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Screening of Sengkuang (*Dracontomelon dao* (Blanco) Merr. & Rofe) as Antibacterial *Escherichia coli*

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Abstract: *Dracontomelon dao* (Blanco) Merr. & Rofe has antimicrobial potential, but *Dracontomelon dao* (Blanco) Merr. & Rofe, originating from Kalimantan, Indonesia, has not been tested for its antibacterial properties and secondary metabolite content. This study aimed to analyze the secondary metabolite content of various *Dracontomelon dao* (Blanco) Merr leaf extracts. & Rofe and their antibacterial effectiveness against *Escherichia coli*. The study used 96% ethanol extract, ethyl acetate, petroleum ether, and chloroform by maceration simplicia leaves. *Dracontomelon dao* (Blanco) Merr. & Rofe is from South Kalimantan, Indonesia. Antibacterial testing was carried out using the agar diffusion method, the Minimum Inhibitory Concentration (MIC) method, and the Minimum Bactericidal Concentration (MBC) method. The results showed that the triterpenoid content was the highest in all extracts, namely ethanol extract 669.8 ± 2,000 mg/ml, ethyl acetate 90 ± 7,638 mg/ml, petroleum ether 792, 800 ± 4,583 mg/ml, chloroform 615, 467 ± 0,577 mg/ml. ml. The antibacterial test using the well diffusion method showed the best inhibition of bacterial growth in ethanol extract, with the largest inhibition zone of 24 mm at a concentration of 500 mg/ml. The study's conclusion was the extract of ethanol, ethyl acetate, petroleum ether and chloroform from the leaves of *Dracontomelon dao* (Blanco) Merr. & Rofe contains the same five secondary metabolites: flavonoids, alkaloids, saponins, tannins and triterpenoids. All types of extracts showed varying abilities to inhibit the growth of *Escherichia coli*. Further research is needed on the antibacterial properties of *Dracontomelon dao* (Blanco) Merr extract. & Rofe, especially on the active substance triterpenoid, which is the largest content in all types of extracts.
Keywords: Antibacterial; *Dracontomelon dao* leaf; *Escherichia coli*

INTRODUCTION

Plant material remains an essential resource for tackling severe diseases in the world. Because plant material's most critical bioactive constituents are alkaloids, tannins, flavonoids and phenolics, advances in identifying sources of new natural products with antimicrobial activity may contribute to new antibiotics (Kamath et al., 2016). Indonesia is a country that is rich in various types of plants, the community empirically uses many plants like herbs and medicines, but most of them have not been scientifically proven. One plant that has not been studied much is *Dracontomelon dao* (Blanco) Merr. & Rofe is a plant belonging to the Anacardiaceae tribe.

Dracontomelon dao (Blanco) Merr. The bark of the *Dracontomelon dao* (Blanco) Merr. & Rofe is one of the medicinal plants for the Dayak community. & Rofe can be used for diarrhoea medicine by pounding, boiling, and drinking mixed with Cananga (Falah et al. 2013). In addition, the flowers and leaves can be cooked and eaten as fresh vegetables (Papua New Guinea) and used as cooking spices (Maluku) (Priya,

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2016). Several biological assays on *Dracontomelon dao* (Blanco) Merr. & Rofe Merrill & Rolf showed that this plant has great potential as an antibacterial (Putri, T. et al., 2022). Treat diarrheal disease caused by *Escherichia coli* can be done using antibacterial drugs (Ningrum, HP., 2011).

The research results of Liu et al. (2014), who conducted an antibacterial test using the microcalorimetric method with n-butanol, ethyl acetate, petroleum ether and chloroform fractions, stated that the most potent antibacterial effect was the ethyl acetate fraction from *Dracontomelon dao* (Blanco) Merr leaves. & Rofe against E. Coli with a concentration of 50%. This was also stated by Zhao et al. (2015) that the antibacterial effect produced by the ethyl acetate fraction of *Dracontomelon dao* (Blanco) Merr leaves. & Rofe inhibits the growth of Staphylococcus aureus because it has the highest flavonoid content of 41.86%. Based on the research of Li et al., it was also stated that Dracontomelo dao leaf extract has antibacterial potential against E. Coli in the presence of flavonoid content in plants (Li et al., 2017). The main composition is *Dracontomelon dao* (Blanco) Merr bark essential oil. & Rofe is n-Hexadecanoic acid (46.13%); Octadecanoic acid (15.44%), (E)-9-Octadecenoic acid (13.73%), and (Z, Z)-9.12-Octadecadienoic acid (7.79%) (Ismail et al, 2015). *Dracontomelon dao* (Blanco) Merr bark essential oil. & Rofe is the first to be widely studied (Fang, 2008), while the research of *Dracontomelon dao* (Blanco) Merr. & Rofe on the leaves has not been widely disclosed.

