

Total Flavonoid Content in the Ethanol Extract Fraction of Carrot (*Daucus carota* L.)

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ABSTRACT

Carrot (*Daucus carota* L.) is a plant that contains flavonoids. Flavonoids are secondary metabolites that play an important role as antioxidants and hold significant potential for utilisation in the health sector. This study aims to determine the differences in total flavonoid content in the n-hexane, ethyl acetate, and aqueous fractions of ethanol extract of carrot. Extraction was carried out using the maceration method with 96% ethanol, followed by successive liquid-liquid fractionation with n-hexane, ethyl acetate, and water. Qualitative testing for flavonoids was conducted using the Wilstater-Cyanidin method, while the total flavonoid content was determined quantitatively using UV-Vis spectrophotometry at a wavelength of 438 nm with quercetin as the standard solution. The qualitative test results indicated that all fractions tested positive for the presence of flavonoids. The highest total flavonoid content was found in the n-hexane fraction, amounting to 267.70 ± 9.54 mg QE/g, followed by the ethyl acetate fraction with 30.99 ± 0.87 mg QE/g, and the aqueous fraction with 4.78 ± 0.08 mg QE/g. The Kruskal-Wallis statistical test showed a significant difference among the three fractions ($p < 0.05$). This study demonstrates that the n-hexane fraction contains the highest level of flavonoids compared to the other fractions, indicating that flavonoid compounds in carrots are more soluble in non-polar solvents.

Keywords: carrot, Fractionation, UV-Vis Spectrophotometry

INTRODUCTION

Indonesia is a country rich in biodiversity, including a wide variety of plants with potential as traditional medicines. The use of plants as a source of medicine has been practiced for generations, in line with the discovery of various bioactive compounds that contribute to pharmacological activities. One group of secondary metabolites commonly found in plants is flavonoids. Flavonoids are known to possess important biological activities, including antioxidant, anti-inflammatory, anticancer, and cardiovascular protective properties. The role of flavonoids as antioxidants is particularly prominent due to their ability to donate hydrogen atoms to neutralize free radicals, thereby contributing to the prevention of cellular damage caused by oxidative stress (1).

Carrot (*Daucus carota* L.) is a root vegetable that is not only consumed as a food source but also possesses pharmacological potential. Carrots contain various bioactive compounds such as carotenoids, limonene, pinene, geraniol, asparagine, and flavonoids. The presence of flavonoid compounds in carrots offers significant potential as a natural source of antioxidants. Several previous studies have shown that carrot extracts contain varying levels of total flavonoids, depending on the extraction method and the part of the plant used. Therefore, further research is needed to investigate the distribution of flavonoids within the fractions produced through the fractionation process (2).

Fractionation is a method of separating active compounds based on their polarity levels using different solvents. This technique is important for obtaining fractions with a higher concentration of specific compounds, thereby enabling identification of which fraction possesses the most potential pharmacological activity. In this study, fractionation was carried out on the ethanol extract of carrot using n-hexane, ethyl acetate, and water as solvents. The choice of solvents was based on their differing polarities: n-hexane for non-polar compounds, ethyl acetate for semi-polar compounds, and water for polar compounds (3).

Based on the aforementioned background, this study was conducted to determine the differences in total flavonoid content among the n-hexane, ethyl acetate, and aqueous fractions of carrot ethanol extract using UV-Vis spectrophotometry. The results of this research are expected to provide scientific information on the flavonoid content of each carrot fraction and serve as a foundation for the development of carrot's potential as a natural antioxidant source in the fields of pharmacy and health.

METHODS

Tool

Glassware (Pyrex), 100 μ L Micropipette (Dragon Lab), Analytical Balance (AND GF-300), Rotary Evaporator (RE-100 Pro), UV-Vis Spectrophotometer (Thermo Scientific).

Materials

The carrot simplicia was obtained from Ngrambe Village, East Java, with a determination certificate number of 407/Lab Bio/B/VI/2025. The materials used included aqua pro injection (IKAPHARMINDO), quercetin (Merck), 96% ethanol (Merck), AlCl_3 (Phydeu Medika), CH_3COOK (Maxlab), ethyl acetate (Merck), and n-hexane (Merck).

