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In Vitro Analysis of Guava (Psidium Guajava Linn) and Papaya (Carita Papaya L.) Leaves Extract Combination Against Escherichia Coli Growth

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KEYWORDS

Antibacterial;
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ABSTRACT:

Introduction: Incident diarrhea is still high in Indonesia; one reason for diarrhea is the existence of infection by Escherichia coli (E. coli). Still, many deep Indonesian societies resolve diarrhea by using herbal medicines such as guava leaves and papaya leaves.

Objectives: The objective of this study is to analyze in vitro analysis of guava and papaya leaf extract as antibacterial.

Methods: Actual experimental post-test only control group design, housed in the Laboratory of biomedical, Faculty of Medicine, Universitas Swadaya Gunung Jati Cirebon. Study this using a petri dish for growth bacteria E. coli, then give treatment study with combination leaf extract with concentrations of 100%, 75%, 50%, 25%, and 12.5%.

Results: The results showed that a combination leaf extract of 75% and 100% effectively inhibited growth comparable to E. coli bacteria with the effectiveness of ciprofloxacin. A 50% concentration can inhibit the growth of E. coli will but is far under the efficacy of ciprofloxacin.

Conclusions: Therefore, combination leaf extract with a minimum concentration of 75% effectively inhibits the growth of E. coli.

1. Introduction

Escherichia coli is a group of gram-negative bacteria enteric (Enterobacteriaceae) i.e normal normal flora found in big human intestines. Bacteria will reduce characteristic pathogens if it is outside the intestine, the standard location of the place being, and other rare places inhabited by bacteria. Escherichia coli can cause an emergency infection of the ducts, urinary tract bile, and other areas in the cavity stomach. Escherichia coli is also the reason for diarrhea and infection channel urinary [1]. Escherichia coli is classified As a type pathogen commensal and diarrhoea or enteric [2]. Diaregenic E. coli (DEC) causes various human diseases, including plague diarrhea, gastroenteritis, and disease-default food, especially in developing countries. About 2 million Dead because E. coli pathogens occur yearly, of which 90% occur in children aged <5 years [3].

Indonesia is one of the countries with diverse culture, including the nation Indonesia often use the traditional plant. Plant traditional could be obtained from various natural power sources like plants [4]. Plant this used to treat different type of disease, between the usual herbal plants worn to treat diarrhea is guava leaves (Psidium guajava Linn) and papaya leaves (Carita Papaya L) [5]. Election

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Table 1. The inhibition zone of combination leaf extract with various concentrations against E. coli (in mm).

Combination Leaf Extract	Research Preparation			Average
	Preparation I	Preparation II	Preparation III	
100%	45	46	46	45.7
75%	36	36	37	36.3
50%	11	12	11	11.3
25%	0	0	0	0
12.5%	0	0	0	0
Positive controls (ciprofloxacin)	50	51	49	50
Negative control	0	0	0	0

ingredient experience or plant as treatment also aims to reduce resistance to antibiotics; resistance multi medicine to use antibiotics is a big problem, so to get over it requires formulating drug antimicrobial new, especially those from source power nature [6].

Guava leaf contains substances that are possibly antibacterial and inhibit the development of E. coli; among others, there is substance Tannins, Flavonoids, oil volatile (E Globulus), and Alkaloids. Guava leaf contains flavonoids which can change the micromorphology of Escherichia coli and Staphylococcus aureus. The antibacterial mechanism of flavonoids in guava leaf on bacteria is by damaging the structure and function of the membrane cell [7,8]. Content tannins in guava could narrow nets and walls of the cell to obstruct permeability cells; alkaloids block part arrangement peptidoglycan in cells bacteria, and oil essential inhibit growth bacteria [9]. Guava leaf effectively inhibits the growth of bacteria E. coli; increasing the concentration will produce more potent power inhibition [10].