Several previous studies have shown the antibacterial effect of *Dracontomelon dao* (Blanco) Merr leaves. & Rofe, but additional data are needed on the variation of the extract solvents and the different lands where *Dracontomelon dao* (Blanco) Merr. & Rofe grows because the content of active plant substances is very dependent on the land where it grows. So this study aims to analyze the content of secondary metabolites in the *Dracontomelon dao* (Blanco) Merr leaves extract. & Rofe from Kalimantan Selatan Indonesia with various solvents and their effectiveness as an antibacterial *Escherichia coli*.

MATERIALS AND METHOD

Sample and Materials

Leaf *Dracontomelon dao* (Blanco) Merr. & Rofe used comes from Hulu Sungai Selatan district, South Kalimantan province, Indonesia. *Dracontomelon dao* tree (Blanco) Merr. & Rofe, whose leaves are taken, is a tree without plant diseases or holes in the trunk. Leaf of *Dracontomelon dao* (Blanco) Merr. & Rofe took clean leaves, not damaged or torn, not young but not too old, hard textured, dark green.

The reagents used were ethanol 96%, ethyl acetate, petroleum ether, and chloroform to extract the leaf metabolites of *Dracontomelon dao* (Blanco) Merr. & Rofe. *Escherichia coli* cultures from the Martapura river, South Kalimantan, Indonesia, were identified and certified by the bacteriology laboratory of Ulin Hospital, South Kalimantan Province. The media used were Mueller Hinton Agar (Merck), EMB Agar (Himedia), TSB (Himedia), Nutrient Agar (Himedia), Mc Farland standard solution 0.5, Propylene glycol, DMSO 10% and NaCl 0.9% solution.

Analysis Instrument

Measurements using a cuvette in which there is already a sample solution, extract and control. A photometer (Rayto) was used in this study as a Minimum Inhibition Concentration (MIC) measuring instrument with a wavelength of 578 nm. This photometer is calibrated and stabilized at 37oC before being used for measurements.

Extract Preparation

Leaf *Dracontomelon dao* (Blanco) Merr. & Rofe is dried and ground into powder, weighed and soaked using four solvents (Ethanol, Ethyl Acetate, Petroleum Ether, Chloroform) with a ratio of 1 part (gram) of *Dracontomelon dao* (Blanco) Merr leaf powder. & Rofe and three parts (mL) of each solvent, allowed to stand for three days, filtered. The filtrate formed was evaporated with a water bath (Thermo) at a temperature of 50o-60oC. The viscous extract used for the agar diffusion antibacterial test was added with DMSO as much as 10% of the total solution, while the extract for the MIC test used propylene glycol.

Phytochemical tests were carried out quantitatively to determine the presence of flavonoid compounds, alkaloids, saponins, tannins, and triterpenoids. Antibacterial test by diffusion was carried out by the well method. Muller Hinton agar medium was smeared with bacterial suspension equivalent to 0.5 mc Farland and allowed to dry for 4-5 minutes. Wells were made, and sengkuang leaf extract was added from various solvents according to variations in concentration. Petri dishes were incubated at 37°C for 18 hours. The inhibition zone formed was measured.

The MIC test was carried out by mixing 1 mL of the test solution and 1 mL of bacterial suspension with three repetitions. The final concentration of the test solution after adding the bacterial suspension was half of the initial concentration so that the concentration of the test solution was 250 mg/mL, 300 mg/mL, 350 mg/mL, 400 mg/mL, 450 mg/mL and 500 mg/mL. MIC results after 24 hours of incubation at 37°C were determined in the solution by measuring the absorbance on a Photometer (Rayto) with a wavelength of 578 nm. The absorbance values before and after incubation were compared. The increase in absorbance value after incubation indicates the growth of live bacterial cells. At the same time, the constant and reduced absorbance value after incubation indicates the absence of live bacterial cell growth (Pajan et al., 2016).

