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Analysis of Total Phenolic and Flavonoid Levels in Carrot (*Daucus carota* L.) Extract with Different Solvents Polarity

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Abstract

Carrot (*Daucus carota* L.) contains phenolic and flavonoid compounds that contribute to various pharmacological activities. Phenolic and flavonoids can be extracted using various solvents. Solvent polarity difference will produce amounts and types of secondary metabolites differently. This research aims to compare the total phenolic and flavonoid content of carrot extract from various solvents. Carrot was extracted using the maceration method with n-hexane, ethyl acetate, and 70% ethanol as solvent to obtain n-hexane, ethyl acetate, and 70% ethanol extract of carrot. Spectrophotometry carried out the determination of total phenolic content by comparing gallic acid and total flavonoids with a comparison of quercetin. The maximum wavelength of gallic acid is 739.60 nm and the operating time of 120 minutes. The maximum wavelength of quercetin is 427.50 nm and an operating time of 30 minutes. Total phenolic and flavonoid levels of carrot extract from the three solvents were analyzed statistically through one-way ANOVA followed by the Bonferroni test at a 95% confidence level. The results showed that the total phenolic contents of the n-hexane extract, ethyl acetate, and 70% ethanol extract of carrot 11.881±0.31; 24.308±1.33 and 39.398±1.65 mg GAE/g extract. The flavonoid content was 2.650±0.13; 20.675±2.58 and 9.063±0.99 mg QE/g extract respectively. The highest total phenolic content was obtained from 70% ethanol solvent and the highest total flavonoid was obtained from ethyl acetate solvent.

Keywords : Phenolics, Flavonoids, Maceration, Various solvents, *Daucus carota* (L.)

INTRODUCTION

Carrot is a type of vegetable from the Apiaceae family that is often used as food and medicine. Carrot extracted with ethanol solvent have pharmacological activity, namely as an antifungal against the growth of Candida albicans (Kalsum & Ayu, 2019), acetylcholinesterase inhibitors (Nuria et al., 2019), anti-inflammatory (Aulia et al., 2013), anthelmintic for ascariasis (Fathnur et al., 2018), and cytoprotective activity in Vero cells (Ginting et al., 2015). Carrot root n-hexane extract activity as a tyrosinase inhibitor (Hasrawati, 2019). Secondary metabolite compounds that contribute to pharmacological activity include phenolic and flavonoid groups.

Research by Haruna et al. (2020) stated that the ethanol extract of carrot contained phenolic and flavonoid compounds. The n-hexane fraction of the ethanol extract of carrot contains alkaloids, phenolics, triterpenoids, and flavonoids (Anisa, 2020). The compounds contained in carrots include flavonols and flavones, namely myricetin, quercetin, kaempferol, and luteolin (Kyslychenko et al., 2022). The results of the identification of compounds by GC-MS carrot extracted with ethanol, namely compounds 3-hydroxybenzoic acid, p-coumaric acid, caffeic acid, and isovanillic acid (Tiveron et al., 2012).

Differences in solvent polarity can produce different amounts and types of secondary

metabolites. Watermelon fruit determined total phenolic content from several solvents and obtained the highest phenolic content in ethanol solvent compared to methanol, water, and hot water (Chitlange et al., 2019). Tomato skins were determined for total phenolic content using methanol, ethyl acetate, chloroform and n-hexane, the highest phenolic content was methanol (Babbar et al., 2012). Papaya fruit was extracted using methanol, ethanol, chloroform, acetone, and water, the highest total phenolic content was water. (Hassan et al., 2020). The results of determining the total flavonoid content of radish (*Raphanus sativus* L.) extracted with several solvents based on polarity obtained the highest levels of flavonoids in ethyl acetate solvent (Evelin & Pasau, 2019). Based on the description above, a study was conducted to determine the total phenolic and flavonoid levels in carrots (*Daucus carota* L.) extracted using 70% ethanol, ethyl acetate, and n-hexane.

METHODS

Equipment

The equipment used in this study was a set of maceration tools, glassware (Pyrex), magnetic stirrer, 40 mesh sieve, electric scales, moisture balance, dropping pipettes, oven, rotating vacuum evaporator (Heidolph), cuvettes, yellow type, blue type, micropipette (Socorex), and UV-vis spectrophotometer 1800 (Shimadzu).

