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Formulation Test of *Dracontomelon dao* (Blanco) Merr. & Rofe Leaf Extract Powder as an Anti-Diarrheal *Escherichia coli* Method In Vivo

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Abstract: *Dracontomelon dao* (Blanco) Merr. & Rofe is one of the medicinal plants used in the community. This study aims to analyze the effectiveness of *Dracontomelon dao* (Blanco) Merr. & Rofe extract powder as an antidiarrheal against *Escherichia coli* in vivo. *Dracontomelon dao* (Blanco) Merr. & Rofe leaf comes from Hulu Sungai Utara, South Kalimantan, Indonesia, and is macerated with 96% ethanol—*Dracontomelon dao* extract powder (Blanco) Merr. & Rofe is made with three parts thick extract, four parts Lactose, and four parts Starch. Experimental rat were conditioned to develop diarrhea and were treated with extract powder with varying doses per kg of body weight, namely 250 mg, 500 mg, and 750 mg. After treatment on day 6, rat recovered from diarrhea in the 750 mg dose treatment group as much as 78%, while the positive control total recovery on day 6 reached 100%. After treatment on day 6, the number of *Escherichia coli* colonies in rat feces decreased, namely the lowest in the 750mg dose treatment group, 276 colonies/gr. This research concludes that *Dracontomelo dao* (Blanco) Merr. & Rofe leaf extract powder is effective as an anti-diarrhea against *Escherichia coli* in experimental rat. Further research is needed on human test subjects to obtain anti-diarrhea drugs with safer and more effective formulations.
Keywords: Anti diarrhea; *Dracontomelon dao* (Blanco) Merr. & Rofe; formulation test; in vivo method.

INTRODUCTION

Dracontomelon dao (Blanco) Merr. & Rofe is a medicinal plant of the Dayak tribe in Indonesia (Falah et al., 2013) and an ingredient in traditional Chinese medicine (Li, Y et al., 2017). Ethnopharmacological studies of medicinal plants show that traditional plants in parts of Indonesia can be an alternative for treating diarrhea problems (Mustofa et al., 2018). There is research that states that medicinal plants, one of which is lotus, contain active compounds that can inhibit the growth of *Escherichia coli*, which causes diarrhea because they contain alkaloids, tannins, saponins, glycosides, flavonoids, triterpenoids, and steroids (Fitrial, Y. et al., 2012).

Previous research showed that the ethanol extract of *Dracontomelon dao* (Blanco) Merr leaves. & Rofe showed inhibitory activity against *Staphylococcus aureus, Bacillus subtilis* (Khan & Omoloso, 2002), and Pseudomonas aerogenosa (Wu et al., 2015). Research by Liu et al. (2014) stated that the most potent antibacterial effect was found in the ethyl acetate fraction of *Dracontomelon dao* (Blanco) Merr leaves. & Rofe against *Escherichia coli* with a concentration of 50%. Meanwhile, research by Zhao et al. (2015) stated that the ethyl acetate fraction of *Dracontomelon dao* (Blanco) Merr leaves produced an antibacterial effect. & Rofe

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inhibits *Staphylococcus aureus* with the highest flavonoid content, namely 41.86%. The main components of these flavonoids are Cianidanol, L-Epicatechin, Quercetin, and Luteolin (Zhao et al., 2015). Research by Li et al. (2017) stated that Dracontomelo dao leaf extract has antibacterial potential against *Escherichia coli* due to flavonoids in the plant (Li et al., 2017).

Dracontomelon dao (Blanco) Leaves Merr. Rofe from Kalimantan, Indonesia, has demonstrated the antibacterial effect of ethanol extract, ethyl acetate, petroleum ether, and chloroform against *Escherichia coli* in vitro (Dwiyanti et al., 2022). The antibacterial test using the 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. Further research in 2023 stated that the active compounds contained in the leaves of *Dracontomelon dao* (Blanco) Merr. & Rolfe are quercetin-3β-D-glucoside, anacardic acid, Dglucosamine, azelaic acid, choline, astragalin, quercetin, luteolin, syringic acid. The active ingredient of ethanol extract of leaves (*Dracontomelon dao* (Blanco) Merr. & Rofe) has the potential as an antibacterial and anti-inflammatory, which can be added to pharmaceutical preparations (Dwiyanti et al., 2023). However, there is still limited research on the effects of *Dracontomelon dao* (Blanco) Merr. & Rolf on experimental animals, especially on antidiarrheal potential, so this research aims to analyze the effectiveness of *Dracontomelon dao* (Blanco) Merr. & Rofe powder as an antidiarrheal against *Escherichia coli* method in vivo.

MATERIALS AND METHODS

This research was carried out at the Bacteriology Laboratory of the Health Analysis Department of the Health Polytechnic of the Ministry of Health, Banjarmasin, and the Biochemistry Laboratory of Lambung Mangkurat University in Banjarbaru and the Banjarbaru Veterinary Center. The method used is experimental. The independent variable used was the dose of *Dracontomelon dao* (Blanco) Merr powder. & Rofe in rat, while the dependent variable is the number of healthy rat returning from diarrhea.