Papaya leaf is one lot of the plants used by the Indonesian people to treat diarrhea [11]. Part of the plant this is often used as a traditional drug is the leaves because they contain papain enzyme [12]. Papaya leaf has several compound antimicrobials, such as papain, tannins, alkaloids, flavonoids, phenols, saponins, and steroids. The compounds could inhibit bacteria Bacillus subtilis, Salmonella typhi, Pseudomonas fluorescens, Clostridium tetani, Escherichia coli, Proteus vulgaris, Staphylococcus aureus, and Shigella dysenteriae [13]. Papaya leaf plays an antibacterial, anticancer, antioxidant, antidiabetic, anti-inflammatory, antimalarial, anti-dengue, and healing process in wounds [14].

2. Material and Methods

This study is quantitative with the experimental post-test-only control group design method. Study this carried out in the Laboratory of biomedical, Faculty of Medicine, Universitas Swadaya Gunung Jati Cirebon.

Implementation pre-research: Preparation of E. coli planting medium was carried out on mac Conkey agar media, and E. coli culture was made with the autoclave's help. The manufacturing process extracts guava leaves and papaya leaf conducted with use method Maceration is an extraction process method cold with the used solvent 96% ethanol. Ciprofloxacin as a control positive was chosen due to research previously antibiotics this still sensitive in inhibiting E. coli growth. Control negative on research this using DMSO.

Implementation research: first of all, made E. coli culture in Mac Conkey agar, E. Coli inoculated in Mac Conkey agar as much as 1 ml into 7 Petri dishes with a diameter of 10 cm, in each Petri dishes made three wells, each 5mm diameter for a given treatment. Each well-given combination leaf extract of 100%, 75%, 50%, 25%, 12.5%, ciprofloxacin, and DMSO as control negative.

3. Results

Table 1 shows the average diameter of the resistance combination of guava leaf and papaya leaf to the growth of E. coli.

Based on the Kolmogorov-Smirnov normality test, the inhibition zone was measured for each group treatment normally distributed because they have p-value = 0.385, so $p > \alpha$, with $\alpha = 0.05$. Whereas Levene's test results for the diameter of the inhibition zone have p-value = 0.021, $p < \alpha$, with $\alpha = 0.05$. This means the inhibition zone diameter data's variance is not homogeneous ($p < 0.05$).

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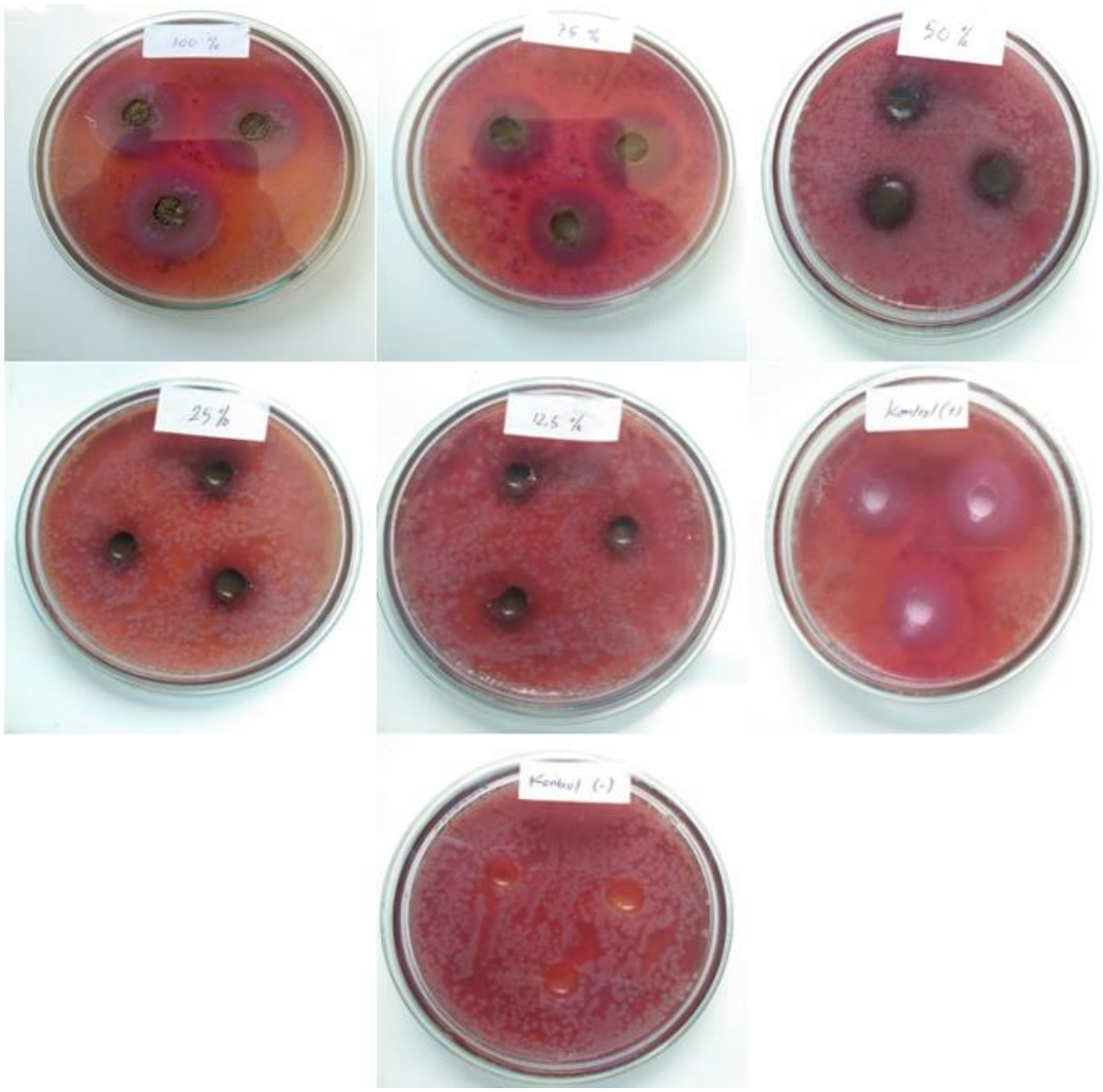

