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Antibacterial Activity Test of Ethanol Extract and Fraction of Carrot Leaf (*Daucus Carota* L.) Against *Staphylococcus Aureus*

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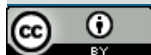
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ABSTRACT

Staphylococcus aureus is one of the bacteria that cause acne and can cause skin and organ damage because bacteria can defeat the body's defences. Carrot leaf extract has antibacterial power or inhibitory activity against *S. aureus*. This study aims to determine the inhibition value and antibacterial activity of ethanol extract and n-hexane fraction of carrot leaves. The research method consists of extraction using 96% ethanol and fractionation using water, ethyl acetate, and n-hexane. The antibacterial activity of carrot leaf extracts and fractions was carried out using the pitting method as well as the use of gentamicin positive control and DMSO negative control. The results showed that 96% ethanol extract of carrot leaves with a concentration of 30% had inhibition against *S. aureus* bacteria with a value of 7.8 mm or weak inhibitory activity, and the ethyl acetate fraction of carrot leaves with a concentration of 20% had inhibition against *S. aureus* bacteria with a value of 11.27 mm or strong inhibitory activity. Therefore, ethyl acetate fraction of carrot leaves have inhibition against *S. aureus* bacteria with strong inhibitory activity compared to other test solutions and is equivalent to gentamicin positive control

INTRODUCTION

Staphylococcus aureus is one of the bacteria that causes acne, because it can clog pores, resulting in excess production of oil glands and causing acne. *S. aureus* can cause skin and organ damage because bacteria can defeat the body's defenses. When bacteria enter the bloodstream, they spread to other organs, such as pharyngitis, tonsillitis, acute middle ear inflammation, pneumonia, heart failure, bone inflammation and heart valve infections which can cause shock and death, as well as can cause infection. One alternative treatment for bacterial infections *S. aureus* is the use of traditional medicine.

Carrot (*Daucus carota*) is a type of tuber plant that comes from the Apiaceae family. Carrots are rich in dietary fiber, antioxidants, anthocyanins, carotenoids, vitamins A, B and C, and minerals. Carrot leaves and tubers contain saponins, besides that, the leaves contain tannins and the tubers contain saponins and polyphenols. The four types of phytochemical compounds that are dominant in carrot leaves are polyacetylene, carotenoids, phenolics and ascorbic acid. Traditionally, parts of the carrot plant have been used as an antidiabetic, diuretic, muscle and back pain reliever, and aphrodisiac. Apart from that, Ahmad et al., (2019) reported that the four main compounds found in carrots also have several other biological activities such as anticancer, antioxidant, anti-inflammatory, antibacterial, plasma lipid modification and serotogenic effects.

Based on research conducted by Hadyarrahman et al., (2017), it is known that carrot leaf extract has antibacterial activity against *S. aureus*. This is proven by the diameter of the inhibitory power of each extract concentration studied. The inhibition zone of carrot leaf extract ranges from 0.7 – 12.15 mm. On the other hand, Ejidike & Clayton, (2022) also reported that carrot leaf extract has antibacterial activity as evidenced by the diameter of the inhibition zone of 9 mm on bacteria *S. aureus*. The compounds that play a role in the antibacterial activity of carrot leaves are phenolic compounds and polyacetylene, so carrot leaf extract

can be used as a traditional medicine to inhibit the activity of carrot leaves. *S. aureus*.

The high incidence of bacterial resistance to antibiotic use can provide opportunities for exploration of the use of traditional medicines from plants that contain compounds or metabolites that have inhibitory activity against *aureus*. This study aims to determine the inhibitory power and antibacterial activity of ethanol extract and n-hexane fraction of carrot leaves (*Daucus carota*) to *S. aureus* through the well method.

METHODS

Tools and Materials

The tools used in this research are maceration vessels, analytical balances, stirring rods, separating funnels, rotary evaporator, Erlenmeyer, beaker, measuring cup, autoclave, test tubes and tube racks, incubators, petri dishes, tube needles and tweezers, micropipettes, calipers, and Laminar air flow. The materials used in this research were aluminum foil, distilled water, bacteria *S. aureus*, ethanol extract of carrot leaves, 70% ethanol, label paper, parchment paper, MHA, MSA and NA media, ethyl acetate, n-hexane, DMSO, and gentamicin.

Preparation of Ethanol Extract and Carrot Leaf Fraction

A total of 500 grams of fine carrot leaf powder was added with 96% ethanol in a ratio of 1:10 g/v, then put into a maceration vessel. *Simplicia* is soaked for the first 6 hours, stirring occasionally, then let sit for 18 hours. The macerate is separated by filtering, the remaceration process is repeated once using the same type and amount of solvent. All the macerate is collected, then evaporated with rotary evaporator until a thick extract is obtained. Calculate the yield weight percentage (w/w). The yield must reach at least the figure specified in each extract monograph.

