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Comparison of phenolic total ethanol extract of 70% and 96% carrot leaves and antibacterial activity test against Staphylococcus aureus

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ABSTRACT

Carrots (Daucus carota L.) are a plant that is widely used by the community, especially the tubers, while the leaves are not widely used and are only used as waste or animal feed. Carrot leaves contain secondary metabolites such as phenols, flavonoids, tannins, saponins, alkaloids, and steroids. Phenolic compounds are secondary metabolite compounds that are most abundant in nature. This compound can be used in the pharmaceutical world as an alternative treatment from natural ingredients, one of which is as an antibacterial. Staphylococcus aureus bacteria can cause skin infections such as boils. If it enters the bloodstream, it can cause meningitis or lung infections. This study aims to determine the total phenolic content in carrot leaves extracted with 70% and 96% ethanol solvents and to determine their antibacterial activity against Staphylococcus aureus. Carrot leaf extract is obtained by the maceration method. Total phenolic content was calculated using the Follin-Ciocalteau method. The antibacterial activity test was carried out using an excellent method. The research results showed that the yield of 70% ethanol extract was 17.235% and 96% ethanol extract was 16.053%. The results of testing the phenolic content of 70% ethanol extract of carrot leaves were 44.586 mgGAE/g and the phenolic content of 96% ethanol extract was 34.939 mgGAE/g. The results of the antibacterial activity test of 70% ethanol extract of carrot leaves had an average measurement of the inhibition zone at concentrations of 20%, 30% and 40%, namely 0.175 cm, 0.226 cm and 0.274 cm.

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1. Introduction

Carrot leaves (Daucus carota L.) are a plant that is widely found in Indonesia. People only use carrots on their tubers, while leaves are not widely used. Carrot leaves contain secondary metabolite compounds including phenols, flavonoids, tannins, saponins, steroids and alkaloids [1]

Phenolic compounds are the most abundant group of secondary metabolite compounds in plants. Phenolic compounds are broken down into subgroups of phenolic acids, flavonoids, and tannins that are sourced from the number of hydroxyl phenolic groups attached to them as well as the structural elements that connect the benzene rings. Phenolic compounds have pharmacological properties of anti-inflammatory, antioxidant, and antibacterial [2].

Bacteria Staphylococcus aureus is one of the most frequent inflammatory germs in the world. The severity of the infection also varies from minor inflammation of the skin and inflammation of the urinary tract to inflammation of the eyes and the Central Nervous System (CNS) [3]. This problem must be overcome, one of which is by utilizing natural sources of medicine.

This study aimed to determine the total phenol content in carrot leaves with variations in ethanol solvent concentrations of 70% and 96% and to determine the antibacterial activity against Staphylococcus aureus.

2. Method

2.1 Research object

The object of this study is a comparison of the total phenolic levels of 70% and 96% ethanol extract in carrot leaves (*Daucus carota* L.) and an antibacterial activity test against *Staphylococcus aureus,* which is indicated by the formation of a inhibition zone around the well.

2.2 Tools and Materials

The tools used in this study are maceration vessels, visible double beam spectrophotometers, blenders, water baths, glass tools, analytical balances, glass chambers and covers, KLT plates, cuvettes, filter paper, petri dishes, bunsen burners, autoclaves, incubators, round osse, Laminar Air Flow (LAF), cylinder cups, autoclaves, tweezers, calipers, gloves, scissors, matches and cola cloth.

The ingredients used are carrot leaves (*Daucus carota* L.), 70% and 96% ethanol. *Staphylococcus aureus bacterial culture*, ammonia 30%, CHCl3, HCl, Dragendrof reagent, Mayer reagent, Aquadest, HCl P, amyl alcohol, FeCl3, MSA media, NB media, NA media, Ciprofloxacin, Dimethyl sulfoxide (DMSO), sterile aquadest, gallic acid raw material, *Folin-Ciocalteu reagent*, Na2CO3.

2.3 Carrot Leaf Ethanol Extract Manufacturing

A total of 200 grams of carrot leaf powder (*Daucus carota* L.) is put into the maceration vessel, then 70% and 96% ethanol solvents are added. The remaging process is carried out for 3x24 hours while occasionally stirring 3 times. After that, it is filtered and 70% and 96% ethanol solvents are added, then let it sit for 1 x 24 hours occasionally stirring. Then filtered, filtrate 1 and filtrate 2 obtained in a rotary evaporator are then evaporated using a water bath with a temperature of 50°C until a thick extract is obtained [4].

