JURNAL KESEHATAN LINGKUNGAN
JURNAL DAN APLIKASI TEKNIK KESEHATAN LINGKUNGAN

ISSN 1829-9407 (Print)
ISSN 2581-0898 (Online)

Volume 22, No. 1, January 2025 ; Page: 69-78 DOI: https://doi.org/10.31964/jkl.v22i1.906

ETHANOLIC EXTRACT GEL OF PLANCHONIA VALIDA LEAVES AS AN ANTIBACTERIAL AGENT AGAINST YERSINIOSIS INFECTION

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Article Info

Article history:

Received February 13, 2024
Revised February 25, 2024
Accepted January 27, 2025

Keywords:

Planchonia valida
Yersinia enterocolitica
Antimicrobial Activity
Ethanol Extract
Inhibition Zone

ABSTRACT

Ethanolic Extract Gel Of Planchonia Valida Leaves As An Antibacterial Agent Against Yersiniosis Infection. *Planchonia valida (puting plant) contains ethanol, which is beneficial for treating skin infections and digestive problems, and is capable of inhibiting the growth of gram-negative bacteria such as Yersinia enterocolitica. This study aims to evaluate the adaptability of P. valida extract to Y. enterocolitica in vitro. This experimental research was statistically analyzed using Statistical Product and Service Solutions (SPSS). The adaptability of Y. enterocolitica was observed on nutrient agar (NA) medium in petri dishes treated with P. valida extract at concentrations of 30%, 40%, 50%, 60%, 70%, and 80%, with aquadest as the negative control and chloramphenicol as the positive control. Observations were conducted over three incubation periods (24, 48, and 72 hours) to monitor bacterial growth rate and adaptation. ANOVA results showed p-values at all incubation periods were below the significance level (α = 0.05), indicating significant differences in bacterial adaptation across treatments. The inhibition zone test revealed that the 40% extract concentration was as effective as higher concentrations (50%, 60%, 70%, and 80%). Although the 80% concentration exhibited the largest inhibition zone, its bactericidal effect was not statistically different from the lower concentrations. These results demonstrate the potential of Planchonia valida leaf extract as an effective antimicrobial agent and support further research into its development for the treatment or prevention of Y. enterocolitica infections.*

INTRODUCTION

Indonesia possesses rich biodiversity, with thousands of plant species, making it one of the world's most plant-diverse archipelagic nations. The tropical rainforests of Indonesia host approximately 30,000 plant species and over 400 species of trees, including wild plants.[1] Among them, around 800 species are known to have medicinal properties, although only about 800–1,200 species have been utilized as traditional medicinal plants. Medicinal plants play a crucial role, especially for rural communities with limited access to healthcare services. For generations, communities have recognized and used medicinal plants to relieve pain, enhance immunity, repair damaged organs, and inhibit bacterial growth. Knowledge about medicinal plants has been passed down through generations[2] and is used to treat various diseases.[3] These plants are natural, relatively safe with minimal side effects, and have scientifically proven benefits, leading to the growing use of natural ingredients for medicine.[4] One such plant is the leaf of *Planchonia valida*.

Planchonia valida is commonly found in humid areas, along riverbanks, and on alluvial plains and mountains at altitudes ranging from 0 to 1000 meters, with annual rainfall between 1,100 mm and 3,800 mm.[5] The plant is reported to contain alkaloids, flavonoids, tannins, saponins, and steroids. This is supported by research.[6] showing that phytochemical screening of powdered leaf tips of *Planchonia valida* tested positive for all these chemical compounds. It is assumed that these compounds can inhibit bacterial growth and are potentially useful in treating gastrointestinal infections such as diarrhea, dysentery, and other digestive tract infections. One such pathogen is *Yersinia enterocolitica*.

Yersinia enterocolitica is a Gram-negative bacterium that causes gastrointestinal infections. According to[7], gastrointestinal infections can be caused by several Gram-negative bacteria, including *Y. enterocolitica*. Yersiniosis is a zoonotic gastrointestinal infection caused by *Yersinia enterocolitica* and transmitted to humans through animal reservoirs such as rabbits, sheep, cattle, horses, dogs, cats, and other rodents, with pigs being the primary reservoir.

Based on the background above, this study aims to investigate the adaptive capacity of *Yersinia enterocolitica* upon exposure to *Planchonia valida* leaf extract at concentrations of 30%, 40%, 50%, 60%, 70%, and 80%, focusing on the bacterial incubation period, with the objective of identifying the antibacterial potential of compounds contained in *Planchonia valida*.[8]

MATERIALS AND RESEARCH METHODS

This study is an experimental research aiming to assess the adaptive capacity of *Yersinia enterocolitica* when exposed to *Planchonia valida* leaf extract at concentrations of 30%, 40%, 50%, 60%, 70%, and 80%, by observing specific growth rates and generation times over a 3 × 24-hour incubation period, thus confirming the antibacterial potential of *Planchonia valida* leaves.

