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Phytochemical Analysis by LC-HRMS and Antibacterial Activity Of the Ethanol Extract of Sengkuang (*Dracontomelon dao* (Blanco) Merr. & Rofe)

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Abstract: *Dracontomelon dao* (Blanco) Merr. & Rofe is one of the medicinal plants for the community. This study aims to analyze the ethanol extract of Sengkuang (*Dracontomelon dao* (Blanco) Merr. & Rofe) using the LC-HRMS Phytochemical Test and determine its antibacterial activity. Leaf *Dracontomelon dao* (Blanco) Merr. & Rofe originates from Hulu Sungai Utara, South Kalimantan, Indonesia, macerated with 96% ethanol. Antibacterial test by diffusion was carried out using the well method. LC-HRMS (Liquid Chromatography - High-Resolution Mass Spectrometry) test using the Instrument Thermo Scientific™ Dionex™ Ultimate 3000 RSLCnano UHPLC coupled with Thermo Scientific™ Q Exactive™ High-Resolution Mass Spectrometer. The content of chemical compounds from the ethanol extract, namely flavonoids 125.5 ± 0.433 mgEQ/g, alkaloids (%) 33.945 ± 0.781, saponins (%) 33.093 ± 0.755, tannins (mg/ml) 0.069 ± 0.003, the highest content was triterpenoids 669.8 ± 2,000(mg/ml). The best inhibition of E coli growth with the largest inhibition zone was 24 mm at a 500 mg/ml concentration. The active compounds contained in the leaves of *Dracontomelon dao* (Blanco) Merr. & Rolfe are quercetin-3β-D-glucoside, anacardic acid, D-glucosamine, azelaic acid, choline, astragalin, quercetin, luteolin, syringic acid. The active ingredient of the ethanol extract of Sengkuang (*Dracontomelon dao* (Blanco) Merr. & Rofe) has the potential as an antibacterial and anti-inflammatory that can be added to pharmaceutical preparations.
Keywords: *Dracontomelon dao* (Blanco) Merr. & Rofe; Phytochemical Analysis; Liquid Chromatography - High-Resolution Mass Spectrometry

INTRODUCTION

Dracontomelon dao (Blanco) Merr. & Rofe is a medicinal plant for the Dayak tribe in Indonesia (Falah et al., 2013) and a traditional Chinese medicine ingredient (Li, Y et al., 2017). Previous research has shown that the ethanol extract of D. dao leaves exhibits inhibitory activity against *Staphylococcus aureus, Bacillus subtilis* (Khan & Omoloso, 2002), Pseudomonas aerogenosa (Wu et al., 2015). Research Liu et al. (2014) stated that the strongest antibacterial effect was the ethyl acetate fraction from the leaves of *Dracontomelon dao* (Blanco) Merr. & Rofe against *E. coli* with a concentration of 50%. Zhao et al. (2015) also stated that the antibacterial effect was produced by the ethyl acetate fraction of *Dracontomelon dao* (Blanco) Merr leaves. & Rofe inhibited *Staphylococcus aureus* with the highest flavonoid content, namely 41.86%, the main component of these flavonoids; Cianidanol, L-Epicatechin, Quercetin, and Luteolin (Liu T. et al., 2013; Zhao et al., 2015). Based on research by Li et al., it was also stated that Dracontomelo dao leaf extract has antibacterial potential against *E. coli* in the presence of flavonoids in plants (Li et al., 2017).

Previous research on the leaves of *Dracontomelon dao* (Blanco) Merr. & Rofe from Kalimantan, Indonesia, has shown the antibacterial effect of ethanol, ethyl acetate, petroleum ether, and chloroform extracts against *E. coli* (Dwiyanti et al., 2022). The antibacterial test using the well-diffusion method 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. However, additional data is needed to analyze other chemical compounds from the ethanol extract of *Dracontomelon dao* (Blanco) Merr leaves. & Rofe, this study aims to analyze the ethanol extract of Sengkuang (*Dracontomelon dao* (Blanco) Merr. & Rofe) with the LC-HRMS Phytochemical Test and determine its antibacterial activity.