2.4 Phytochemical Screening and Thin-Layer Chromatography of Phenol Compounds.

A sample of 0.5 grams is added 10 mL of aquadest heated in a test tube added with 5% FeCl3. If a blackish-green or blue color is formed, it indicates the positive presence of tannins (Ningsih et al., 2015). Identification by thin-layer chromatography using the n-hexane motion phase: ethyl acetate (2 : 8) and 10% FeCl3 spot visibility. Positive samples contain phenol if they produce green or blackish-blue stains [5].

2.5 Determination of Phenolic Levels

2.5.1 Determining the Operating Time

Pipetted as much as 0.6 mL of 30 ppm medium standard in a test tube. Reagents added *Folin-Ciocalteu* 10% as much as 3 mL and let stand for 3 minutes, add 7.5% Na2CO3 as much as 2.4 mL, then incubate for 30 minutes. Absorbance is read every minute in the range of 0 – 60 minutes with a wavelength of 760 nm [6].

2.5.2 Wavelength Determination

Pipette as much as 0.6 mL of 30 ppm middle standard in a test tube. Added with reagents *Folin-Ciocalteu* 10% as much as 3 mL and let it sit for 3 minutes, add 7.5% Na2CO3 as much as 2.4 mL and let it sit at room temperature for 60 minutes, read the absorption wavelength 400 – 800 nm [7].

2.5.3 Manufacture of Gallic Acid Raw Solution

The parent raw solution of 500 ppm is made by weighing 50 mg of gallic acid put into a 50 mL measuring flask and dissolved with ±0.5 ml of ethanol p.a, ad aquadest to the limit mark, then made with a concentration of 10, 20, 30, 40, 50 ppm.

2.5.4 Determination of Total Phenolic Levels of Carrot Leaf Extract

A sample of carrot leaf ethanol extract weighed 10 mg was put into a 10 mL measuring flask and dissolved with ±0.5 ethanol 70% and 96%, ad aquadest until the limit mark, then pipetted 0.6 mL, added 10% *Folin-Ciocalteu* as much as 3 mL and let it sit for 3 minutes, added 7.5% Na2CO3 as much as 2.4 mL. The mixture was shaken and let stand according to the OT results, measured by the wavelength obtained using a visible spectrophotometer.

2.5.5 Antibacterial Activity Test of Carrot Leaf Ethanol Extract

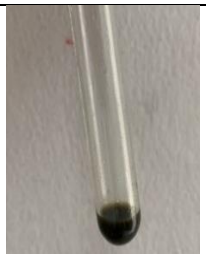
Place the *cylinder cup* on top of the first layer of 10 mL of solidified MSA media, then pour 0.5 µL of *Staphylococcus aureus* suspension mixture into 15 mL of MSA media, and the second layer of MSA media, let it solidify. *The cylinder cup* was lifted, and carrot leaf ethanol extract was inserted at each concentration of 20%, 30%, and 40%, positive dick and negative control. Petri dishes were incubated for 1 x 24 hours at a temperature of 37°C and then the diameter of *Staphylococcus aureus* growth inhibition was measured using a caliper.

3. Results and Discussion

The manufacture of ethanol extract of carrot leaves uses the maceration method. This is a simple method that produces more yields. Carrot leaf extract yield was obtained at 70% ethanol at 17.235% and 96% ethanol at 16.053%. The highest yield is at a concentration of 70%, meaning that more chemical compounds in the sample are absorbed in the solvent. 70% ethanol solvent is more polar compared to 96% ethanol because the water concentration in 70% ethanol solvent is more, which is 30%. Phenolic compounds are polar compounds, so more is concentrated in 70% ethanol, which is more polar [8].

Phenolic compounds can be identified by the FeCl3 reagent forming a blue or blackish-green color. This color occurs due to the reaction of FeCl3 with a hydroxyl group in phenol compounds [9]. Phytochemical screening tests were carried out to determine the phenol compounds contained in the ethanol extract of carrot leaves. The results of the screening can be seen in Table 1.

Table 1. Results of identification of phenol compounds from ethanol extract of carrot leaves

Compound Test	Reagents	Result	Information
Phenolic	Aquadest + FeCl3 5%		(+) A blackish-green solution is formed

	Eluene = n-hexane : ethyl acetate (2:8) Spot spot spot = FeCl3		(+) Several blackish-green stains are formed after spraying with FeCl3. Rf 1=0.93 Rf 5=0.40 Rf 6=0.25

Next is an analysis of total phenolic content in carrot leaf extract using the Follin-Ciocalteau method with visible spectrophotometry instrument. The initial stage of testing is determined by operating time. The purpose of determining the operating time (OT) is to obtain the measurement time when the reaction has run optimally, characterized by stable absorbance, to maximize the measurement [10]. The stage after the determination of OT is carried out, the maximum wavelength measurement is obtained, which is 754 nm. From the results of the measurement of the maximum wavelength, a linear regression of y=0.01187x + 0.0509 with an r-value of 0.9992 was obtained. The result of the total phenolic content in carrot leaf extract is in 70% ethanol extract with a value of 44.586 ±3.009. This is in line with the extraction results that phenol compounds are more concentrated in ethanol 70%. The results of determining the sample level can be seen in Table 2.