Figure 1. The diameter of the inhibition zone (Incubated at 30°C for 24 hours).

Then to know whether or not the difference between group treatments Kruskal Wallis test was performed. Based on Kruskal Wallis test results, inhibition zone diameter data show a difference in mean between-group treatment with sig. 0.000 (sig. < 0.05).

Three of the 5 Groups' treatments (combination leaf extract of 100%, 75%, and 50%) show different results by significance with group control positive and control negative with p-value = 0.000. So that p-value <, with = 0.005. Whereas two groups with a concentration of 25% and 12.5% were no different by significance with group control because p-value = 1.0000, so that p >.

4. Discussion

The result study data obtained shows that combination leaf extract can inhibit the growth of E. coli. As seen in Table 1, the combination of concentration high 50%, 75% and 100% can hinder the growth of E. coli. Whereas combination concentrations low 25% and 12.5% can inhibit the growth bacteria E. coli. This result is also suitable with research by Zhang et al [8]. Stating that flavonoid extract from guava is an agent with natural

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Table 2. Normality Test.

Variable	Mean Different (SD)	p-value	Homogeneity
Concentration 100%	45.67 (0.577)	0.385	
Concentration 75%	36.33 (0.577)	0.385	
Concentration 50%	11.33(0.577)	0.385	
Concentration 25%	0	n/a	0.021
Concentration 12.5%	0	n/a	
Ciprofloxacin	50.00(1.000)	0.175	
Control	0	n/a	

antimicrobial potential [8,15]. Tuntun [12], in his research, also obtained data that extracted papaya leaf influenced E.Coli growth.

Combination leaf extract in high concentrations (100% and 75%) can approach obstacles to the antibiotic ciprofloxacin. In comparison, a combination leaf extract of 50% has low power inhibition to the E. coli growth. Whereas combination leaf extract with low concentrations (25% and 12.5%) no have the ability power resistor to grow bacteria E. coli. Condition thereby by study [16,17], obtained research data that the more significant concentration, the more substantial diameter power inhibition formed in the extracted single nor extract combination [18].