Material

The materials used in this study were carrot (*Daucus carota* L.) harvested from Magelang, Central Java., Technical n-hexane, technical ethyl acetate, technical 70% ethanol, ethanol p.a (Merck), aqua dest, gallic acid (Sigma), Na_2CO_3, Folin-ciocalteu, Quercetin (Sigma), $AlCl_3$ reagent (Merck), and potassium acetate (Merck).

Method

1. Plant Determination

   The determination was carried out at the Ecology and Biosystematics Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang.

2. Preparation of Carrot Powder

   The carrots to be used are fresh, not pockmarked, not rotten, smooth, and ready to harvest. The carrots that have been wet sorted are then washed using clean water to remove impurities in the carrots after which they are drained. The carrots were cut obliquely to increase the surface area and dried in an oven at 40°-50°C. The dried carrots were powdered with a pollinator and then sieved using a 40 mesh sieve and checked for water content using a moisture balance. Requirements for good powder moisture content, namely less than 10% (Depkes, 1985).

3. Carrot Extraction

   Carrot extraction was carried out by maceration using 70% ethanol, ethyl acetate, and n-hexane. Carrot powder was weighed as much as 100 grams and then added 750 mL of each solvent into the maceration vessel. The soaking process was carried out for 3 days while stirring occasionally every 8 hours, then the mixture was filtered to obtain macerate 1. The dregs were soaked again with 70% ethanol, 250 ml of ethyl acetate, and n-hexane for 2 days then filtered again to obtain macerate (2). The results of macerate (1) and macerate (2) are mixed and then concentrated using a rotating vacuum evaporator at 45°C until a thick extract is obtained.

   The yield of carrot extract is calculated using the following formula:

   $$\text{Extract yield} = \frac{viscous\ extract\ weight}{dry\ powder\ weight}\ x\ 100\% \qquad (1)$$

4. Preparation of carrot extract sample solution

   Carrot n-hexane, ethyl acetate, and 70% ethanol extract were weighed as much as 100 mg and then put into a 10 mL beaker glass added with sufficient ethanol p.a. The extract solution was stirred for 1 hour using a magnetic stirrer at 500 rpm, filtered and the filtrate obtained was put in a 10 mL measuring flask and ethanol p.a up to the mark. The concentration of the carrot extract sample solution was 10,000 µg/mL.

5. Determination of Total Phenolic Content

   a. Preparation of 7% Na_2CO_3 solution

   Seven grams of sodium carbonate was weighed and then put into a 10 mL beaker, dissolved with

enough distilled water then put in a 100 mL measuring flask and added distilled water up to the mark.

b. Preparation of 1000 µg/mL gallic acid stock solution

Gallic acid was weighed as much as 100 mg and then dissolved in ethanol p.a sufficiently until dissolved. The solution was put into a 100 mL measuring flask and ethanol p.a was added to the mark and shaken until completely dissolved.

c. Preparation of gallic acid concentration series

The gallic acid stock solution was taken as much as 100, 200, 300, 400, and 500 µL respectively, then put into a 10 mL measuring flask and added ethanol p.a up to the limit mark and shaken until completely dissolved in order to obtain a series of gallic acid concentrations of 10, 20, 30, 40 and 50 ppm.

d. Determination of the maximum wavelength of gallic acid

Gallic acid solution with a concentration of 30 µg/mL was taken 1 mL and then put in a 10 mL measuring flask. The solution was added by 0.4 mL of Folin Ciocalteu and allowed to stand for 8 minutes, then 4 mL of 7% Na_2CO_3 and ethanol p.a were added to the mark, and then shaken until completely dissolved. The solution was poured into a cuvette and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 550-800 nm.

e. Determination of operating time (OT)

The same solution as step (d), was poured into a cuvette and the absorbance was measured using a UV-Vis spectrophotometer at maximum wavelength with measurement times of 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 135, and 150.

f. Preparation of gallic acid standard curve

Gallic acid solution with concentration series of 10, 20, 30, 40, and 50 ppm was taken as much as 1 mL each and then put in a 10 mL measuring flask. The solution was added 0.4 mL of Folin Ciocalteu and allowed to stand for 8 minutes, then 4 mL of 7% Na_2CO_3 and ethanol p.a were added to the mark, and then shaken until completely dissolved. The absorbance of the solution was read using a UV-Vis spectrophotometer at the maximum wavelength and operating time.

g. Determination of total phenolic content of carrot extract

The sample solution for n-hexane, ethyl acetate, and 70% ethanol extract of carrot was taken as much as 1 mL and put in a 10 mL measuring flask. The solution was added by 0.4 mL of Folin Ciocalteu and allowed to stand for 8 minutes, then 4 mL of 7% Na_2CO_3 solution and ethanol p.a were added to the mark and then shaken until completely dissolved. The absorbance of the solution was read using a UV-Vis spectrophotometer at the maximum wavelength and operating time. Replication was carried out 3 times. The total phenolic content is expressed as the number of mg of gallic acid (GAE) equivalent in each gram of extract.