Detailed Procedure

Preparation of Carrot Extract

The obtained carrots were cleaned, cut into small pieces, and then dried under sunlight followed by oven drying at 50°C. Once dried, the carrots were ground into a fine powder to produce simplicia and weighed. A total of 418.503 g of powdered simplicia was extracted using the maceration method with 96% ethanol in a ratio of 1:10, resulting in a total volume of 4.21 L of 96% ethanol used. The maceration process was carried out for 3 days at room temperature with occasional stirring. Afterward, the macerate was filtered to separate the extract from the residue, which was then remacerated using the same solvent and duration. All collected macerates were evaporated using a rotary evaporator, followed by heating in a water bath at 60°C until a thick, solvent-free extract was obtained. The extract yield was calculated as the ratio of extract weight to the weight of the dried simplicia.

Fractionation

A total of 100 g of carrot ethanol extract was dissolved in ethanol at a 1:1 ratio and stirred until fully dissolved. Subsequently, n-hexane was added in a 1:1 ratio, and the mixture was partitioned using a separating funnel, shaken gently until the solution became clear. The partitioning process produced two layers: the n-hexane fraction and the extract layer. The extract layer was then further extracted using ethyl acetate at a 1:1 ratio, resulting in the ethyl acetate fraction and the aqueous fraction. The three obtained fractions — n-hexane,

ethyl acetate, and water — were then evaporated using a water bath until thick extracts were obtained. These fractions were subsequently used for further analysis.

Identification of Total Flavonoid Compounds (Colour Test)

A total of 100 mg of the ethanol extract fraction sample from carrot was dissolved in 10 mL of solvent, then filtered and placed into a test tube. Two drops of hydrochloric acid (HCl) and powdered magnesium metal were added, followed by observation of any colour change. A positive result for flavonoids is indicated by a colour change to dark red.

Determination of Total Flavonoid Content

A quercetin standard solution was prepared by weighing 25 mg of quercetin and dissolving it in a 250 mL volumetric flask using analytical grade ethanol (ethanol p.a.) up to the mark, resulting in a 100 ppm solution. For wavelength determination, 6 mL of the 100 ppm quercetin standard solution was pipetted into a 10 mL volumetric flask, followed by the addition of 0.4 mL of 10% AlCl_3 , 0.4 mL of 1 M CH_3COOK , and distilled water up to the 10 mL mark, yielding a 60 ppm quercetin solution. The absorbance of this solution was measured over the wavelength range of 350–500 nm. The same procedure was used to determine the operating time, with the optimal stable time found to be 60 minutes. A standard calibration curve series was prepared by diluting the 100 ppm stock solution to concentrations of 10, 20, 30, 40, 50, and 60 ppm, each placed in a 10 mL volumetric flask. To each, 0.4 mL of 10% AlCl_3 , 0.4 mL of 1 M CH_3COOK , and distilled water were added up to the mark. The solutions were mixed thoroughly and incubated at room temperature for 30 minutes before measurement using a UV-Vis spectrophotometer. For the flavonoid content measurement, 100 mg of each fraction, n-hexane, ethyl acetate, and aqueous, was weighed and dissolved in a 10 mL volumetric flask with analytical grade ethanol. From these solutions, 100 μL of the n-hexane fraction, 500 μL of the ethyl acetate fraction, and 4 mL of the aqueous fraction were each taken and added with 0.4 mL of 10% AlCl_3 , 0.4 mL of 1 M CH_3COOK , and distilled water up to 10 mL. The mixtures were then left to stand for the previously determined operating time and measured at the maximum wavelength of quercetin. Absorbance measurements were performed in triplicate.