MATERIALS AND METHODS

Leaf *Dracontomelon dao* (Blanco) Merr. & Rofe originates from Hulu Sungai Utara South Kalimantan, Indonesia, dried and ground into powder, weighed, and soaked using 96% ethanol with a ratio of 1 part (gr) of *Dracontomelon dao* (Blanco) Merr leaf powder. & Rofe and three parts (mL) of solvent, allowed to stand for three days, filtered off. The formed filtrate was evaporated with a water bath (Thermo) at 50º-60ºC. The thick extract used for the diffusion antibacterial test added DMSO as much as 10% of the total solution. Phytochemical screening of Sengkang leaf extract (*Dracontomelon dao* (Blanco) Merr. & Rofe) quantitatively to determine the presence of flavonoids, alkaloids, saponins, tannins, and triterpenoids. Antibacterial test by diffusion was carried out using the well method. Muller Hinton (Merck) agar medium was smeared with E coli suspension equivalent to 0.5 mc Farland and dried for 4-5 minutes. Wells with a diameter of 6mm were filled with ethanol extract of Sengkang leaves with various concentrations of 250-500 mg/ml. Petri dishes were incubated at 37°C for 18 hours. The inhibition zone formed was measured.

LC-HRMS (Liquid Chromatography - High-Resolution Mass Spectrometry) Test using Instrument: Thermo Scientific™ Dionex™ Ultimate 3000 RSLCnano UHPLC coupled with Thermo Scientific™ Q Exactive™ High-Resolution Mass Spectrometer. Mobile Phase included 2 Solutions; Solution A contains water and 0.1% Formic Acid, and Solution B contains Acetonitrile + 0.1% Formic acid. Analytical Column contains Phenyl Hexyl 100 mm x 2,1. Specifications are instrument flow: 0,20 mL/min, Sample injection volume: 5 µL, analysis running time: 65 minutes, with full MS at 70,000 FWHM Resolution, Dependent data MS2 at 17,500 FWHM, for dividing electron type as positive and negative with heated Electrospray Ionization (H-ESI). Compound Identification by Thermo Scientific™ Compound Discoverer Software.

The sample preparation and inspection procedure was to take ± 0.1 mg of extract, which was dissolved in ethanol for LC-Ms, homogenized with a vortex mixer, then filtered with 0.2 µM miles. Inject 5 µL into HRMS, and finally, wait until the inspection process is complete and read the results.

RESULTS AND DISCUSSION

The content of chemical compounds from the ethanol extract, namely flavonoids 125.5 ± 0.433 mgEQ/g, alkaloids (%) 33.945 ± 0.781, saponins (%) 33.093 ± 0.755, tannins (mg/ml) 0.069 ± 0.003, the highest content was triterpenoids 669.8 ± 2,000(mg/ml). Different from the results of a study conducted by Kurniawan et al. (2020), who stated that the highest content of phytochemical compounds in Sengkang leaves was flavonoids. The diffusion test results showed the best inhibition of bacterial growth with the largest inhibition zone of 24 mm at a concentration of 500 mg/ml (Table 1).

Table 2 shows Identified Compounds from *Dracontomelon dao* (Blanco) Merr. & Rofe with Structure, Name, Retention time (Rt), and m/z Values Using LC-HRMS. Table 3 shows an image of the Compound Chromatogram of *Dracontomelon dao* (Blanco) Merr. & Rofe Using LC-HRMS. The results of the compounds found in the LC-HRMS test were 79 compounds, and those that affected bacteria, especially E coli, there were 9, namely Quercetin-3β-D-glucoside, Anacardic acid, D-Glucosamine, Azelaic acid, Choline, Astragalin, Quercetin, Luteolin, Syringic acid.

Tabel 1. Phytochemical ScreeningTest and Sensitivity Test of Ethanol Extract *Dracontomelon dao* (Blanco) Merr. & Rofe

Ethanol Extract *Dracontomelon dao* (Blanco) Merr. & Rofe			
Quantitative Phytochemical Examination Test		Agar Diffusion Test	
Parameter	Content	Concentration	Average Diametre
Flavonoid (mgEQ/g)	125,5 ± 0,433	500 mg/ml	24,00
Alkaloid (%)	33,945 ± 0,781	450 mg/ml	21,75
Saponin (%)	33,093 ± 0,755	400 mg/ml	21,00
Tanin (mg/ml)	0,069 ± 0,003	350 mg/ml	19,00
Triterpenoid (mg/ml)	669,8 ± 2,000	300 mg/ml	18,25
		250 mg/ml	16,75

The results of this study showed that the ethanol extract of *Dracontomelon dao* (Blanco) Merr. & Rolfe leaves showed the best inhibition of E coli growth with the largest inhibition zone of 24 mm at a concentration of 500 mg/ml (Table 1); other studies also showed the antibacterial effect of *Dracontomelon dao* (Blanco) Merr. & Rolfe against *E. coli*, but with different solvents, namely petroleum ether, chloroform, ethyl acetate, n-butanol, and water (Liu T. et al., 2013; Zhao et al., 2015).

