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The Effectiveness of Ethyl Acetate Extract From Breadfrui (*Artocarpus Altilis*) Leaves to Inhibit Diarrhea-Causing Bacteria

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ABSTRACT

Diarrhea is a health problem that commonly occurs in developing countries. Bacteria that cause diarrhea are among others *Escherichia coli*, *Salmonella typhosa* and *Staphylococcus aureus*. This research aimed to investigate the diameters of inhibition zones of breadfruit leaf (*Artocarpus altilis*) ethyl acetate extract in different concentrations against the growth of *Escherichia coli*, *Salmonella typhosa* and *Staphylococcus aureus*. The research applied an experimental laboratory by using a post-test control group design. This research was performed at the Bacteriology Laboratory of STIKES Nasional by using the diffusion disk method. The research showed the radical zone diameters against *Escherichia coli* with the concentrations of 20%, 40%, 60%, 80%, and 100%, were 6.16 mm, 6.41 mm, 6.74 mm, 7.49 mm, and 7.79 mm, respectively. The inhibition zones against *Staphylococcus aureus* were 8.15, mm 9.43 mm, 10.29, 10.38 mm and 11.42 mm, while against *Salmonella typhosa* were 7.94 mm, 8.87 mm, 10.15 mm, 10.26 mm, and 11.23 mm, respectively. The results of the ANOVA test showed the *p*-value=0.00 and the results of the LSD test revealed the differences in the inhibition effects of *Artocarpus altilis* leaf extract against the growth of *Escherichia coli*, *Salmonella typhosa* and *Staphylococcus aureus*. This study concludes that concentration variations of *Artocarpus altilis* leaf ethyl acetate extract can inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhosa*.

Keywords:
extract of ethyl acetate,
Artocarpus altilis leaves,
Escherichia coli,
Salmonella typhosa,

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INTRODUCTION

Diarrhea is a health problem that can cause extraordinary events in developing countries (WHO, 2013). The survey conducted by the Ministry of Health of the Republic of Indonesia (2017) reported 7,077,299 people suffering from diarrhea in Indonesia (Kementrian Kesehatan RI, 2018). This disease can be caused by a number of bacteria, including *Escherichia coli*, *Salmonella typhosa* and *Staphylococcus aureus*.

Qu, et al., (2016) found that 10.7% of diarrhea cases in children were caused by a bacterial infection and the two most common causing bacteria were *Escherichia coli* (4.6%) and *Salmonella* (4.3%). The research conducted by Sung et al. (2014) investigated that 31.7% of gastroenteritis cases in children were attributed to *Staphylococcus aureus*. The presence of bacteria in the body is due to food contamination. The bacteria get into the food in various ways, making the food unhealthy for consumption (Hussain, 2016).

Diarrhea attributed to bacteria can be treated with antibiotics. However, the uncontrolled use of antibiotics produces a negative impact, such as triggering an increase in bacterial resistance (CDC, 2013). According to Qu et al., (2016) *Salmonella* showed 40-60% resistance to ampicillin, nalidixic acid, streptomycin and sulfisoxazole. The research by Dwidjoyono (2018) reported that *Staphylococcus aureus* identified in the samples obtained from Dr. Soeradji Tirtonegoro General Hospital had a high level of resistance to penicillin antibiotics. Meanwhile, Sumampouw (2018) found that *Escherichia coli* bacteria in the samples of diarrhea patients were resistant to chloramphenicol, ampicillin, amoxicillin, and tetracycline. The increasing pattern of bacterial resistance to antibiotics demands an improvement in the development of alternative treatments for bacterial infection.

One alternative to natural ingredients potential to have antibacterial activity is the breadfruit (*Artocarpus altilis*) leaf. *Artocarpus altilis* leaf is used to treat various diseases, including hepatitis, enlarged spleen and diabetes. The antibacterial contents of *Artocarpus altilis* leaf are steroid, phenol and flavonoid compounds that can be used as antibacterial agents (Bempa, 2011). The research conducted by Retnaningsih (2016) concluded that the ethanol extract of *Artocarpus altilis* leaf can inhibit the growth of *S. dysentriae* with the largest inhibitory diameter of 8.81 mm at a concentration of 100%, and *E coli* with the diameter of 11.3 mm. Ethyl acetate is a semi-polar solvent that can attract polar and nonpolar compounds. Ethyl acetate is expected to maximize the withdrawal of secondary metabolite compounds (Putri, 2013). The present study used ethyl acetate solvent in breadfruit leaf extract to obtain tannins, flavonoids and saponins. Based on the above background, the study was conducted to investigate the effectiveness of the antibacterial activities of ethyl acetate extract in breadfruit (*Artocarpus altilis*) leaf against *Escherichia coli*, *Salmonella typhosa* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Research Design

This study was carried out in the Bacteriological Laboratory of STIKES Nasional with an experimental-analytical method using a post-test control group design.