Data Analysis

Statistical analysis in this study was performed using SPSS version 27.0. The total flavonoid content data from the n-hexane, ethyl acetate, and aqueous fractions of the carrot ethanol extract were first tested for normality using the Shapiro-Wilk test and for homogeneity using Levene's test. Subsequently, the analysis proceeded with a parametric one-way ANOVA. However, if the data did not meet these assumptions, a non-parametric Kruskal-Wallis test was employed instead.

RESULT AND DISCUSSION

Extraction

The result of the maceration of carrot weighing 418.503 g with 96% ethanol solvent can be seen in Table 1. In this study, the extraction process of carrot (*Daucus carota* L.) was carried out using the maceration method, a technique that separates compounds by soaking the sample in an organic solvent at a certain temperature. This method is considered advantageous due to its relatively low cost, simple procedure, and ease of implementation (4). 96% ethanol was chosen because it is universal, polar, readily available, and has high

selectivity, good absorption capacity, and high extraction efficiency, enabling it to extract compounds with various polarity levels, including non-polar, semi-polar, and polar (5). The extraction yield was calculated as the percentage ratio of the dry extract weight to the initial sample weight, where a high yield indicates a high content of bioactive components (6). Based on the results of this study, the ethanol extract yield of carrot was 43.72% w/w, which meets the criteria for a good thick extract yield, i.e., not less than 10% (7). Compared to the journal (8), where carrot extraction at a ratio of 1:4.5 yielded 18.107%, this study's results are considered better as they produced a higher yield, indicating a greater potential content of bioactive compounds, including flavonoids.

Table 1. Yield Results of Carrot Ethanol Extract

Simplicia	Dry Simplicia Weight (grams)	Weight of 96% Ethanol Extract (grams)	Yield (%) (w/w)
Carrot (<i>Daucus carota</i> L.)	418.503	182.855	43.72

Fractionation

The results of the fractionation of carrot ethanol extract in the n-hexane, ethyl acetate, and aqueous fractions can be seen in Table 2.

Table 2. Yield Results of the Fractions from Carrot Ethanol Extract

96% Ethanol Extract	Weight of Thick Extract (grams)	Fractions	Weight of Thick Fraction (grams)	% Yield (w/w)
Carrot (<i>Daucus Carota</i> L.)	100	N-Hexane	2.18	2.18
		Ethyl Acetate	2.63	2.63
		Water	16.73	16.73

In this fractionation process, three types of solvents were used, n-hexane, ethyl acetate, and water, selected to separate compounds based on their polarity in order to enhance selectivity and facilitate the identification and evaluation of the biological activity of each fraction (9). The solvents were chosen sequentially from non-polar to polar, so that non-polar secondary metabolites could be extracted first using non-polar solvents, as polar solvents have a broader range and are capable of extracting compounds across all levels of polarity. This fractionation technique was carried out using a liquid-liquid extraction method through shaking, which relies on differences in solvent polarity and density. Based on the results, the yield of the concentrated extract was 2.18% w/w for the n-hexane fraction, 2.63% w/w for the ethyl acetate fraction, and 16.73% w/w for the aqueous fraction. The higher yield of the aqueous fraction compared to the others is attributed to the more polar nature of water, which allows it to extract a greater number of polar compounds from carrots, such as sugars, organic acids, and pigments (α -carotene and β -carotene), which are polar or semi-polar in nature (10).

Identification of Total Flavonoid Compounds

The identification of total flavonoid compounds was carried out using the Wilstater-Cyanidin method by adding magnesium powder and concentrated hydrochloric acid, with the resulting colour change observed as shown in Table 3.