6. Determination of Total Flavonoid Levels

a. Preparation of 10% $AlCl_3$ solution

$AlCl_3$ was weighed as much as 500 mg and put into a 5 mL measuring flask and ethanol p.a was added to the mark and then shaken until completely dissolved.

b. Preparation of 1 M potassium acetate solution

Five hundred mg of potassium acetate was weighed and put into a 5 mL measuring flask and ethanol p.a was added to the mark and then shaken until completely dissolved.

c. Preparation of 1000 µg/mL quercetin stock solution

Quercetin as much as 100 mg was weighed and dissolved in ethanol p.a until dissolved in a glass beaker. The solution was put into a 100 mL measuring flask added with ethanol solvent p.a to the mark and then shaken until completely dissolved.

d. Preparation of quercetin concentration series

The quercetin stock solution was taken as much as 5, 10, 15, 20, and 25 µL respectively, then put into a 10 mL measuring flask and added ethanol p.a to the boundary mark and shaken until completely dissolved to obtain a series of quercetin concentrations of 5, 10, 15, 20, and 25 ppm.

e. Determination of the maximum wavelength of quercetin

One mL of quercetin solution with a concentration of 15 µg/mL was taken and then put into a 10 mL measuring flask. Into the solution, 200 µL $AlCl_3$ 10%, 200 µL potassium acetate 1 M, and ethanol p.a were added to the mark and then shaken until completely dissolved. The solution was poured into a cuvette and the absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 400-800 nm.

f. Determination of operating time (OT)

The same solution as Step (e), was poured into a cuvette and the absorbance was measured using a UV-Vis spectrophotometer at a maximum wavelength with measurement times of 5, 10, 15, 20, 25, 30, 45, and 60 minutes.

g. Preparation of quercetin standard curve

Quercetin solution with concentration series of 5, 10, 15, 20, and 25 ppm was taken as much as 1 mL each and then put into a 10 mL measuring flask. Into the solution, 200 µL $AlCl_3$ 10%, 200 µL potassium acetate 1 M, and ethanol p.a were added to the mark and then shaken until completely dissolved. The absorbance of the solution was read using a UV-Vis spectrophotometer at the maximum wavelength and operating time.

h. Determination of total flavonoid content of carrot extract

The sample solution for the n-hexane, ethyl acetate, and 70% ethanol extract of carrots was taken as much as 1 mL and put in a 10 mL measuring flask. Into the solution, 200 µL $AlCl_3$ 10%, 200 µL potassium acetate 1 M, and ethanol p.a were added to the mark and then shaken until completely dissolved. The absorbance of the solution was read using a UV-Vis spectrophotometer at the maximum wavelength and operating time. Replication was carried out 3 times. Total flavonoid content was expressed as the number of mg quercetin (QE) equivalent in each gram of extract.

Data analysis

The absorbance data from the gallic acid and quercetin concentration series were then made into standard curve equations. The standard curve equation is y= bx + a with y = absorbance, x = gallic acid or quercetin content in µg/mL. The absorbance value of the n-hexane, ethyl acetate, and 70% ethanol extract of carrots was entered into the equation of the standard curve of gallic acid and standard curve of quercetin so that the equivalence of phenolic and total flavonoids of carrots (µg/mL) was obtained. Total phenolic and flavonoid levels were calculated using the following formula:

$$TPC/TFC = \frac{C \; x \; Fp \; x \; V}{g} \quad (2)$$

Information:

TPC = Total Phenolic Content
TFC = Total Phenolic Content
C = Sample concentration
Fp = Dilution factor
V = Extract volume (mL)
g = Sample weight used (gram)

Phenolic and flavonoid contents obtained from n-hexane, ethyl acetate, and 70% ethanol extract of carrot were then analyzed using the Shapiro-Wilk/Kolmogorov Smirnov test with a 95% confidence level to see normality while the Levene test to see homogeneity. If the data is normally distributed and homogeneous then it is tested with the Oneway ANOVA test with the Bonferroni test and if the data is not normally distributed or not homogeneous then it is continued with the Kruskal Wallis test to see its significance. Mann-Whitney was used for further tests, to determine differences in various solvents.