Research Population and Samples

The population in this research was *Artocarpus altilis* leaves obtained from Sawahan, Karanganyar, using a quota sampling technique.

Research Procedure

Extraction of Artocarpus altilis leaves

The dried *Artocarpus altilis* leaves were ground and sieved with 20 mesh. 500 grams of *Artocarpus altilis* leaf powder was immersed using ethyl acetate solvent with a ratio of 1:9 for five days. The residue yielded was extracted again using ethyl acetate with a ratio of 1: 2.5 for two days. The filtrate was concentrated using a rotary evaporator at 50°C until a thick extract was produced.

Isolation of the test bacterial culture

Escherichia coli, *Salmonella typhosa*, and *Staphylococcus aureus* bacteria were obtained from the isolation of patients with diarrhea. Feces samples of people suffering from diarrhea were fertilized using brain heart infusion media and blood peptone broth and then incubated at 37°C for 24 hours. The fertilization results were isolated using a blood agar plate and Mac Conkey media and incubated at 37°C for 24 hours. Colonies of *Escherichia coli*, *Salmonella typhosa* and *Staphylococcus aureus* suspects were subjected to biochemical tests and culture using nutrient agar media.

Phytochemical test of Artocarpus altilis leaf ethyl acetate extract

Flavonoid test: 2 ml of thick extract was measured and added with 0.5 g powder of Magnesium powder. 1 ml of HCl positive result was indicated by the formation of red, yellow, or orange colors (Zohra et al., 2012). *Saponin test:* 0.5 ml of extract measured, added with 20 ml of distilled water, and then shaken for 15 minutes. The positive result was indicated by the emergence of foam as high as 1 cm (Begum et al., 2014). *Tannin test:* A total of 1 ml of extract was added with 2 drops of 1% $FeCl_3$ solution. A positive result was obtained at the temperature of 37°C. After 15 minutes, a sterile antimicrobial disc paper was prepared and added with 20 µl of ethyl acetate extract of *Artocarpus altilis* leaves, negative control (DMSO4), and positive control (*Ciprofloxacin* 5 µg). It was then incubated at the temperature of 37°C for 24 hours. A positive result was indicated by the presence of a bluish-green to black color (Putri, 2013). *Inhibition test Artocarpus altilis against Escherichia coli, Salmonella typhosae and Staphylococcus aureus*

Pure bacterial samples of *Escherichia coli*, *S. dysentriae*, *Salmonella typhosa*, *Staphylococcus aureus* were inoculated into 0.9% NaCl and compared for the turbidity by using Mc Farland no. 0.5 standard. The suspension of bacteria and NaCl was inoculated into Muller Hinton agar plate media using the flattening method and then incubated for 15 minutes at the temperature of 37°C. The inhibition zone diameter was observed and the radical zone was measured using calipers.

Data analysis

The data obtained from the results of the inhibition test on ethyl acetate extract obtained from breadfruit (*Artocarpus altilis*) leaves against *Escherichia coli*, *S. thyposa* and *Staphylococcus aureus* were analyzed using one-way ANOVA and then the LSD test was performed to determine the significant differences.

RESULTS AND DISCUSSION

In this study, ethyl acetate was used as a semi-polar solvent that was able to attract polar, semi-polar and non-polar secondary metabolites (Putri, 2013). The maceration process was carried out twice to obtain more secondary metabolites. Stirring in the maceration process was done repeatedly to equalize the concentration of the solution to avoid too quick saturation (Wardhani & Sulistyani, 2015). The results of the phytochemical test can be seen in Table 1. Table 1 shows that ethyl acetate extract from *Artocarpus altilis* contains flavonoids, saponins, and tannins. This is in line with the result of the study by Bempa (2016) that *Artocarpus altilis* leaf extract contains flavonoids, saponins, and tannins. Flavonoids can destroy bacterial cell walls by inhibiting DNA gyrase synthesis and bacterial metabolism (Dewi, Joharman, & Budiarti, 2013). Saponins can inhibit cell membrane permeability so that the bacterial cell components come out and the cells become lysis (Kurniawan & Aryana, 2015). Tannins damage the membrane of bacterial cells. The binding of tannins with iron in bacterial cells can interfere with the process of DNA precursor reduction (Rahman et al., 2017).