The *Escherichia coli* bacteria used were cultures isolated from the Martapura River until colonies were obtained that were metallic green in color and had biochemical test characteristics in accordance with Murray's 2017 book (Murray, P. R., 2017). The rats used were white Winstar (Rattus norvegicus) (Irfayanti, N. A, 2023). *Dracontomelon dao* (Blanco) Leaves Merr. & Rofe comes from Banjar Regency, South Kalimantan, Indonesia.

The leaves are dried and ground into 500 gr powder and soaked using 96% ethanol with a ratio of 1 part (gr) of *Dracontomelon dao* (Blanco) Merr. & Rofe powder and three parts (mL) of solvent, left for three days, then filtered (Prayoga, T., 2020). The filtrate formed is evaporated using a water bath (Thermo) at a temperature of 50-60^{o}C. The extraction results in a thick extract (Sani, R.N., 2013) amounting to 81 grams with a total content of 16.2%—*Dracontomelon dao* (Blanco) Merr. & Rofe Powder is made by mixing three parts thick extract, four parts Lactose, and four parts Starch. For a thick extract weighing 6 grams, add 1 ml of 96% alcohol, 8 grams of Lactose, and Starch, each mixed into the thick extract; add little by little while grinding until evenly mixed and the size of the powder is made the same—dosage of *Dracontomelon dao* (Blanco) Merr. & Rofe powder in rats per Kg of body weight is 250 mg, 500 mg, and 750 mg. Rats were grouped based on the dose given, namely groups A (250mg), B (500mg), C (750mg), D (positive control / given

amoxicillin), E (negative control / no treatment) with the number of rats in each group being nine rats.

Rats were conditioned to have diarrhea by administering 108 doses of *Escherichia coli* bacterial suspension with a probe directly into the rats' abdomens for six consecutive days until all the rats had diarrhea. After diarrhea occurred, each group was given an extract formulation drug according to a predetermined dose, with the provisions that group A was given a dose of 250 mg/Kg of rat body weight, group B was given a dose of 500 mg/Kg of rat body weight, group C was given a dose of 750 mg /Kg rat body weight, group D was given Amoxicillin 250 mg/Kg rat body weight, group E was given distilled water, each dose and drug, and distilled water were diluted to a volume of 1 ml, and given three times a day using a probe directly into the abdomen for six days, the number of rat that recovered from diarrhea after treatment was observed and recorded. The number of *Escherichia coli* bacteria in the rat feces (/gr) was measured using the spread plate method using Eosin Methylen Blue agar media (Merck), counting the metallic green colonies. The feces taken are all the feces that are collected in filter paper, which is placed as a base in the rat's cage. Approval for this research was obtained from the Ethics Commission of the Banjarmasin Health Polytechnic, Ministry of Health, with No.XX/EC/KEPK-S1/05/2021.

RESULTS AND DISCUSSION

Research results in observation data on rats treated with *Dracontomelon dao* (Blanco) Merr. & Rofe powder and observation data on the average number of *Escherichia coli* colonies of rat faeces on days 1 to 6 after treatment can be seen in Table 1 and Table 2.

Table 1. Observation Data on Rats Treated with *Dracontomelon dao* (Blanco) Merr. & Rofe Powder

Dracontomelon dao (Blanco) Merr. & Rofe Powder Concentration (/Kg Body Weight)	Number of Rats /group	Number of Rats Recovering from Diarrhea				Note
		Day 3	Day 4	Day 5	Day 6	
250mg	9	3 (33%)	6 (67%)	4 (44%)	4 (44%)	
500mg	9	1 (11%)	3 (33%)	4 (44%)	4 (44%)	
750mg	9	4 (44%)	6 (67%)	5 (56%)	7 (78%)	Day 6: 1 subject died
Positive control	9	3 (33%)	5 (56%)	6 (67%)	9 (100%)	
Negative control	9	3 (33%)	2 (22%)	3 (33%)	2 (22%)	Day 3: 1 subject died

Research results after administering *Dracontomelon dao* (Blanco) Merr powder. & Rofe, on days 0, 1, and 2, all experimental animals still experienced diarrhea. On days 3 to 6, some rat recovered from diarrhea. On day 6, the highest

number of rat recovered from diarrhea in the 750 mg dose treatment group was 78%, while the total number of rat recovered from diarrhea on day 6 reached 100%.