RESULTS AND DISCUSSION

Determination of carrot plants aims to determine the identity of these plants and to avoid mistakes in collecting plants to be used in research. The results of the determination stated that the plant samples used in the study were: 1b-2b-3b-4b-6b-7b-9b-10b-11b-12b-13b-14b-16a- (Goal 8. Single leaf plants scattered)-109b- 119b-120b-129b-135b-139b-140b-142b-143b-146a-147a-146a- (Family 98 Umbeliferae (Apiaceae) – (Genus Daucus) – (*Daucus carota*). The results of plant determination showed that the plants used in the study really carrot plants.

Fresh carrots used in the study were 2 kg. Carrots that had been dried were 1.18 kg with a yield of 59% and a moisture content of powder of 7.8%. Based on the drying results, the simplicia is considered good because the water content is below 10% so the quality of the simplicia is maintained (Depkes RI, 1995). Simplisia water content that is too high can result in the growth of microorganisms such as bacteria, fungi, or mold. Low water content simplicia will be more durable to be stored for a

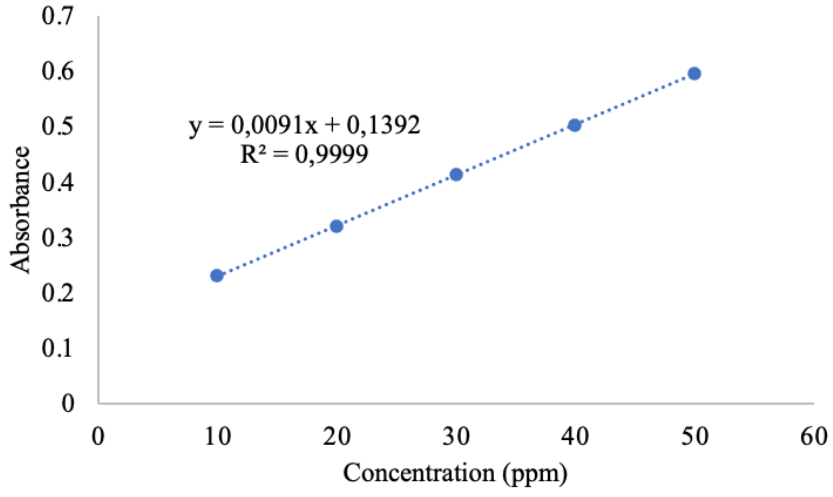
long time. The extraction method used in this study was maceration because it was to extract the active substances in the simplicia, both heat-resistant and non-heat-resistant. The purpose of using three solvents with different polarities (n-hexane, ethyl acetate, and ethanol) is to find out which phenolic and flavonoid compounds are attracted based on their polarity level. During the maceration process, stirring was carried out several times. The goal is to even out the concentration of the solution so that the concentration of the solution inside and outside the cell is maintained (Ahwan, 2018). The second maceration process is called re-maceration, aiming so that the active compounds can be maximally attracted and the desired amount of macerate is obtained. Concentration was carried out using a rotary vacuum evaporator with a temperature of 50 ^{o}C with a rotation of 60 rpm. The active substance must be concentrated at a temperature below its boiling point, which is to avoid the active compound content in carrot tubers which are not damaged by high temperatures (Aslah et al., 2019). The results of each yield of carrot extract obtained can be seen in Table 1.

Table 1. Yield of Carrot Extract from Several Solvents

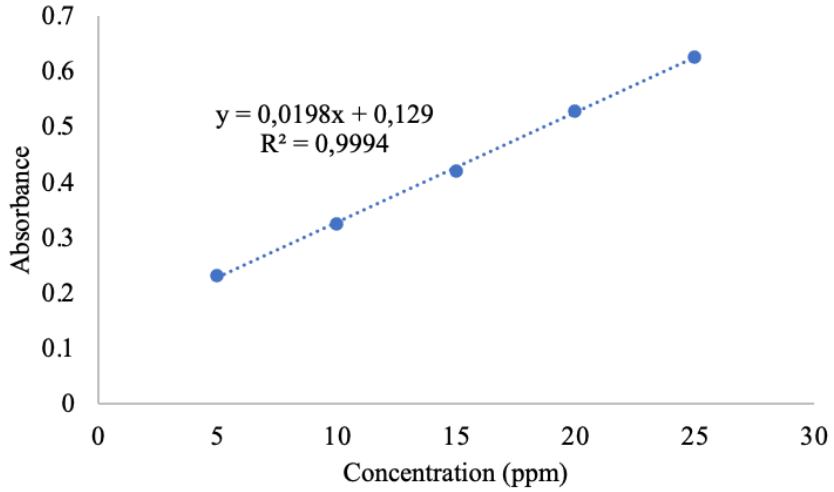
Solvent	Yield (%)
n-hexane	3
Ethyl acetate	3
Ethanol 70%	38