The effectiveness of antibacterial effects of *Artocarpus altilis* leaves on *Escherichia coli, S. thyposa,* and *Staphylococcus aureus* is presented in Tables 2, 3, and 4. Table 2 demonstrates that the average inhibition zone was 7.73 mm with 100% concentration. This was far lower than the average inhibition zone diameter of positive control. Table 3 presents that the average inhibition zones of ethyl acetate extract from *Artocarpus altilis* leaves against the growth of *S. thyposa* was 11.23 mm at 100% concentration. This result was also far lower than the inhibition zone diameter of the positive control group.

Table 1. The results of the phytochemical test on ethyl acetate extract from *Artocarpus altilis* leaves

No	Active Compound	Result	Conclusion
	Flavonoids	The orange-yellow color was formed.	+
	Saponins	The stable foam was formed for five minutes.	+
	Tannins	The brownish-green color was formed.	+

Table 2. Inhibition zone diameter of ethyl acetate extract from *Artocarpus altilis* leaves against the growth of *Escherichia coli*

Replication	Inhibition zone diameter (radical) in each concentration (mm)					Negative control (mm)	Positive control (mm)
	20%	40%	60%	80%	100%		
1	6.00	6.00	6.47	7.22	7.06	6.00	25.71
2	6.00	6.00	6.62	7.47	7.24	6.00	23.43
3	6.00	6.31	6.70	7.50	7.72	6.00	23.25
4	6.21	6.69	6.76	7.53	7.98	6.00	25.11
5	6.30	6.73	6.92	7.60	8.14	6.00	24.21
6	6.47	6.74	6.98	7.64	8.21	6.00	25.12
Average	6.16	6.41	6.74	7.49	7.73	6.00	24.72

Table 3. Inhibition zone diameter of ethyl acetate extract from *Artocarpus altilis* leaves against the growth of *Salmonella typhosa*

Replication	Inhibition zone diameter (radical) in each concentration (mm)					Negative control (mm)	Positive control (mm)
	20%	40%	60%	80%	100%		
1	8.35	8.92	10.57	10.36	11.18	6	24.86
2	7.64	8.73	9.84	9.94	10.93	6	23.73
3	7.43	8.51	9.93	10.12	11.05	6	23.74
4	7.95	8.86	10.14	10.27	11.28	6	24.13
5	8.07	9.03	10.37	10.69	11.39	6	24.63
6	8.17	9.19	10.03	10.17	11.57	6	24.53
Average	7.94	8.87	10.15	10.26	11.23	6.00	24.27

Table 4. Inhibition zone diameter of ethyl acetate extract from *Artocarpus altilis* leaves against the growth of *Staphylococcus aureus*

Replication	Inhibition zone diameter (radical) in each concentration (mm)					Negative control (mm)	Positive control (mm)
	20%	40%	60%	80%	100%		
1	8.56	9.95	10.72	10.84	12.43	6.00	29.28
2	8.70	9.74	10.69	10.72	12.65	6.00	29.19
3	8.10	9.40	10.57	10.68	11.69	6.00	28.84
4	7.91	9.18	10.39	10.50	11.11	6.00	29.67
5	7.89	9.18	9.79	9.95	10.56	6.00	28.81
6	7.73	9.08	9.52	9.55	10.03	6.00	27.98
Average	8.15	9.43	10.29	10.38	11.42	6.00	28.97

As presented in Table 4, the average inhibition zone of ethyl acetate extract in *Artocarpus altilis* leaves on the growth of *Staphylococcus aureus* was 11.42 mm at 100% concentration, which was far lower than the inhibition zone diameter of positive control. The difference in inhibition effects of ethyl acetate extract of *Artocarpus altilis* leaves can be seen from the results of the one-way ANOVA test illustrated in Table 5. Table 5 presents the differences in the inhibition effect of ethyl acetate extract of *Artocarpus altilis* leaves against *E.coli, Salmonella typhosa* and *Staphylococcus aureus.* Further, the identification results of groups having different significance levels using the LSD test are demonstrated in Table 6. Table 6 demonstrates the significantly different inhibition effects of ethyl acetate extract of *Artocarpus altilis* leaves between *E.coli* and both *Salmonella typhosa* and *Staphylococcus aureus* but the inhibition effects between *Salmonella typhosa* and *Staphylococcus aureus* did not appear to be significantly different.