Control-positive antibiotics ciprofloxacin can inhibit E. coli growth; where is it? Antibiotics are still sensitive to inhibition growth bacteria. Whereas control-negative DMSO no has the ability inhibition E. coli growth. Ciprofloxacin is still susceptible to E. coli growth. While ampicillin, amoxicillin, and tetracycline are already resistant to E. coli growth [19].

Table 3. Post Hoc Test 5 Groups Treatment

Group 1	Group 2	Mean Difference (IJ)	SE (Standard Error)	P-Value	95% Confidence Intervals	
					Lower Bound	Upper bound
concentration100	Concentration 75	9,333 *	,436	,000*	8.40	10,27
	concentration50	34,333 *	,436	,000*	33,40	35,27
	concentration25	45,667 *	,436	,000*	44,73	46,60
	concentration12.5	45,667 *	,436	,000*	44,73	46,60
	ciprofloxacin	-4,333 *	,436	,000*	-5.27	-3.40
	control negative	45,667 *	,436	,000*	44,73	46,60
concentration75	concentration100	-9,333 *	,436	,000*	-10.27	-8.40
	concentration50	25,000 *	,436	,000*	24.06	25.94
	concentration25	36,333 *	,436	,000*	35,40	37.27
	concentration12.5	36,333 *	,436	,000*	35,40	37.27
	ciprofloxacin	-13,667 *	,436	,0008	-14.60	-12.73
	control negative	36,333 *	,436	,000*	35,40	37.27
concentration50	concentration100	-34,333 *	,436	,000*	-35.27	-33.40
	concentration75	-25,000 *	,436	,000*	-25.94	-24.06
	concentration25	11,333 *	,436	,000*	10,40	12,27
	concentration12.5	11,333 *	,436	,000*	10,40	12,27
	ciprofloxacin	-38,667 *	,436	,000*	-39.60	-37.73
	control negative	11,333 *	,436	,000*	10,40	12,27
concentration25	concentration100	-45,667 *	,436	,000*	-46.60	-44.73

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Group 1	Group 2	Mean Difference (IJ)	SE (Standard Error)	P-Value	95% Confidence Intervals	
					Lower Bound	Upper bound
	concentration75	-36,333 *	,436	,000*	-37.27	-35,40
	concentration50	-11,333 *	,436	,000*	-12.27	-10.40
	concentration12.5	,000	,436	1,000	-,94	,94
	ciprofloxacin	-50,000 *	,436	,000*	-50.94	-49.06
	control negative	,000	,436	1,000	-,94	,94
concentration12.5	concentration100	-45,667 *	,436	,000*	-46,60	-44.73
	concentration75	-36,333 *	,436	,000*	-37.27	-35,40
	concentration50	-11,333 *	,436	,000*	-12.27	-10.40
	concentration25	,000	,436	1,000	-,94	,94
	ciprofloxacin	-50,000 *	,436	,000*	-50.94	-49.06
	control negative	,000	,436	1,000	-,94	,94
ciprofloxacin	concentration100	4,333 *	,436	,000*	3.40	5.27
	concentration75	13,667 *	,436	,000*	12.73	14.60
	concentration50	38,667 *	,436	,000*	37.73	39.60
	concentration25	50,000 *	,436	,000*	49.06	50.94
	concentration12.5	50,000 *	,436	,000*	49.06	50.94
	control negative	50,000 *	,436	,000*	49.06	50.94
control negative	concentration100	-45,667 *	,436	,000*	-46,60	-44.73
	concentration75	-36,333 *	,436	,000*	-37.27	-35,40
	concentration50	-11,333 *	,436	,000*	-12.27	-10.40
	concentration25	,000	,436	1,000	-,94	,94
	concentration12.5	,000	,436	1,000	-,94	,94
	ciprofloxacin	-50,000 *	,436	,000*	-50.94	-49.06

* The mean difference is significant at the p-value < 0.05.

5. Conclusion

A combination leaf extract in high 75% and 100% can inhibit E. coli growth and is comparable with the ability of ciprofloxacin antibiotic. A combination leaf extract of 50% can inhibit E. coli growth, but when compared with ciprofloxacin still far lower in inhibiting growth bacteria E. coli. A low 25% and 12.5% combination could inhibit E. coli growth.

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Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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