 515 nm.

2.4.3. Determination of Antioxidant Activity of Water and Ethyl Acetate Fractions of Qust Al Hindi Root

Each as much as 10 mg of ethyl acetate fraction and water qust al hindi was put in a 10 mL volumetric flask and each added as much as 10 mL of 96% ethanol while stirring and homogenized, then the volume was sufficient to the limit mark to obtain a stock solution with a concentration of 1000 ppm. The 1000 ppm stock solution was made at concentrations of 100, 150, 200, 250 and 300 ppm. Each concentration series was pipetted as much as 1 mL into the vial after that, added 3 mL of 35 ppm DPPH standard solution. Each mixture of the solution was incubated for 30 minutes in a dark place. Then the absorbance was measured at a maximum wavelength of 515 nm. The same was done for the ethyl acetate fraction sample. The antioxidant activity of the fraction obtained was then calculated the amount of DPPH free radical absorption inhibition by calculating the percentage of DPPH absorption inhibition.

2.5. Data Analysis

Calculation of % inhibition of DPPH absorption using the formula (Molyneux, 2004) :

$$\% \text{ Inhibisi} = \frac{\text{Abs Blanko -Abs Sampel}}{\text{Abs Blanko}} \times 100\%$$

The IC_{50} value of each sample concentration was calculated using the linear regression equation formula. Sample concentration as x-axis and % inhibition as y-axis. From the equation Y = a + bx. To determine the IC_{50} value, it can be calculated using the formula:

$$IC_{50} = \frac{(50 - a)}{b}$$

Description:
Y = % Inhibition
x = Concentration
a = Intercept (the intersection of the line on the Y axis)
b = Slope

3. RESULTS AND DISCUSSION

This study was conducted to determine the antioxidant activity contained in the water and ethyl acetate fractions of just al Hindi roots to determine the ability of water and ethyl acetate fractions of just al Hindi roots to capture radical compounds. The parameter used to determine the magnitude of the compound as an antioxidant is the IC_{50} value. The acquisition of Inhibition Concentration 50% (IC_{50}) and data analysis on the sample and quercetin as a comparison obtained the results below (**Tables 1** and **2**):

Table 1. Extraction Results and Percent Yield of Ethanol Extract of Qust Al Hindi Plant Root.

Sample	Amount of Solvent (mL)	sample weight (g)	weight of extract (g)	Extract Yield (%)
Root qust al hindi	300	50	3.6851	7.3702

Table 2. Fractionation Results and Percent Yield of Ethanol Extract of Qust Al Hindi Plant Root.

Sampel	weight of extract (g)	Amount of Solvent (mL)	weight of fraction (g)	Fraction Yield (%)
Water fraction	3.6851	30	0.8888	24.1187
Ethyl acetate fraction	3.6851	30	1.7418	47.2660

Prior to the antioxidant activity test on the quercetin comparator and the sample, the maximum wavelength was determined, to determine the maximum absorption. **Figure 1.** shows the maximum wavelength of 35 ppm DPPH measured using a UV-Vis spectrophotometer in the wavelength range of 400-800 nm so that the maximum wavelength of 515 nm is obtained.

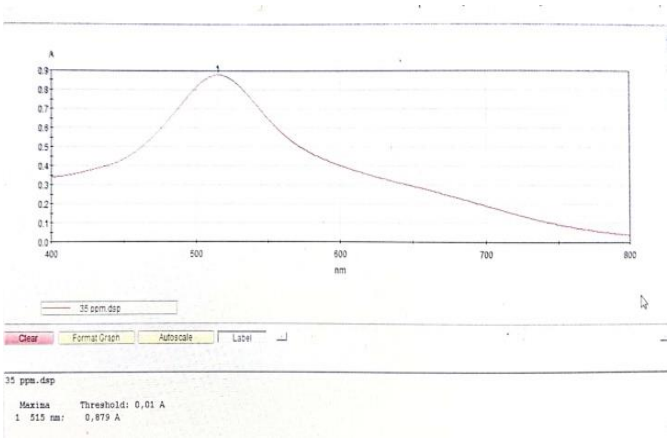

Figure 1. Maximum Wavelength Determination Result of DPPH Solution.