Based on the yield value, it was found that 70% ethanol solvent produced more extract yield than ethyl acetate and n-hexane. This shows that the compounds contained in carrots are maximally attracted to polar solvents. 70% ethanol solvent is a polar solvent. Based on the results of the phytochemical screening of the ethanol extract of carrot, it was found that the ethanol extract of carrot contained phenolic and flavonoid compounds (Haruna et al., 2020). Phenolic compounds tend to dissolve easily in polar solvents because they generally often bind to sugars as glycosides in nature, while flavonoids have several groups, of which the flavonoid group found in carrot tubers, namely the flavonol and flavones groups, dissolves in polar and semipolar solvents.

The maximum wavelength measurement result between gallic acid with folin-ciocalteu reagent and 7% Na_2CO_3 is 739.6 nm. The results of this study were reinforced by Amin's research (2019), namely, the maximum wavelength of gallic acid obtained was 743 nm. Stable absorbance from the gallic acid solution with Folin-Ciocalteu reagent occurred in the 120th minute. These results were reinforced by Amin's research (2019), namely, the operating time of gallic acid was obtained in the 125th minute. The absorbance is said to be stable if the absorbance value at a certain time does not decrease or stays the same. Meanwhile, the maximum wavelength measurement results for the $AlCl_3$ quercetin complex were 427.50 nm. The results of this study are strengthened by research by Safitri et al. (2018) namely the maximum wavelength of quercetin obtained is 428.9 nm. The results of the operating time between quercetin and $AlCl_3$ occurred in the 25th minute. This result is reinforced by Amin's research (2019), namely, the operating time is obtained in the 25th minute. The graph of the standard curve for gallic acid and quercetin can be seen in Figure 1.



(a)



(b)

Figure 1. Standard curve (a) Gallic acid, (b) Quercetin

Determination of the total phenolic content of n-hexane, ethyl acetate, and 70% ethanol extract of carrot aims to determine the amount of total phenolic content contained in carrot extract. Gallic acid is used as a reference solution because it is one of the natural phenolics, is stable and is most often used as a standard in determining the total phenolic content of plant extracts. Gallic acid is a derivative of hydroxybenzoic acid which is classified as a simple phenolic acid (Pamungkas et al., 2017). Determination of the total phenolic content of n-hexane extract, ethyl acetate extract, and 70% ethanol resulted in different average levels according to the polarity of the solvent. The results of determining the total phenolic content of n-hexane, ethyl acetate, and ethanol in 70% of carrot can be seen in Table 2.

Table 2. Determination of Total Phenolic Content of N-Hexane Extract, Ethyl Acetate, and 70% Ethanol Carrot

Solvent	Absorbance value	Dilution	Phenolic content (mgGAE/gram)	Average phenolic content (mgGAE/gram)
n-hexane	0.381	5	11.949	11.881±0.31
	0.373		11.540	
	0.385		11.154	
Ethyl acetate	0.398	10	25.639	24.308±1.33
	0.372		22.979	
	0.385		24.309	
Ethanol 70%	0.535	10	39.662	39.398±1.65
	0.547		40.890	
	0.515		37.615	

Total phenolic data of n-hexane, ethyl acetate, and 70% ethanol extract of carrots were analyzed statistically. Based on the Shapiro-Wilk normality test it shows that the data is normally distributed and the Levene Statistics homogeneity test shows that the variance of the data is homogeneous with a significance value of $p > 0.05$. Then an analysis was carried out using One-Way ANOVA. The test results showed a significance value of 0.000 ($p < 0.05$). Follow-up test using the Bonferroni post hoc test. The Bonferroni test was chosen based on the relatively small number of samples used in the study. The Bonferroni test results obtained with sig 0.000 showed that there were significant differences between all the extract groups, which means that different solvents in the carrot extract produced different phenolic levels.