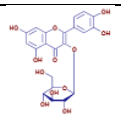
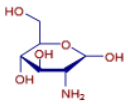
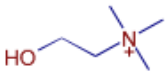
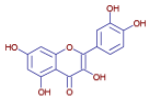
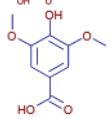
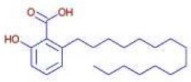
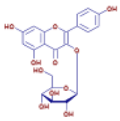
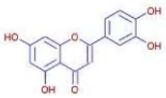
The active compound content in *Dracontomelon dao* (Blanco) Merr.& Rolfe leaves. & Rolfe is quercetin-3β-D-glucoside, anacardic acid, D-Glucosamine, azelaic acid, choline, astragalin, quercetin, luteolin, syringic acid (Table 2 & Figure 1) . Research by Li has proved the potential for a synergistic effect between Luteolin and Quercetin, Li Y. et al. (2017), which has an antibacterial effect by microcalorimetric analysis.

Luteolin is the active compound found in this study. Another study showed that the Mentha longifolia ethanol extract containing the Luteolin component was the most active fraction against the tested bacteria (Akroum et al., 2009). The combination of Luteolin and Amoxicillin shows synergistic activity against *E. coli* (Eumkeb et al., 2012). In addition, the new Luteolin derivative showed beneficial antibacterial activity in vitro against *B. subtilis, S. aureus, P. fluorescens*, and *E. coli* (Lv et al., 2009).

Quercetin-3β-D-glucoside, Quercetin is the active compound found in this study, besides Luteolin. Luteolin and Quercetin have a synergistic effect as antibacterial, Flavonoids have different types of chemical structures, but Luteolin and Quercetin have a similar structural framework; only Quercetin has one more hydroxyl than Luteolin, so the possible sites of antibacterial action of these two compounds may be combined (Li, Y. et al., 2017). The active compound found in this study is D-glucosamine. A study stated that 6-Sulfo-6-deoxy-D-glucosamine (GlcN6S), 6-sulfo-6-deoxy-D-glucosaminitol (ADGS), and their N-acetyl and methyl ester derivatives are inhibitors of enzymes that catalyze the reaction of the UDP-GlcNAc pathway, on bacteria. Both compounds showed antimicrobial activity in vitro, with MICs in the 0.125-2.0 mg mL-1 (Skarbek K et al., 2017).

Another active compound found in this study is choline. The combination of choline: germanic acid can be an ideal antimicrobial for human use. A study using molecular dynamics simulations has identified the mechanism of action of choline: germanic acid on *E. coli* membranes, namely that choline is attracted to the negatively charged cell membrane and consequently incorporates germanic acid into the lipid bilayer (Ibsen KN et al., 2018).