Fractionation was carried out using the liquid-liquid extraction method using water, ethyl acetate and n-hexane as solvents. A total of 20 grams of thick carrot leaf extract was dissolved in 40 mL of distilled water and put into a separating funnel. After that, 40 mL of n-hexane solvent was added, then shaken and allowed to stand until 2 layers were formed and then separated. Next, the water fraction was extracted liquid-liquid by adding 40 mL of ethyl acetate solvent, shaking and letting it sit until two

layers were formed and then separated. The fractions obtained were evaporated using rotary evaporator.

Media Creation

Mueller Hinton Agar (MHA) is dissolved in 250 ml of distilled water and heated until dissolved, pour the medium into a test tube. Dissolve 10.8 grams of Mannitol Salt Agar (MSA) media in an Erlenmeyer containing 100 mL of distilled water, then heat until boiling, pour the media into a test tube. Nutrient Agar (NA) 10 grams, put it in a 1L Erlenmeyer then add 500 ml of distilled water. then each suspension was heated until boiling while

stirring thoroughly for 15 minutes, then sterilized using autoclave at a temperature of 121°C [7].

Preparation of Test Solution Concentration

Varying concentrations of test solutions were made at 20%, 30%, 40% and 50% from ethanol extract, water fraction, ethyl acetate fraction and hexane fraction of carrot leaves which were then dissolved in 10% DMSO until the volume was 10 mL.

Table 1. Concentration of Test Solutions for Carrot Leaf Extracts and Fractions

Test Solution Concentration (%)	g/mL	10% DMSO Solvent Volume (mL)
20	0,2	ad 10
30	0,3	ad 10
40	0,4	ad 10
50	0,5	ad 10

In table 1 it can be seen that based on calculations, a 20% test solution concentration contains 0.2 g of extract/fraction in 1 mL of DMSO solvent, at a 30% concentration it contains 0.3 g of extract/fraction in 1 mL of DMSO solvent, at a concentration of 40% contains 0.4 g of extract/fraction in 1 mL of DMSO solvent and at a concentration of 50% contains 0.5 g of extract/fraction in 1 mL of DMSO solvent.

Antibacterial Test Aureus

The antibacterial activity test of ethanol extract and carrot leaf fraction was carried out using the well method. Bacterial suspension *S. aureus* put into 3 petri dishes containing MHA media by taking 15 mL and leveling it with an L rod. then add the extract with various concentrations. Apart from that, 1 petri dish was divided into 2 parts for positive control and negative control. The treatment was carried out 3 times on each cup. positive control used gentamicin and negative control used 10% DMSO. Carrot leaf extracts and fractions with various concentrations of 20%, 30%, 40% and 50% were put into 20 µL wells of MHA media using a micropipette with sterile processing. Next, the petri dishes containing bacteria with various extract concentrations were placed in the refrigerator at a

temperature of 4°C for 24 hours so that the compounds diffused in the medium. The incubation process continues in an incubator at a temperature of 37°C for 24 hours [8]. After the inhibition zone is formed, the diameter of the inhibition zone is measured using a caliper.

RESULTS AND DISCUSSION

Carrot leaves used in this research, was determined at the Botany Laboratory, Biology Department, Jember University, East Java. This determination is carried out to obtain the truth regarding the clear identity of the plants studied and to avoid errors in collecting the main materials used in the research. The determination results showed that the samples used in this research were confirmed to be leaves from carrot plants *Daucus carota* L. (Family – Apiaceae; Vernacular name – Carrot, (eng).). The results of the determination of carrot leaves were identified based on Backer, C. A., & Van Den Brink, R. C. B. 1963. *Flora of Java; Spermatophytes only*, Vol. 2. (pp. 178) .

96% ethanol extract of carrot leaves is obtained from the extraction of carrot leaf powder. Extraction is carried out using the cold soaking or maceration method using 96% ethanol solvent. The solvent

ethanol 96 is used because this solvent is a semi-polar universal solvent that can dissolve polar and

non-polar parts of the extract. The yield results of carrot leaf extract can be shown in table 2.

Table 2. Yield of Ethanol Extract of Carrot Leaves

Powder weight (g)	Extract weight (g)	Yield (%)
500	50,8	10,2
500	51,6	10,3
500	52	10,4
Rata-Rata ± SD		10,3 ± 0,1

The thick extract of carrot leaves obtained was 154.4 grams and the yield of ethanol extract of carrot leaves showed a yield of >10%, this is in accordance with the requirements for a good yield according to the Indonesian Ministry of Health [9]. The thick extract that has been obtained is then fractionated. Fractionation using n-Hexane, ethyl acetate and water solvents. N-hexane, which is non-polar, works to attract non-polar compounds such as steroids, chlorophyll and terpenoids found in carrot leaf extract. Fractionation with ethyl acetate has the aim

of separating polyphenol or flavonoid compounds. Fractionation of ethyl acetate and water serves to separate the flavonoid compounds in the ethanol extract into two parts of different polarity and solubility, namely in the aglycone form and the form bound to sugar. Flavonoid compounds in the form of aglycones will be more easily distributed into the ethyl acetate fraction, while flavonoids in the form of glycosides will be more easily distributed into the water phase. The fractionation results of the ethanol extract of carrot leaves are in table 3.