From the solution that shows the MIC, 20 ul is taken and spread on the surface of the Nutrient Agar media for 24 hours of incubation at 37°C. MBC was shown with Nutrient Agar plate media where there was no bacterial colony growth.

Data Analysis

The data obtained from the test was then tested for normality and homogeneity. The distribution of the MIC and MBC test data in this study was abnormal, so the data analysis used the Kruskal-Wallis test using a computer application.

RESULTS AND DISCUSSION

Tabel 1. Qualitative and Quantitative Phytochemical Examination Test of *Dracontomelon dao* (Blanco) Merr. & Rofe Leaf Extract Results Table

Parameter	Solvent			
	Ethanol	Ethyl Acetate	Petroleum Ether	Chloroform
Flavonoid (mgEQ/g)	125,5 ± 0,433	75,083 ± 1,258	7,500 ± 0,500	52,250 ± 0,289
Alkaloid (%)	33,945 ± 0,781	21,391 ± 1,258	16,823 ± 0,345	5,033 ± 0,530
Saponin (%)	33,093 ± 0,755	17,376 ± 0,889	26,489 ± 0,989	6,416 ± 0,318
Tanin (mg/ml)	0,069 ± 0,003	0,031 ± 0,003	0,016 ± 0,005	0,612 ± 0,008
Triterpenoid (mg/ml)	669,8 ± 2,000	90 ± 7,638	792, 800 ± 4,583	615, 467 ± 0,577

Tabel 2. Agar Diffusion Test Average Diametre

Concentration	Ethanol (mm)	Ethyl Acetate (mm)	Petroleum Ether (mm)	Chloroform (mm)
500 mg/ml	24	11	6,5	10,25
450 mg/ml	21,75	10,75	6,5	7,5
400 mg/ml	21	9,75	6	6,75
350 mg/ml	19	8,75	6	6,5
300 mg/ml	18,25	7,5	6	6,25
250 mg/ml	16,75	6,75	6	6
Control (-)	6	6	6	6
Control (+)	29,25	29,5	30	30,25

Table 3. Minimal Inhibitory Concentration (MIC)

Extract Concentration (mg/L)	Absorbance Value on the Repetition			
	Ethanol	Ethyl Acetate	Petroleum Ether	Chloroform
500	Increased	Increased	Increased	Decreased
450	Decreased	Increased	Decreased	Decreased
400	Decreased	Increased	Decreased	Decreased
350	Decreased	Increased	Increased	Increased
300	Increased	Decreased	Decreased	Decreased
250	Decreased	Increased	Decreased	Decreased
Control (+)	Decreased	Increased	Increased	Increased
Control (-)	Increased	Increased	Increased	Increased
Extract Control	Increased	Decreased	Increased	Increased

Tabel 4. Minimal Bactericidal Concentration (MBC) Test

Concentration	Solvent			
	Ethanol	Ethyl Acetate	Petroleum Ether	Chloroform
500	0	0	∞	0
450	0	0	750	0
400	0	0	287	0
350	0	0	1	0
300	0	9	1	0
250	0	8	0	0

Tabel 5. Kruskall-Wallis MIC Test Results

	Pelarut	N	Mean Rank	Asymp Sig.
Kategori_Abs	Ethanol	6	13.50	0.116
	Ethyl Acetate	6	7.50	
	Chloroform	6	15.50	
	Petroleum Ether	6	13.50	
	Total	24		

Tabel 6. Kruskall-Wallis MBC Test Results

	Pelarut	N	Mean Rank	Asymp Sig.
Kategori Jumlah Koloni Kuman	Ethanol	24	55.00	0.001
	Ethyl Acetate	24	47.00	
	Chloroform	24	49.00	
	Petroleum Ether	24	43.00	
	Total	96		

Based on the results of secondary metabolite tests on sengkuang leaf extract using four solvents (Table 1), the highest flavonoid content in ethanol extract was 125.5 ± 0.433 mgEQ/g compared to other types of extracts. Meanwhile, the triterpenoid content was the highest metabolite in all extracts. In contrast to research conducted by Kurniawan et al. (2020) which stated the highest content of phytochemical compounds in sengkuang leaves were flavonoids.