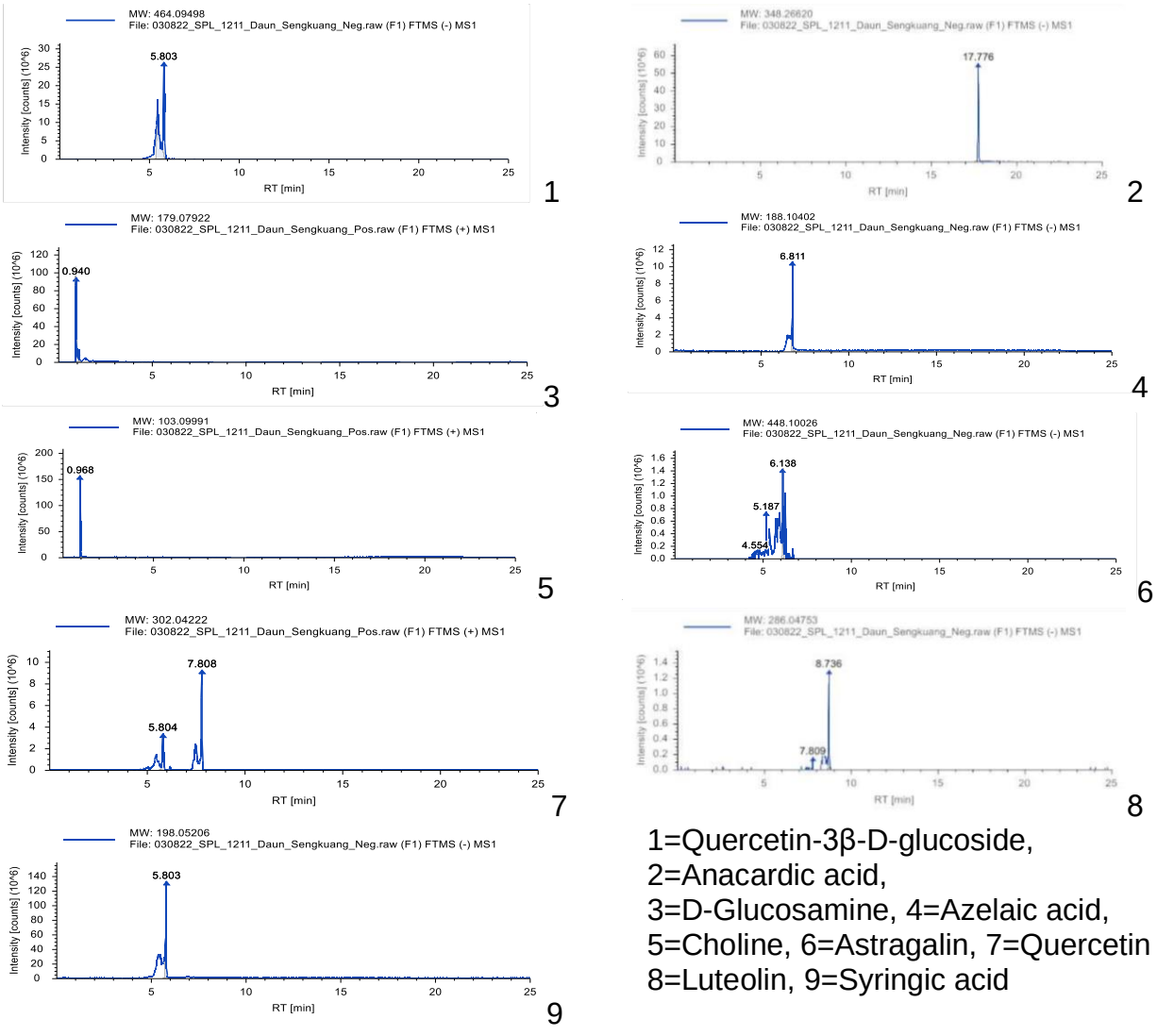
Table 2. Identified Compounds from *Dracontomelon dao* (Blanco) Merr. & Rofe with Structure, Name, Retention time (Rt), and m/z Values Using LC-HRMS

No	Structure	Name	Retention time (Rt)	Formulas	m/z Values
1		Quercetin-3β-D-glucoside	5,48	C21 H20 O12	463,08777
2		D-Glucosamine	0,94	C6 H13 N O5	180,08649
3		Choline	0,96	C5 H13 N O	104,10719
4		Quercetin	5,81	C15 H10 O7	303,04987
5		Syringic acid	5,79	C9 H10 O5	197,04472
6		Anacardic acid	17,77	C22H36O3	412,96613
7		Azelaic acid	6,81	C9 H16 O4	187,09674
8		Astragalin	6,15	C21 H20 O11	447,09302
9		Luteolin	8,73	C15H10O6	285,04025

Another active compound found in this study is Anacardic Acid. Research on the antibacterial activity of five phenolic lipids derived from anacardic acid related to their physicochemical properties, transport across the epithelial monolayer, cytotoxicity, and antibacterial activity compared to standard antibiotics. Results demonstrated no cytotoxicity in cell lines derived from fibroblasts, liver, colorectal, or kidney. Two phenolic lipids significantly improved survival in the Galleria mellonella animal model infected with Meticillin-resistant Staphylococcus aureus (MRSA) and Vancomycin-resistant Enterococci (VRE) compared to the untreated control group (Saedtler M et al., 2020).