Furthermore, the antioxidant activity of quercetin and sample was measured using UV-Vis spectrophotometer with a maximum wavelength of 515 nm. The measurement results can be seen in **Tables 3** and **4**.

Tabel 3. IC_{50} Calculation Result of Quercetin Comparator

Sample	Concentration (ppm)	DPPH Absorbance	Sample Absorbance	% Inhibition	IC_{50} (ppm)
quercetin	2	0.754	0.447	40.716	5.728
	4	0.754	0.407	46.021	
	6	0.754	0.371	50.795	
	8	0.754	0.335	55.570	
	10	0.754	0.294	61.007	

Figure 2. shows the results of linear regression analysis of the relationship between the concentration of quercetin comparator standard with the percent attenuation of DPPH absorbance obtained regression equation y = 2.5066x + 35.782 with a correlation coefficient value (r) = 0.9996. 0191 The value (r) obtained close to 1 indicates that the regression equation is linear, so it can be said that the absorbance and concentration have a very strong correlation.

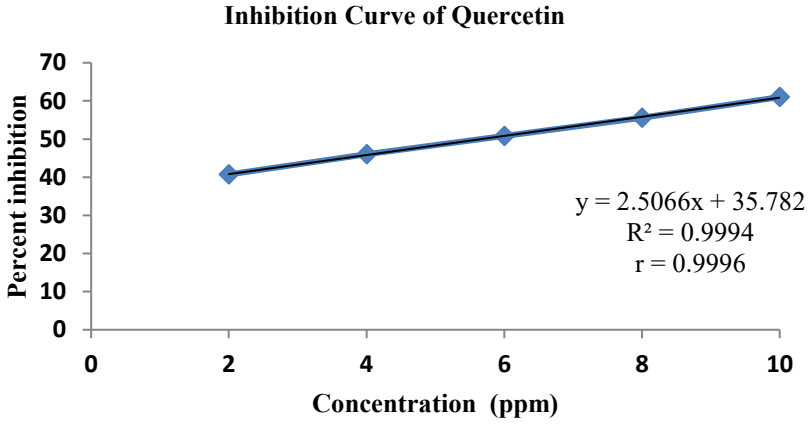

Figure 2. Percent Inhibition Curve of Quercetin Comparator Standard

Table 4. IC_{50} Calculation Results of Qust Al Hindi Root Samples Water and Ethyl Acetate Fractions

Sample	Concentration (ppm)	DPPH Absorbance	Sample Absorbance	% Inhibition	IC_{50} (ppm)
Water fraction	100	0,757	0,520	31,307	203,5
	150	0,757	0,499	34,081	
	200	0,757	0,466	38,441	
	250	0,757	0,441	41,743	
	300	0,757	0,406	46,367	

Ethyl acetate fraction	100	0,902	0,608	32,594	193,3
	150	0,902	0,574	36,363	
	200	0,902	0,541	40,022	
	250	0,902	0,505	44,013	
	300	0,902	0,475	47,339	

Figure 3. shows the results of linear regression analysis of the relationship between the concentration of water and ethyl acetate fractions with the percent absorbance of DPPH obtained regression equation y = 0,0756x + 23,275 and y = 0.0743x + 25.21 with a correlation coefficient (r) value of 0.9969; 0.9996, respectively.

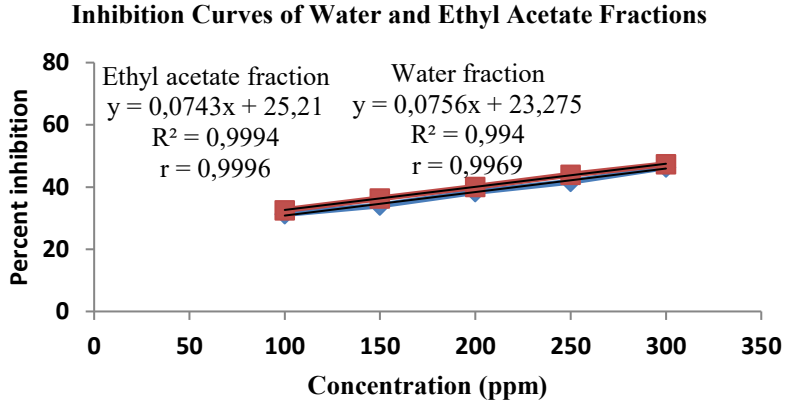

Figure 3. Percent Inhibition Curve of Qust Al Hindi Root Samples Water and Ethyl Acetate Fractions