Table 3. Table of Flavonoid Compound Identification

Sample	Reagent	Result	Explanation
N-hexane Fraction	Magnesium powder + concentrated HCl		Deep red +++
Ethyl Acetate Fraction	Magnesium powder + concentrated HCl		Orange ++
Aqueous Fraction	Magnesium powder + concentrated HCl		Orange ++

Explanation

(+) : Positive for flavonoid content

(-) : Does not contain flavonoids

In this study, a colour change to deep red was observed, indicating the formation of flavylium salts as a result of the reduction of the benzopyran core in the flavonoid structure by magnesium powder and concentrated hydrochloric acid. This deep red colour serves as a positive indicator of the presence of flavonoids. In the ethyl acetate and aqueous fractions, the resulting colour was orange, which also indicates the presence of total flavonoids, as the magnesium powder acts as a reducing agent in a redox reaction with flavonoids, producing flavylium salts that range in colour from orange to red. These results indicate that each fraction contains flavonoid compounds. This is consistent with the findings of (11), where the identification of flavonoids using HCl resulted in a colour change from orange to brick red as a positive indicator. It is also supported by the journal (12), which reported that the Wilstätter test using magnesium powder and concentrated HCl produced

a reddish-yellow colour change due to the formation of benzopyrylium salts from the reduction of flavonoid polyhydroxy groups by magnesium in a hydrochloric acid solution.

Total Flavonoid Content Analysis

Determination of the Maximum Wavelength of Quercetin

The UV-Vis spectrum of quercetin at a concentration of 60 ppm shows a maximum wavelength of 438 nm with an absorbance of 0.611. In this study, the wavelength obtained was 438 nm, which falls within the range specified in journal (13), stating that the wavelength for total flavonoid analysis lies within the relevant range of 400–800 nm.

Determination of Stability Time (Operating Time)

The stable time required for the reaction between quercetin, AlCl_3 reagent, and CH_3COOK was achieved between the 26th and 32nd minute, with maximum stability observed at the 30th minute.

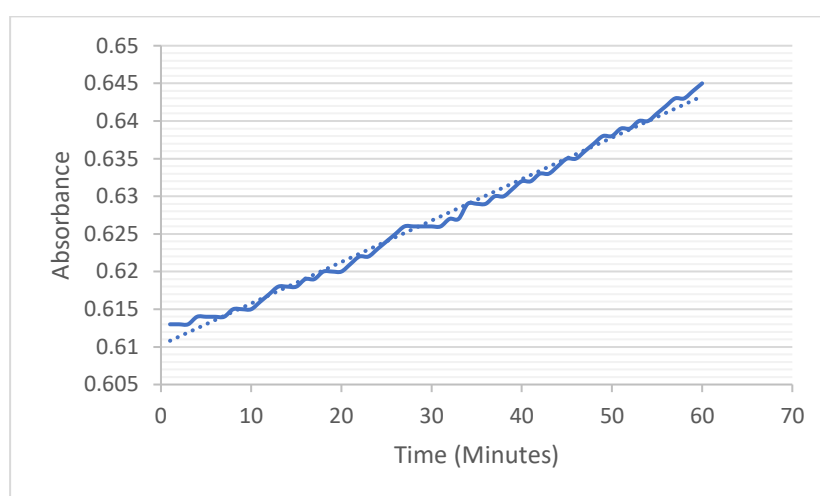


Figure 1. operating time

The operating time aims to determine the optimal measurement time of a compound, specifically when the absorbance value is at its most stable. In this research, the operating time was determined to be at the 30th minute, indicating compliance with the requirements.

Quercetin Standard Curve

Total flavonoid content was determined using a series of concentrations of 10, 20, 30, 40, 50, and 60 ppm. The results can be seen in Table 4.

Table 4. Absorbance Measurement Results of Quercetin Standard Solution

Number	Concentration	Average Absorbance
1	10	0,254
2	20	0,338
3	30	0,462
4	40	0,590
5	50	0,697
6	60	0,875

From the data, a standard curve was obtained with the equation $y = 0.0125x + 0.099$ and a coefficient of determination (R^2) of 0.9921, which was used to calculate the total flavonoid content.

This concentration range was chosen based on Lambert-Beer's Law, which states that the appropriate absorbance values should fall within 0.2–0.8. This is intended to minimize photometric errors, thereby keeping analytical error within the acceptable range of 0.5–1% (14). The determination of total flavonoid content in the ethanol extract of carrot for the n-hexane, ethyl acetate, and aqueous fractions was carried out using colorimetry, with aluminium chloride ($AlCl_3$) as the reagent. The principle of this colorimetric method is based on the formation of a complex between $AlCl_3$ and flavonoid compounds. $AlCl_3$ forms a stable acid complex with the keto group at position C-4 and the hydroxyl group at position C-3 or C-5 of flavones and flavonols (15).

