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Total Flavonoid Content (TFC) and Antioxidant Activity of Carrot Extract Isolate (*Daucus carota* L.)

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Abstract: Antioxidants are antiradical compounds that can neutralize reactive free radicals into non-reactive ones that are relatively more stable so as to protect cells from the harmful effects of free radicals. Carrots have antioxidant activity due to the presence of carotenoid compounds. This study aims to determine the antioxidant activity of carrots. Extraction research method with maceration method. Isolation of flavonoids by column chromatography method. Antioxidant activity test using FRAP method. The results of research conducted from 60 extract fractions obtained 10 flavonoid fractions with a %TFC value 5,332%; 5,242%; 6,742%; 7,475%; 6,829%; 5,896%; 5,282%; 6,191%; 5,450%; and 5,498%. IC50 3,920; 1,960; 2,096; 2,065; 1,846; 0,867; 3,517; 1,179; 3,383; dan 3,570 ppm. In conclusion, the antioxidant activity of the ten fractions was very strong (<50 g/mL).

Keywords: Carrot, Isolate, Antioxidant, FRAP, TFC

INTRODUCTION

Antioxidants are antiradical compounds that can neutralize reactive free radicals into non-reactive which is relatively more stable so that it can protect cells from the harmful effects of free radicals (Soebagio et al., 2007). Carrots (Daucus carota L.) contain several chemical compounds such as phenolics, carotenoids, polyacetylene and ascorbic acid. They are bioactive compounds known for their dietary supplements and health benefits. These chemicals help prevent cancer and cardiovascular disease with antioxidants, anti-inflammatory, plasma lipid modification, and effectiveness as antitumor (Maria et al., 2017).

The total flavonoid content is measured based on the presence of quercetin in plant extracts. Analysis of flavonoid content is carried out with the addition of FeCl3 reagent. As a Lewis acid, AlCl3 will form complex bonds with the hydroxyl groups of flavonoid compounds. This change is identified through absorbance in the visible light region through a spectrophotometer (Neldawati, 2013).

The FRAP method is a simple, fast method, with the reagents used quite simple and does not use special tools to calculate the total antioxidants. This method is based on the increase in absorption of the reaction mixture. An increase in uptake indicates an increase in antioxidant activity. In this method antioxidants form colored complexes with potassium ferricyanide, trichloroacetic acid, and iron(III) chloride measured at a wavelength of 700 nm. The increase in the absorption of the reaction mixture indicates the reducing power of the sample (Amelia, 2011). Based on this description, so researchers are interested in conducting research to analyze the antioxidant activity of carrots (Daucus carota L.).

METHODS

Type and Design of Research

The type of research used in this study is experimental research. This research was conducted at the Laboratory of pharmaceutical technology and pharmaceutical biology STIKES Telogorejo Semarang. This study was conducted to determine the Total Flavonoid Content (TFC) and antioxidant activity of carrot extract isolate (Daucus Carota L.).

Research Tools and Materials

The tools used in this study were filter paper, silica gel GF254, ointment pot packaging, cuvettes, drip pipettes, volume pipettes, glassware [pyrex, pyrex], pyrex, chamber, stirring rod, porcelain cup, glass funnel, test tube (Pyrex), tube rack, mortar, stamper, measuring cup, water bath, maceration vessel, object glass, watch glass, pH indicator, scaled glass, analytical balance (Ohaus), chromatographic column, adhesion test equipment, rotary evaporator (DLAB RE 100-pro), incubator, oven, and UV-VIS spectrophotometer (Shimadzu 190).

The material used is carrot (Daucus carota L.) obtained from UD. Carrot nutrition located in Jimbaran Village, Bandungan, Semarang Regency, Virgin Coconut Oil (VCO) obtained from the cottage industry of Islamic College Tremas Islamic Boarding School Pacitan East Java, stearate acid, tea, tween 80, span 80, cetyl alcohol, liquid paraffin, glycerin, methyl paraben, propyl paraben, aquadest, C_6H_{14} (n-hexane), CH_3OH (methanol), $AlCl_3$ (aluminum chloride), KCH_3COO (potassium acetate), phosphate buffer (pH 6.6), 1% potassium ferricyanide solution, 10% TCA (Trichloroacetic acid) solution, 0.1% FeCl3 (ferric chloride), concentrated Mg (magnesium) powder, concentrated HCl (hydrogen chloride), 2N HCl (hydrogen chloride), 70% C_2H_5OH (ethanol), dragendroff reagent (bismutsubnitrate and potassium iodide), libermann burchad reagent [CH_3COOH (anhydrous acetic acid) and H_2SO_4 (concentrated sulfuric acid)], sulfuric acid vanillin reagent, and quercetin raw.