Table 2. Observation Data on the Average Number of *Escherichia coli* Colonies of Rat Faeces on Days 1 to 6 After Treatment

Dracontomelon dao (Blanco) Merr. & Rofe Powder Concentration (/Kg Body Weight)	Average Number of *Escherichia coli* Colonies in Rat Faeces (/gr)						
	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
250mg	4685	4480	4018	2168	1447	752	665
500mg	5634	1963	1930	1650	1218	819	328
750mg	4582	1469	1417	1386	1302	538	276
Positive Control	6803	2912	2357	858	825	243	192
Negative Control	6850	4375	4867	4422	4164	3977	3704

Research results before administering *Dracontomelon dao* (Blanco) Merr. & Rofe powder saw that the average number of *Escherichia coli* colonies in rat feces exceeded 4000; however, after administering *Dracontomelon dao* (Blanco) Merr. & Rofe powder saw that the number of *Escherichia coli* colonies that grew decreased, namely the lowest in the 750mg dose treatment group at 276 colonies/gr.

Dracontomelon dao (Blanco) Merr. & Rofe leaf powder, in this study, used ethanol extract of *Dracontomelon dao* (Blanco) Merr. & Rofe Leaf. It was known in our previous research that the chemical compound content of the ethanol extract of *Dracontomelon dao* (Blanco) Merr. & Rofe 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 is triterpenoids. 669.8 ± 2,000(mg/ml). Our previous research showed that the ethanol extract of *Dracontomelon dao* (Blanco) Merr. & Rolfe leaves showed the best inhibition against the growth of *Escherichia coli*, with the largest inhibition zone of 24 mm at a concentration of 500 mg/ml. After administration of *Dracontomelon dao* (Blanco) Merr. & Rofe powder in experimental rat on the sixth day, the highest number of rat recovered from diarrhea in the 750mg dose treatment group, 78%, while the positive control total recovery on day 6 reached 100% (Table 1) after treatment with *Dracontomelon dao* (Blanco) Merr. & Rofe powder , in experimental rat after day 6, the number of *Escherichia coli* colonies in the feces decreased, namely the lowest in the 750 mg dose treatment group, 276 colonies/g (Table 2).

The presence of phytochemical components that have antibacterial activity in the powder is thought to play a role in inhibiting the growth of *Escherichia coli* in the digestive tract of experimental animals. The presence of tannins in the extract can inhibit protease activity and degrade mucin, which functions to prevent the attachment of pathogens to intestinal epithelial cells (Budiarti & Mubarik, 2007)

Apart from its antibacterial role, phytochemical components also play a role in the defense of the intestinal mucosa, as saponins are known to have anti-ulcer activity through the formation of protection on the surface of the intestinal mucosa (Aguwa & Lawal, 1988). Tannin and tannic acid denature proteins by forming a complex (protein-tannate); this complex forms a layer on the intestinal mucosa and makes it more resistant, so gastric secretion is simultaneously reduced (Aniagu et al., 2005).

Flavonoids have anti-inflammatory activity (Otshudi et al., 2000) and antioxidants and can strengthen the mucosal defense system by stimulating gastric mucus secretion (Aniagu et al., 2005). Apart from that, flavonoids also have anti-diarrhea activity by inhibiting contractions in the ileum by inhibiting the influx of extracellular Ca2+ and releasing intracellular Ca2+ (Wang et al., 2005). Flavonoids can also inhibit the production of prostaglandins, which are known to cause fluid accumulation in the intestines and digestive tract motility (Agunu et al., 2005; Gunakkunru et al., 2005). Agunu et al. (2005) concluded that the antidiarrheal mechanism of a medicinal plant could be through the antimicrobial activity contained in the plant or through the inhibition of prostaglandins.

Apart from that, the experimental animal's body itself has defenses to fight pathogens that enter the body. Cell secretions such as mucus, lysozyme, and phospholipase influence the viability of bacteria in the digestive tract, which can have both negative and positive impacts. The intestinal epithelial cells of experimental animals can also excrete antimicrobials in the form of peptides/proteins, which can make bacterial cell membranes permeable and leak (Wilson, 2005).

Another research on medicinal plant extracts with phytochemical content which also functions as an anti-diarrhea is beluntas leaves. Beluntas leaf extract provides an antidiarrheal effect at doses of 150 and 300 mg/kg body weight; at a dose of 600 mg/kg body weight, it provides an effect comparable to loperamide. (Nurhalimah, H., et al, 2015). Meanwhile, our research used *Dracontomelon dao* (Blanco) Merr. & Rofe powder provides an antidiarrheal effect at 250 mg and 500 mg/kg bw at a dose of 750 mg/kg bw, almost comparable to amoxicillin. A limitation of this study is that the number of *Escherichia coli* in the cecum (lumen) of rats was not measured. However, the number of *Escherichia coli* in rats feces describes the intestinal microbes' condition.

CONCLUSION

The results of this study were that *Dracontomelo dao* (Blanco) Merr. & Rofe Leaf extract powder was effective as an anti-diarrhea drug against *Escherichia coli*. This was proven by the 78% recovery of rats from diarrhea on the 6th day after treatment (dose 750 mg/kg body weight). These results require further research on human test subjects as an anti-diarrhea drug with a safer and more effective formulation.

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CONFLICT OF INTEREST

The author and team have declared no conflict of interest.

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