Anacardic acid (6-alkyl salicylic acid) inhibits the metabolism of *Escherichia coli*, as well as other bacteria such as *Streptococcus faecalis, Bacillus subtilis*, and

Pseudomonas fluorescens. The sensitivity of Gram-negative bacteria increases with increasing alkyl chain unsaturation in anacardic acids (Joanne L et al., 2011).



1=Quercetin-3β-D-glucoside,
2=Anacardic acid,
3=D-Glucosamine, 4=Azelaic acid,
5=Choline, 6=Astragalin, 7=Quercetin
8=Luteolin, 9=Syringic acid

Figure 1. Chromatogram Compound *Dracontomelon dao* (Blanco) Merr. & Rofe Using LC-HRMS

Another active compound found in this study is azelaic acid. The antimicrobial potential of azelaic acid was demonstrated by the Minimum Inhibitory Concentration against *E. coli* ATCC 25922 measured under aerobic conditions, µg/mL. (MIC = 16000 µg/mL) (Blaskovich MAT et al, 2019). azelaic acid exerts a bacteriostatic effect on aerobic and anaerobic bacteria (Nazzaro-Porro M et al., 1983). A clinical trial recorded a 224-fold decrease in the population of Micrococcaceae and a 30-fold decrease in the density of Propionibacterium sp. on the skin after the application of 20% azelaic acid cream (Bladon PT et al., 1986)

Another active compound found in this study is Astragalin. Research on the effect of Astragalin and chlorogenic acid on the inflammatory model of sheep endometrial epithelium cells (SEECs) induced by Escherichia coli showed that Astragalin and chlorogenic acid could reduce the inflammatory response caused by *E. coli* by inhibiting the activation of the toll-like receptor 4 (TLR4) signaling pathway. /nuclear factor-kappa B (NF-κB) (Hu X et al., 2020).

Astragalin (kaempferol 3-glucoside) is a well-known natural flavonoid for its various pharmacological applications such as anti-inflammatory, antioxidant, neuroprotective, cardioprotective, anti-obesity, anti-osteoporotic, anti-cancer, anti-ulcer and anti-diabetic properties. Astragalin carries out the activities mentioned above by regulating and modulating various molecular targets such as transcription factors (NF-κB, TNF-α, and TGF-β1), enzymes (iNOS, COX-2, PGE2, MMP-1, MMP-3, MIP-1α, COX-2, PGE-2, HK2, AChe, SOD, DRP-1, DDH, PLCγ1, and GPX), kinases (JNK, MAPK, Akt, ERK, SAPK, IκBα, PI3K, and PKCβ2), cell adhesion proteins (E-cadherin, vimentin PAR-2, and NCam), apoptotic and antiapoptotic proteins (Beclin-1, Bcl-2, Bax, Bcl-xL, cytochrome c, LC3A/B, caspase-3, caspase -9, procaspase-3, procaspase-8, and IgE), and inflammatory cytokines (SOCS-3, SOCS-5, IL-1β, IL-4, IL-6, IL-8, IL-13, MCP-1, CXCL -1, CXCL-2, and IFN-γ) (Riaz A et al., 2018).

Another active compound found in this study is syringic acid. Other studies have shown that syringic acid affects Staphylococcus epidermidis by reducing the biofilm mass by 50%–70%, depending on the strain's genotype. The effect of anti-biofilm inhibition was analyzed using crystal violet staining against three S. epidermidis strains with different biofilm genotypes (Minich A et al., 2022).

The limitation of this study is that the antibacterial effect of the active compounds found in the LC-HRMS test has not been carried out directly in vitro or in vivo but only based on the literature. The results of this study can be used as the basis for further research at the in vivo stages of experimental animals and clinical trials.

CONCLUSION

Based on the LC-HRMS test, the active compounds that have an effect on bacteria in the leaves of *Dracontomelon dao* (Blanco) Merr. & Rolfe are quercetin-3β-D-glucoside, anacardic acid, D-Glucosamine, azelaic acid, choline, astragalin, quercetin, luteolin , syringic acid. The active ingredients have the potential as antibacterial and anti-inflammatory, which can be added to pharmaceutical preparations.

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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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