Carrot extraction (Daucus Carota L.)

From 4 kg of carrots, simplisia weighing 150 g was obtained, from 150 g of simplisia, 1.6 g of extract was obtained with a yield of 1.06% (Hasrawati, 2019). Carrot simplisia powder weighed 1 kg and then put in a maceration vessel and macerate with n-hexane solvent 1: 5 (Anggraeni et al, 2020). Soaking is carried out for 84 hours, this time is the time of saturation of solvents to extract beta-carotene compounds (Sarindang, 2018), then re-maceration is carried out for 24 hours. Then the maserat results are concentrated using a rotary evaporator and continued with concentration in a vapor dish above the water bath then count the yield value

Phytochemical screening

Phytochemical screening was carried out on flavonoids, alkaloids, tannins, and saponins using color reaction tests and thin-layer chromatography (TLC) tests.

Isolation of Flavonoid Compounds

The most optimal eluent results from the TLC test are then used for the separation of flavonoid compounds by column chromatography. Preparation begins by activating 50 g of silica gel powder by heating in an oven at 110ºC for 60 minutes and then dissolving with eluents until it forms like a slurry. The silica gel slurry is slowly inserted into the column while stirring so that there is no air cavity in the middle of the column, then the eluent is added and the top of the column is covered with aluminum foil during saturation. Next, the column is left overnight (Mabruroh et al., 2019). A total of 2 grams of extract samples were dissolved in 3 ml of methanol and then put into a saturated column. The fraction is accommodated in vial bottles. The chromatographic process is stopped after all metabolites are thought to have been eluted. (Asih et al., 2015).

***Total Flavonoid Content* (TFC)**

Samples of the extract were diluted with methanol to 100 ppm. The calibration curve is made by diluting quercetin in methanol (10, 15, 20, 25 and 30 ppm). Aqueous extract or quercetin (2.0 mL) was mixed with 0.1 mL of 10% aluminum chloride solution (w/v) and 0.1 mL of 0.1 mM potassium acetate solution. The mixture is kept at room temperature for 30 minutes. Maximum absorbance measured at 415 nm using UV-Vis spectrophotometer (Do *et al*., 2014).

FRAP method antioxidant activity test

Samples were prepared with a concentration of 1000 ppm dissolved with ethanol as the parent solution, then made concentration series (2, 4, 6, 8, and 10 ppm). Next, 0.5 ml of each was taken and then added with 2.5 mL of phosphate buffer pH 6.6 (0.2 M) and 0.5 mL of 1% potassium ferricyanide solution. The mixed solution was incubated at 50°C for 20 minutes and then supplemented with 0.5 mL of 10% TCA (Trichloroacetic acid) solution. When two layers occur, it is centrifuged (3,000 rpm for 10 minutes). The top layer is taken as much as 0.5 mL added 0.5 mL aquades and 0.5 mL FeCl3 0.1%. The solution mixture was then measured at a wavelength of 700 nm.

Method validation

Precision

The sample was measured 3 times. From the results of sample measurements, data processing is carried out through statistical calculations so that cv% is obtained to obtain precision. Account:

Presisi $= \frac{SD}{X}$ x 100%

Accuracy

The sample was measured 3 times. From the results of sample measurements, data processing is carried out through statistical calculations so that %recovery is obtained to obtain accuracy. Account:

%Recovery $= \frac{Cf}{CA}$ x 100%

Linearity

To determine linearity, a standard with 5 different concentrations is made. The measurement results are made a curve that shows the relationship between concentration and absorption, then a simple linear regression test and correlation test are carried out to calculate the linear coefficient. Linear regression equation :

y = bx + a (Miller, 2010).

RESULT AND DISCUSSION

Preparation of carrot extract (Daucus carota L.)

20 kg of fresh carrots are washed clean, then cut crosswise with a thickness of 1 cm, then drying is done by oven with a temperature of 50^{o}C for 32 hours, then 1,249 g of dry simplisia is obtained, then grinding is carried out using a grinder until simplisia powder is obtained, simplisia powder is sifted with sieve number 40. The powder obtained is 1,823 g. Extraction is carried out by maceration method by weighing 1kg of simplisia powder then soaked using 5 L of n-Hexan for 84 hours because that time is the time of solvent saturation to extract beta-carotene compounds (Sarindang, 2018) with occasional stirring then remaceration for 24 hours to maximize the results of digestion. After obtaining maserat then concentrated using a Rotary Evaporator with a temperature of 50^{o}C then concentrated again on a waterbath with a temperature of 50^{o}C so that a thick extract of 19 g was obtained so that the yield obtained was 1.9%.