Table 5. The results of the one-way ANOVA test on the differences in the inhibition effects of ethyl acetate extract of *Artocarpus altilis* leaves in *E.coli, Salmonella typhosa* and *Staphylococcus aureus*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	169.183	2	84.591	73.496	.000
Within Groups	100.134	87	1.151		
Total	269.317	89			

Table 6. The results of the LSD test on the differences in the inhibition effects of ethyl acetate extract of *Artocarpus altilis* in *E.coli*, *Salmonella typhosa* and *Staphylococcus aureus*

(I) Bacteria	(J) Bacteria	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Escherichia coli	*Salmonella typhosa*	-2.78233*	.27700	.000	-3.3329	-2.2318
	Staphylococcus aureus	-3.02000*	.27700	.000	-3.5706	-2.4694
Salmonella typhosae	*Escherichia coli*	2.78233*	.27700	.000	2.2318	3.3329
	Staphylococcus aureus	-.23767	.27700	.393	-.7882	.3129
Staphylococcus aureus	*Escherichia coli*	3.02000*	.27700	.000	2.4694	3.5706
	Salmonella typhosa	.23767	.27700	.393	-.3129	.7882

This study used 10% DMSO negative control, which was selected from the solvent used to produce variations in extract concentrations and ensure the result of bacterial inhibition effects obtained from the active material of *Artocarpus altilis.* The inhibition zone diameter produced from negative control was 6 mm (Tables 2, 3, and 4), which was similar to the diameter of the blank disk. Thus, it can be concluded that 10% of DMSO do not have antibacterial effects.

The positive control used *Ciprofloxacin* 5 µg to compare the inhibition effect produced by *Artocarpus altilis.* Tables 2, 3, and 4 present the average inhibition zones of *Ciprofloxacin 5 µg* against *Escherichia coli, Salmonella typhosa,* dan *S.aureus* that reached 24.72 mm, 24.27 mm, and 28.97 mm, respectively. The average diameters of the inhibition zones produced by *Artocarpus altilis* in 100% concentration were 7.73 mm, 11.23 mm, and 11.42 mm. According to CLSI (2018), the inhibition effects of *Ciprofloxacin* 5 µg against *Salmonella typhosa* dan *S.aureus* (Tables 3 and 4) were categorized as sensitive, while the inhibition effect of *Ciprofloxacin* 5 µg against *Escherichia coli w*as considered intermediate (Babii et al., 2018).

As shown in Table 6, the inhibition effects of *Artocarpus altilis* leaf ethyl acetate extract against *Escherichia coli* were different than that against *Salmonella typhosa* dan *S.aureus*. *Escherichia coli* are Gram-negative bacteria having complex cell wall structures and the ability to produce colonies. Colonies created by Escherichia coli consist of CFA (Colonization Factor Antigen), CS (*Coli* Surface Antigen), or PCF (Putation Colonization Factor). This ability increases the formation of quorum sensing that causes the bacteria to be resistant to antibiotics (Silviani, Puspitaningrum, 2015). The Escherichia coli resistance is also influenced by the ability to produce β-lactamases, carbapenemases, 16S rRNA methylases, plasmid-mediated quinolone resistance (PMQR) genes and MCR genes. Thus, the bacteria are resistant to cephalosporins, carbapenems, aminoglycosides, fluoroquinolones, and polymyxins (Poirel et al., 2018). Mussa & Al-mathkhury (2018) found that *E.coli* is 44% resistant against *Ciprofloxacin*.

The inhibition effects of *Artocarpus altilis* leaves against *Staphylococcus aureus* were higher than against *Escherichia coli*. The flavonoids contained in *Artocarpus altilis* leaves were polar so that the antibacterial activity in Gram-positive was higher than that in Gram-negative. The compound plays a role in destroying the phospholipid layer of the cytoplasmic membrane. The H^+ ions in the flavonoid work in the polar group of bacteria. *Staphylococcus aureus* is a Gram-positive bacterium with a polar cell membrane that makes *Artocarpus altilis* easier to penetrate than Gram-negative bacteria (Lutpiatina, 2015).

CONCLUSION

This study concludes that ethyl acetate extract obtained from *Artocarpus altilis* leaves can inhibit the growth of *Escherichia coli* and *Staphylococcus aureus*. The inhibition effects of ethyl acetate extract of *Artocarpus altilis* against *Escherichia coli* and *Staphylococcus aureus* appear to be different.

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