The results of this study stated that the phenolic compounds in 70% ethanol extract of carrots were soluble in polar solvents because the highest phenolic content was produced in 70% ethanol solvent. It is suspected that the phenolic compounds in the carrots are perfectly extracted with polar solvents. Based on the identification of the ethanol extract of carrots using GC-MS, the compounds obtained were 3-hydroxybenzoic acid, p-coumaric acid, caffeic acid and isovanillic acid. Some of these compounds may be polar so they were maximally attracted to 70% ethanol (Tiveron et al., 2012).

Phenolics are compounds found in all plants, where almost all types of phenols have polar (OH^-) properties. In determining the total flavonoid content, the sample extract solution was added with $AlCl_3$ which can form a complex, resulting in a shift in the wavelength towards the visible which is indicated by the solution producing a more yellow color. The addition of potassium acetate aims to maintain the wavelength in the visible region (Chang et al., 2002). Quercetin is used as a comparison because quercetin can form complexes between $AlCl_3$ and the keto group on the C-4 atom and with the hydroxyl groups on the C-3 or C-5 atoms which are neighbors of flavones and flavonols (Chang et al., 2002). Determination of the total flavonoid content of n-hexane, ethyl acetate, and 70% ethanol extract in carrots aims to determine the amount of total flavonoid content contained in the extract. The results of determining the levels of flavonoids from n-hexane, ethyl acetate, and 70% ethanol extract produced different average levels according to the polarity of the solvent. The results of determining the total phenolic content of n-hexane, ethyl acetate, and 70% ethanol extract of carrots can be seen in Table 3.

Table 3. Determination of Total Flavonoid Content of Carrot N-Hexane Extract, Ethyl Acetate, and Ethanol 70%

Solvent	Absorbance value	Dilution	Flavonoid content (mgGAE/gram)	Average flavonoid content (mgGAE/gram)
n-hexane	0.212	10	2.668	2.650±0.13
	0.214		2.777	
	0.209		2.505	
Ethyl acetate	0.555	10	21.309	20.675±2.58
	0.584		22.885	
	0.491		17.831	
Ethanol 70%	0.336	10	9.407	9.063±0.99
	0.309		7.940	
	0.344		9.842	

Data on total flavonoids from n-hexane, ethyl acetate, and 70% ethanol extract of carrots were analyzed statistically. The results of the Shapiro-Wilk normality test showed that the data were normally distributed and the results of the Levene Statistics homogeneity test showed that the variance of the data was homogeneous with a significance value of $p > 0.05$. Statistical results that the data are normally distributed and have homogeneous data variants are then statistically analyzed using One-way ANOVA. The results of statistical tests showed that the significance value of 0.000 ($p < 0.05$) that the total flavonoid content of n-hexane, ethyl acetate, and ethanol 70% carrot extract had a significant difference with a significance value of $p < 0.05$. The post hoc test was continued using the Bonferroni test. The selection of the Bonferroni test based on the number of samples used in the study is relatively small. Bonferroni test results showed that there were significant differences between all extract groups, which means that different solvents resulted in different levels of total flavonoids. The results showed that the total flavonoid content of carrot with ethyl acetate solvent obtained the highest levels compared to 70% ethanol and n-hexane solvents. This is because the flavonoid compounds in carrot extract are maximally attracted to ethyl acetate. Based on the research of Kyslychenko et al. (2022) the results of the identification of flavonoid compounds in carrots using GC-MS obtained aglycone flavonoid compounds namely quercetin, kaemferol, luteolin, and myricetin. Some of these compounds are aglycone flavonoids which are easily soluble in semi-polar solvents such as ethyl acetate, chloroform, and ether but are insoluble in water (Markham, 1988). The results showed that it was suspected that the aglycone flavonoids (flavones) contained in the ethyl acetate extract of carrot were maximally attracted to semi-polar solvents.

CONCLUSION

The total phenolic content of 70% ethanol extract, ethyl acetate, and n-hexane of carrot (*Daucus carota* L.) were 39.398±1.65; 24.308±1.33 and 11.881±0.31 mgGAE/gram extract while total flavonoids 9.063±0.99; 20.675±2.58 and 2.650±0.13 mgQE/gram extract respectively. Different solvents produce different levels of total phenolics and flavonoids. The highest total phenolic content of carrot was obtained in 70% ethanol solvent and the highest total flavonoids in ethyl acetate solvent.

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