Determination of Total Flavonoid Content

The results of the determination of total flavonoid content in the n-hexane, ethyl acetate, and aqueous fractions can be seen in Table 5.

Table 5. Results of Total Flavonoid Content Determination

Fraction	Total Flavonoid Content (mg QE/g)			Average	SD	RSD
	R1	R2	R3			
N-Hexane	278.37	260.00	264.74	267.70	9.536	3.57
Ethyl Acetate	30.09	31.04	31.84	30.99	0.876	2.82
Water	4.72	4.77	4.87	4.78	0.076	1.60

The total flavonoid content obtained in this study was, respectively: 267.70 ± 9.53 mg QE/g in the n-hexane fraction, 30.99 ± 0.87 mg QE/g in the ethyl acetate fraction, and 4.78 ± 0.076 mg QE/g in the aqueous fraction, with the highest flavonoid content found in the n-hexane fraction. The n-hexane fraction showed the highest concentration because n-hexane is a non-polar solvent that effectively dissolves flavonoid compounds which are also non-polar or weakly polar, such as certain types of flavonoid aglycones, in accordance with the principle of "like dissolves like" (16). The non-polar flavonoid content in the ethanol extract of carrot is more readily soluble in n-hexane, and this is supported by the chemical structure of flavonoids, which often contain methoxy groups or aromatic rings that are soluble in non-polar solvents. The stepwise fractionation following the initial ethanol extraction also plays a role in separating compounds based on their polarity, allowing the n-hexane fraction to retain a greater quantity of non-polar compounds. Furthermore, journal (9) also reports that the highest flavonoid content is found in the n-hexane fraction. Although flavonoids are generally considered polar due to their glycoside forms, certain types such as polymethoxylated flavonoids or isoflavones may undergo the removal of glycone groups, resulting in a more non-polar structure that is more soluble in n-hexane.

Data Analysis

The flavonoid content results were then analysed using statistical analysis, which can be seen in Table 6. The research data were subsequently analysed using statistical analysis, specifically One-way ANOVA. Prior to applying this test, it was necessary to assess the normality and homogeneity of the data. If the data are found to be non-normal and non-homogeneous, a non-parametric test such as the Kruskal-Wallis test is required. In this study, the normality test produced a result of p-value > 0.05 , indicating that the data were normally distributed. However, the homogeneity test resulted in a p-value < 0.05 , indicating that the data were not homogeneous. Therefore, although the data were normal, the lack of homogeneity necessitated the use of a non-parametric test, namely the Kruskal-Wallis test. The Kruskal-Wallis test produced a result of $p = 0.027$,

meaning $p < 0.05$, which indicates a significant difference in flavonoid concentrations among the n-hexane, ethyl acetate, and aqueous fractions.

Table 6. Results of Normality, Homogeneity, and Kruskal-Wallis Tests

Fraction	Normality Test (Shapiro-Wilk)	Homogeneity Test (Levene's Test)	Kruskal-Wallis
N-Hexane	0.480	0.015	0.027
Ethyl Acetate	0.906		
Water	0.637		

Note:

p-value > 0.05 = data is normally distributed

p-value > 0.05 = data is homogeneous

p-value < 0.05 = data shows a significant difference

CONCLUSION

Based on the study, it can be concluded that the total flavonoid content obtained was 267.70 ± 9.54 mg QE/g for the n-hexane fraction, 30.99 ± 0.87 mg QE/g for the ethyl acetate fraction, and 4.79 ± 0.076 mg QE/g for the aqueous fraction. The highest total flavonoid content was found in the n-hexane fraction. Statistical analysis showed that there were significant differences in total flavonoid content among the n-hexane, ethyl acetate, and aqueous fractions.

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