Phytochemical screening

Flavonoid testing on carrots extract showed positive results (contains flavonoids). This is shown in the results of the color reaction formed in red. These results are in accordance with research conducted by Hasrawati (2019) with the results of testing flavonoid compounds on n-Hexan extract of carrot positive for flavonoid compounds.

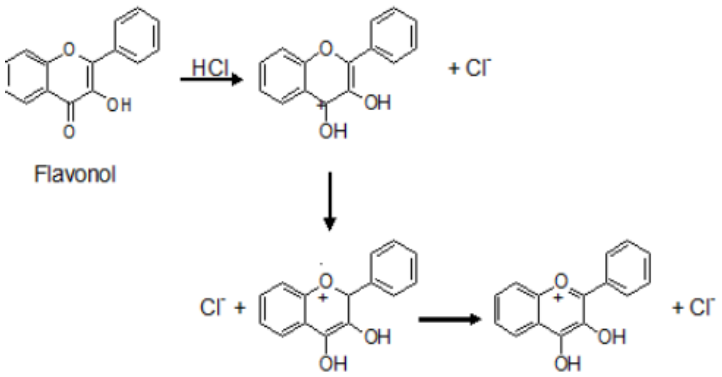


Figure 1. Chemical Reactions of Flavonoid Test

Saponin testing on carrot extract showed positive results (containing saponins). This is shown by the formation of stable foam after adding water and shaken. These results are in accordance with research conducted by Hasrawati (2019) with the results of testing saponin compounds on n-Hexan extract of carrot positive for saponin compounds. The reaction of foam formation and the color test results of saponin compounds can be seen in figure 2.

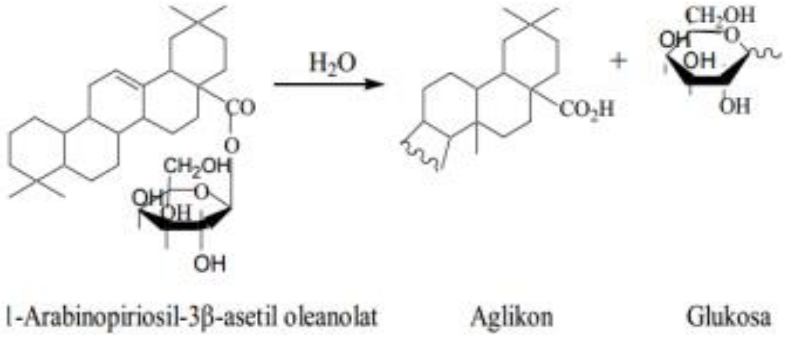
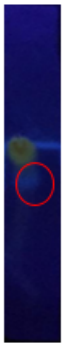


Figure 2. Chemical Reactions of Saponin Test

The results of the alkaloid and tannin tests on carrot extract (Daucus carota L.) showed negative results so that the Thin Layer Chromatography did not form stains, while the flavonoid eluent test used was n-Hexan: ethyl acetate (6: 4) showed positive results with the occurrence of greenish-yellow color after spraying the appearance of citroborate spots and ovened at a temperature of 110oC for 5 minutes which aimed to emphasize the color then observed under 366nm UV light. The results of the expansion of flavonoid compounds obtained an Rf value of 0.688. These results are in accordance with the existing literature where after heating in a 110oC oven for 5 minutes gives a yellow color (Pramono, 1989).

In the eluent saponin test used is N Butanol: Water (1: 1) shows positive results with the occurrence of fluorescent blue color after spraying the appearance of citroborate spots observed under 366nm UV light, these results are in accordance with the existing literature where the results are tested positive if there are fluorescent blue spots with an Rf range of 0.4-0.66 (Sopianti & Sary, 2018).

Table 1. Thin Layer Chromatography Test Results

compound	Rf (cm)	Before spraying			After spraying			Reference	Conclusion
		Visual	UV 254 nm	UV 366 nm	Visual	UV 254 nm	UV 366 nm		
flavonoid	0,425	- 	yellow 	Blue 	Yellow 	Yellow 	purple 	**quercetin UV 366nm** Fluorescent purple **Rf** 0,538	Positive
Saponin	0.625	- 	- 	Blue 	-	-	-	**UV 366nm** fluorescent blue Rf 0,4-0,66 (Sopianti & Sary,2018).	Positive

Isolation of flavonoids by column chromatography

From the separation of 2g of samples by column chromatography yields 60 fractions. Then layer chromatography was carried out with the mobile phase n-Hexan: ethylacetate (6: 4) KLT test results which showed purple single spots then the KLT results were adjusted to the KLT results on quercetin standards with the Rf obtained obtained 10 fractions that had Rf values close to the Rf values of quercetin standards, namely with quercetin standard Rf values were 0.538 and RF values in vials number 33-42 sequentially 0.475; 0,5; 0,538; 0,525; 0,475; 0,463; 0,538; 0,5; 0,475; and 0.438.