Table 2. Results of sample rate determination

Replication	Ethanol 70%		Ethanol 96%	
	Sample weight (mg)	Total phenols (mg GAE/g)	Sample weight (mg)	Total phenols (mg GAE/g)
I	10,4	42,212	9,8	34,051
II	10,1	47,970	10,0	35,560
III	9,9	43,578	10,1	35,208
Average		44,5863,009±		34,9390,790±

The results of the SPSS data test that was carried out, the normality test, with a significance result of 0.437>0.05, show that the data is normally distributed. The homogeneity test obtained a result of 0.080>0.05, stating that the extract was homogeneous. In the results of the independent sample T-test, the results of 0.080>0.05 stated that the data did not differ significantly, meaning that the results of the total phenolic levels obtained from 70% and 96% ethanol were not much different.

Ethanol extract with the largest phenolic content was used to test the antibacterial activity against Staphylococcus aureus using the well method. The results of the activity test are in Table 3.

Table 3. Inhibition zone diameter

Replication	Average Table of Inhibition Zones of Ethanol Extract of Carrot Leaves				
	Inhibition Zone Diameter (cm)				
	20%	30%	40%	Control +	Control-
1	0,103	0,161	0,156	1,053	0
2	0,106	0,158	0,217	1,570	0
3	0,198	0,218	0,232	1,112	0
4	0,200	0,257	0,275	1,116	0
5	0,269	0,337	0,494	1,712	0
Average	0.175±0.071	0.226±0.074	0.274±0.129	1,312±0,305	0±0

From the table above, it can be seen that carrot leaf extract with each concentration has antibacterial power against the growth of Staphylococcus aureus. Based on the measurement of the diameter of the inhibition zone, which is carried out 5 times replication for each sample concentration. The average diameter of the inhibition zone of 20% concentration is 0.175 cm ±0.071, 30% concentration is 0.226 cm ±0.074, 40% concentration is 0.274 cm±0.129. The results of the antibacterial test of carrot leaf extract showed that at the smallest concentration, which is 20%, it provides the smallest inhibition zone, this shows that The higher the concentration of the extract, the greater the size of the diameter of the inhibition zone. The mechanism of phenol compounds as antibacterial is the reaction of hydrogen compounds in phenol compounds and the disruption of the cytoplasmic membrane of bacterial cells [11]. The positive control in this study is Ciprofloxacin, which is known to have antibacterial properties. This control is used to determine the pattern of inhibition activity from the sample, while the negative control is used for dimethyl sulfoxide. This compound is used as a solvent for samples. As a result of the antibacterial activity test, DMSO does not confer an inhibition zone, which means that DMSO as a solvent does not affect the bacterial inhibition activity of the sample.

The normality test aims to determine whether the distribution of data is normally distributed or not. The normality test results of all concentrations showed a Sig value of > 0.05, so the data of all ethanol extracts of carrot leaves were normally distributed. The results of the homogeneity test of all concentrations showed a value of sig. <0.05, the data of all concentrations are not homogeneous. In the non-parametric results of Kruskal-Wallis, the results were 0.310 > 0.05, it was stated that the data were not significantly different, meaning there was no difference in each concentration of ethanol extract of carrot leaves.

4. Conclusion

The yield of 70% ethanol extract of carrot leaves was 17.235% and the yield of 96% ethanol extract was 16.053%. The average phenolic content of total ethanol extract of carrot leaves with a solvent concentration of 70% was 44.587 mg GAE/g of the sample and the solvent concentration of 96% was 34.939 mg GAE/g of the sample. The ethanol extract with the largest total phenolic content is 70% ethanol extract of carrot leaves. The results of the antibacterial activity test of 70% ethanol extract of carrot leaves had an average inhibition zone measurement at concentrations of 20%, 30%, and 40% of 0.175 cm, 0.226 cm and 0.274 cm. The diameter of the positive control is 1,312 cm. It is necessary to do further using different bacteria and fungi as well as different extraction methods and solvents. It is necessary to do further by using preparations of carrot leaf extract.

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