Table 3. Results of Fractionation of Ethanol Extract of Carrot Leaves

Test Fraction	Viscous fraction weight (grams)	Yield (%)
Ethyl acetate	6,53	5,44
N-Hexane	25,07	20,89
Air	61,43	51,19

The yield of the ethyl fraction is less than the n-Hexane fraction and the water fraction, the results of the fractionation are different due to differences in the ability to attract compounds from each solvent [10]. Based on this, it is suspected that the content of polar compounds in carrot leaf extract is greater than that of semi-polar and non-polar compounds. The total yield was 77.52%, this result was far from the amount that should have been 100%. This was due to

a lack of silence during separation and caused the compounds to not be separated completely.

Actibacterial activity test *S. aureus* with the well method using MHA media. The well method is used because the results of measuring the area of the inhibition zone formed are easier because bacterial isolates will form down to the bottom [11]. The results of the antibacterial test of extracts and fractions using the well method are shown in table 4.

Table 4. Antibacterial Test Results of Carrot Leaf Extracts and Fractions

Preparation	20% (mm)	30% (mm)	40% (mm)	50% (mm)
Carrot leaf extract	2,87 ± 0,33 ^b	7,8 ± 0,51	3,8 ± 0,24	3,47 ± 0,21
Ethyl acetate fraction	11,27 ± 0,71 ^a	5,67 ± 0,33	4,73 ± 0,29	3,2 ± 0,24
N-hexane fraction	4,9 ± 0,62 ^b	3,1 ± 0,33	2,43 ± 0,34	1,4 ± 0,29
Water fraction	1,13 ± 0,26 ^b	1,07 ± 0,21	0,47 ± 0,17	0,23 ± 0,12
Control (+)	13,5 ^a	14,3 ^a	12,7 ^a	0
Control (-)	0	0	0	0

Information:

Control + used the antibiotic gentamicin 10µg/ml

Control – using 10% DMSO

a = not significantly different

b = significantly different

Results of antibacterial testing against bacteria *S. aureus* showed that the ethyl acetate fraction of carrot leaves with a concentration of 20% had an inhibitory power of 11.27 mm. In general, the diameter of the inhibition zone tends to increase in proportion to increasing extract concentration. However, in this study there was a decrease in the diameter of the inhibition zone when the concentration of the extract and fraction was increased. This value is similar to Hadyarrahman's research et al., (2017), where the diameter of the inhibition zone does not always increase in proportion to increasing extract concentration [5]. According to Jawetz et al., (2008), the formation of the antibacterial inhibition zone is influenced by factors such as incubation temperature, incubation time, homogeneity and microbial density [12].

The inhibitory power of the 20% ethyl acetate fraction is in the strong category compared to the inhibitory power produced by other carrot leaf fractions. Data from antibacterial tests on extracts and fractions were subjected to normality analysis to see the distribution of the data. Based on normality data, the antibacterial activity of carrot leaf extracts and fractions was normally distributed ($p > 0.05$). Subsets analysis showed that the results of the antibacterial test on the 20% ethyl acetate fraction were close to the positive control compared to the extract, n-Hexane fraction and water fraction of carrot leaves. The antibacterial activity of the 20% ethyl acetate fraction is thought to be due to the flavonoid content. Flavonoids play a direct role by interfering with the cell function of microorganisms and inhibiting the microbial cell cycle [13], [14].

CONCLUSION

1. 96% ethanol extract of carrot leaves with a concentration of 30% has inhibitory power against bacteria *S. aureus* with a value of 7.8 mm or weak inhibitory activity.
2. The ethyl acetate fraction of carrot leaves with a concentration of 20% has inhibitory power against bacteria *S. aureus* with a value of 11.27 mm or strong inhibitory activity.
3. The ethyl acetate fraction of carrot leaves has inhibitory power against bacteria *S. aureus* with strong inhibitory activity compared with other test solutions and equivalent to the positive control gentamicin.

Declaration/Statement

Funders

This research was not funded from any source.

Author Contributions

The first author prepared the conceptual framework of the research and drafted the manuscript in full. The second author conducted research on the extraction and fractionation of carrot leaves, as well as carrying out inhibitory activity against bacteria *S. aureus*. The third author provided direction regarding research procedures and manuscript writing.

Conflict of Interest

There is no conflict of interest in this research.

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