The results of antioxidant activity test of water and ethyl acetate fractions of qust al hindi root using DPPH inhibition method at a wavelength of 515 nm can be seen in Table 3. The results obtained that the comparison of IC_{50} of quercetin with IC_{50} of water and ethyl acetate fraction samples is 5,728 µg/mL: 203,5 µg/mL: and 193,3 µg/mL. so it can be interpreted that the antioxidant activity in the water and ethyl acetate fractions of qust al hindi roots is still low compared to the antioxidant activity of quercetin. The measurement results obtained showed that the higher the concentration of the solution, the lower the absorbance. This is because the higher the concentration of the solution, the higher the content of antioxidant substances, so that more DPPH will be inhibited by the solution. (Fitriana, *et al*., 2019).

In this study, the samples were extracted by the soxhletation method using 96% ethanol solvent. The reason for choosing the soxhletation method is because the time used is more efficient, organic solvents can attract organic compounds in natural materials repeatedly and the extraction process runs continuously as needed without increasing the volume of solvent. This is very beneficial because in addition to being economical, a more concentrated extract will be obtained. In other words, less solvent is required compared to maceration or percolation methods (Depkes RI, 1985). The use of 96% ethanol as a solvent because it is selective, non-toxic, good absorption and high ability to extract non-polar, semi-polar and polar compounds (Fauzi, 2021). The sample was then evaporated using a rotary vacuum evaporator to obtain a thick extract. The results of extraction and fractionation can be seen in tables 1 and 2.

In this study, the method used was the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method. The DPPH method is a method that can be used to determine the antioxidant activity in the sample to be tested by looking at its ability to counteract DPPH free radicals (Fawwaz et al., 2023). The advantages of this method are that the method is simple, easy, fast, sensitive, and requires a small amount of sample and the DPPH radical compound used is relatively stable compared to other methods. The principle of this method is the donation of hydrogen atoms (H+) from the sample tested to the DPPH radical into a non-radical compound diphenyl picrylhydrazine which will be indicated by a color change. The color change that occurs is a color change from purple to yellow, where the intensity of the DPPH color change is directly proportional to the antioxidant activity to reduce the free radicals (Rahmawati, *et al*., 2016). Antioxidant activity test using DPPH with quercetin as the comparator. The reason for using quercetin as a comparator is because quercetin is a single flavonoid compound of the flavonol group and has been shown to have very strong antioxidant activity (Simanjuntak, 2012).

The results of measuring the antioxidant activity of the water and ethyl acetate fractions of qust al hindi root compared with quercetin showed that the antioxidant activity of the water fraction was very weak and the ethyl acetate fraction was weak compared to quercetin. Many factors can affect the results of this study. In several journals that the author has reviewed, it is explained that there are several factors that can cause differences in the results of the % inhibition and IC50 values in each study on antioxidant activity. One of them is due to the process of solvent evaporation using heating which has the potential to damage and remove antioxidant compounds in qust al hindi root

samples, especially for compounds that are thermolabile, this is what makes the levels of antioxidant compounds contained in the water and ethyl acetate fractions of qust al hindi root less.

4. CONCLUSION

Based on the discussion that, the results of antioxidant activity in the water and ethyl acetate fractions of qust al hindi root show that it is still in the very weak and weak antioxidant group, this is thought to be due to the solvent evaporation process using heating which has the potential to damage and remove antioxidant compounds in qust al hindi root samples, especially for compounds that are thermolabile, this is what makes the levels of antioxidant compounds contained in the water and ethyl acetate fractions of qust al hindi root less.

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