The four extracts have been tested for antibacterial using the well diffusion method. The results showed the best inhibition of bacterial growth in the ethanol extract, with the largest inhibition zone of 24 mm at a concentration of 500 mg/ml (Table 2). The antibacterial test also used the MIC and MBC methods; based on table 3; it is known that the MIC test results on ethyl acetate extract almost all showed an increase; the other three types of extracts showed varied results, so in the statistical test there was no significant difference in the four treatments (Table 5). From the measurement results using a photometer, it was found that the MIC of the ethanol extract of sengkuang leaves as an antibacterial *Escherichia coli* occurred at a concentration of 250mg/mL; the MIC of the ethyl acetate extract occurred at a concentration of 300mg/mL; MIC of petroleum ether extract and chloroform extract at a concentration of 250mg/mL. This concentration is the minor concentration that decreases the absorbance value after incubation for 24 hours.

The results of statistical tests for MBC (Table 6) are known from the four types of extracts that there is a significant difference with the Asymp.Sig value of 0.001 (less than 0.05). Based on the results of statistical tests, it is known that among four types of sengkuang leaf extract, ethanol extract has the highest mean rank value of 55, chloroform 49, ethyl acetate 47 and petroleum ether 43. This shows that ethanol extract is the best in killing *Escherichia coli* bacteria. Because ethanol solvent is polar, it can take up secondary metabolites maximally; according to Wulandari et al. (2021), polar solvents can easily attract the content of polar-type metabolites. Sani et al. (2014) stated that ethanol could dissolve phytochemical compounds more optimally because ethanol can attract sugars, amino acids, and several phytochemical compounds such as flavonoids, alkaloids, flavonoid glycosides and chlorophyll.

In contrast to research by Liu et al. (2013), which showed that ethyl acetate extract of sengkuang leaves had the best antibacterial activity of *Escherichia coli* compared to chloroform, butanol, and petroleum ether solvents. The difference in the results of this study is because the research of Liu et al. (2013) used the microcalorimetric method so that with a small concentration of ethyl acetate extract (98.5 g mL), it was able to inhibit the growth of *Escherichia coli*.

The triterpenoid content was the highest in all extracts, namely ethanol extract 669.8 ± 2,000 mg/ml, ethyl acetate 90 ± 7,638 mg/ml, petroleum ether 792, 800 ± 4,583 mg/ml, chloroform 615, 467 ± 0,577 mg/ml. Triterpenoid compounds are widely distributed in nature, in plants and animals, in their free form, esters and glycosides. The action of antibacterial terpenoid compounds is to react with porins (transmembrane proteins) on the outer membrane of the bacterial cell wall and reduce

the permeability of the bacterial cell wall so that bacterial cells lack nutrients. Bacterial growth will be inhibited or terminated (Arlofa, 2015).

The limitation in this research is that the MBC value has not been found for all types of extract solvents; the solubility of the extract at all concentrations for the MIC test may affect the MIC and MBC values. A better method of dissolving the extract with a suitable solvent is needed so that the active substance in the extract can dissolve and function optimally to inhibit bacterial growth in the MIC test.

CONCLUSION

Extracts of ethanol, ethyl acetate, petroleum ether and chloroform from the leaves of *Dracontomelon dao* (Blanco) Merr. & Rofe contains the same five secondary metabolites: flavonoids, alkaloids, saponins, tannins and triterpenoids. Ethanol extract can inhibit the growth of the largest *Escherichia coli*, producing an inhibition zone of 24 mm at a concentration of 500 mg/ml in the well diffusion method. The ethanol extract contains flavonoids, alkaloids, saponins, tannins and triterpenoids respectively 125.5 ± 0.433 mgEQ/g, 33.945 ± 0.781 %, 33.093 ± 0.755 %, 0.069 ± 0.003 mg/ml, and 669.8 ± 2,000 mg/ml . Further research is needed on the antibacterial properties of *Dracontomelon dao* (Blanco) Merr extract. & Rofe, especially on the active substance triterpenoid, which is the largest content in all types of extracts.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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