Total Flavonoid Content (TFC)

The results of measuring the fraction of carrot n-hexan extract from 10 fractions that showed positive flavonoid results in KLT obtained absorbance of 0.333 each; 0,323; 0,511; 0,603; 0,522; 0,405; 0,328; 0,442; 0,349; and 0.355 then from the absorbance results calculated based on the %TFC formula, the %TFC value of each fraction is 5.332%; 5,242%; 6,742%; 7,475%; 6,829%; 5,896%; 5,282%; 6,191%; 5,450%; and 5.498%.

Table 2. Total Flavonoid Content (TFC)

Number fraction	Absorbance	%TFC
33	0,333	5,332%
34	0,323	5,242%
35	0,511	6,742%
36	0,603	7,475%
37	0,522	6,829%

38	0,405	5,896%
39	0,328	5,282%
40	0,442	6,191%
41	0,349	5,450%
42	0,355	5,498%

FRAP antioxidant activity test

In determining the maximum wavelength in this study, a maximum wavelength of 688.8 was obtained. In this study, an average IC_{50} value of 10 fractions of 0.867-3.920ppm was obtained. which shows that carrot tubers (Daucus carota L.) have very strong antioxidant activity, which is shown by the result of IC_{50} value <50 µg / mL. The IC_{50} value is said to be very strong if it has a value of <50 µg / mL, Strong if it has a value in the range of 50-100 µg / mL, medium if it has a value in the range of 100-150 µg / mL, and weak if it has a value in the range of 151-200 µg / mL (Purwanto *et a*., 2017).

Table 3. IC Value 50 Antioxidant Activity Test

nomor	Y=bx+a			IC_{50}			Average IC_{50}
	1	2	3	1	2	3	
33	y = 16,212x - 13,827	y = 15,959x - 12,546	y = 15,026x - 8,4617	3,922	3,904	3,935	3,920
34	y = 7,8806x + 32,422	y = 7,0529x + 36,012	y = 6,7035x + 36,865	2,199	1,663	2,018	1,960
35	y = 8,6927x + 32,09	y = 9,0944x + 30,646	y = 9,0005x + 30,988	2,032	2,101	2,155	2,096
36	y = 8,7936x + 31,95	y = 8,8332x + 31,574	y = 8,6407x + 32,896	2,024	2,058	2,114	2,065
37	y = 6,8361x + 36,011	y = 6,5699x + 38,404	y = 6,4795x + 37,925	2,010	1,727	1,803	1,846
38	y = 5,9437x + 43,828	y = 5,1757x + 45,848	y = 5,6271x + 43,036	0,996	0,754	0,850	0,867
39	y = 13,068x + 2,9251	y = 13,097x + 4,3712	y = 12,968x + 4,8093	3,583	3,465	3,503	3,517
40	y = 6,1666x + 42,732	y = 6,1635x + 42,602	y = 5,6748x + 44,228	1,138	1,160	1,241	1,179
41	y = 13,928x + 2,4556	y = 13,952x + 2,8974	y = 13,495x + 4,6051	3,396	3,358	3,394	3,383
42	y = 11,675x + 8,2317	y = 11,636x + 8,3799	y = 11,634x + 8,7377	3,556	3,555	3,598	3,570

%TFC value against IC_{50} per fraction

High total flavonoid levels will result in lower IC_{50} so that antioxidant activity becomes stronger (Hilma et al., 2020). Based on the test results in this study, the results were not in accordance with the literature, where the highest %TFC was fraction number 36%TFC 7.475% with IC_{50} 2.065ppm. The lowest IC_{50} value or very strong antioxidant activity is fraction number 38 which shows an IC_{50} value of 0.867 ppm with %TFC 5.896%. The ability of flavonoids and phenols as antioxidants is likely due to the presence of hydroxy groups in its basic framework (Asih et al., 2015). The antioxidant activity of phenol and flavonoid components by reducing free radicals depends on the number of hydroxy groups in the molecular structure so that there is a relationship between the structure of flavonoid and phenol compounds on antioxidant activity (Zuraida et al., 2017). Based on this research so that this is thought to be caused because flavonoids do not have much effect on the IC_{50} value, which affects the IC_{50} value is phenol compounds. This flavonoid compound is included in the phenol group but all phenols are not included in the flavonoid group so it is suspected that this phenol compound has a considerable contribution in antioxidant activity in carrot extract isolate.

CONCLUSION

The results of testing antioxidant activity using the FRAP method on the isolation fraction are very strong with the IC_{50} value obtained is 0.867-3.920ppm.

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