The *Planchonia valida* leaves were cleaned and air-dried in open air without direct sunlight, then ground into powder using a blender. Equipment used includes incubator, laminar air flow (LAF), Petri dishes, test tubes, 100 mL measuring cylinders, inoculating loop, Bunsen burner, autoclave, hot plate, dropper pipette, blender, rubber bands, cover paper, tweezers, magnifying glass, vortex mixer, calipers, matches, stove, tray, scissors, pipette pump, magnetic stirrer, jam jar, metal stirrer, and writing tools.

To prepare the *Planchonia valida* leaf extract: clean and dry the leaves, chop into small pieces, weigh, and blend into powder. Soak the powder in methanol for about 3 hours. Filter the suspension through clean cloth and filter paper, then collect the filtrate in a beaker glass. Evaporate the extract using a controlled-temperature evaporator until no alcohol remains. The resulting extract serves as the mother stock solution.

Pure cultures of *Yersinia enterocolitica* were isolated on NA medium and incubated for 24 hours. The experiment included eight groups: positive control using chloramphenicol, negative control using distilled water, and treatments with *Planchonia valida* extract at concentrations of 30%, 40%, 50%, 60%, 70%, and 80%. All samples were incubated for 3 × 24 hours. Inhibition zones were measured every 24 hours.

NA agar plates were prepared and labeled. Paper discs (2 cm in diameter) were soaked in the bioherbal extract for 15 minutes. Microbes were inoculated onto NA media using cotton swabs. The soaked discs were placed on the inoculated media according to each treatment concentration. All plates were incubated at 37°C, and inhibition zones were observed and measured every 24 hours for 4 days. All observations were recorded.

The growth ability of *Yersinia enterocolitica* was assessed based on the presence and size of inhibition zones. A larger zone indicates stronger antibacterial activity of *Planchonia valida* extract against the microorganism. The inhibition zones varied due to differences in active compound concentrations in the extract.[8]

RESEARCH RESULTS AND DISCUSSION

The results of the fungistatic efficacy test of Planchonia valida leaf extract against Yersinia enterocolitica in vitro can be seen in the following figure:

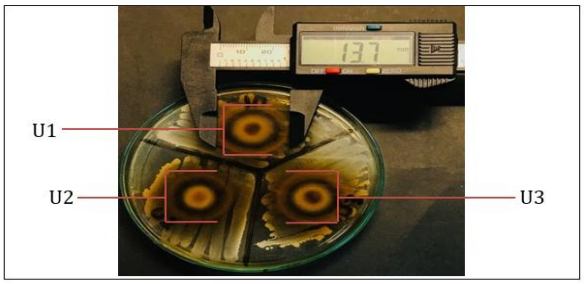

Figure 1. *Planchonia valida*

Figure 1 illustrates the formation of an inhibition zone, with the highest antibacterial activity observed at 80% concentration of Planchonia valida extract, resulting in inhibition diameters of 4.7 mm (U1), 5.0 mm (U2), and 5.2 mm (U3). The clear zones formed are influenced by several factors, particularly the properties of the antimicrobial compounds. The ability of antimicrobial agents to inhibit microbial growth is crucial. Chemical properties such as molecular structure, polarity, and solubility can significantly affect interactions with microorganisms. The concentration of antimicrobial compounds in the plant extract is a critical factor.[4]

As shown in Figure 1, variations in Planchonia valida extract concentration affect the size of the inhibition zones. Each microorganism exhibits different sensitivity levels to antimicrobial substances—some may be more resistant or more susceptible to specific compounds. The 80% concentration of Planchonia valida extract produced the largest inhibition zone, indicating that at this concentration, the antimicrobial compounds within the extract exert the most potent inhibitory effect on the tested microorganism. The clear zones depicted in Figure 1 are further supported by the observation data summarized in Table 2.

The research data on the adaptive response of Yersinia enterocolitica to putat (Planchonia valida) leaf extract, as indicated by the diameter of the inhibition zones at each concentration over a 3 × 24-hour incubation period, is presented in Table 1.

Table 1. Adaptive Capacity of Yersinia enterocolitica

Inhibition Zone Diameter of *Yersinia enterocolitica* Growth (mm)									
		Treatment (%)							
times	**N**	(-)	(+)	30	40	50	60	70	80
	1	0	9.9	4.2	5.1	6.3	4.8	6.3	7.2
	2	0	10.8	5.3	6.2	6	6.4	5.4	5.9
24 hours	3	0	10.9	4.3	6	3	5.3	7	6.6
	averange	0	10.53	4,6	5.76	5,1	5.5	6.23	6.56
	1	0	8.7	4.1	5.0	6.2	4.2	5.1	6.7
	2	0	9.4	4.3	6	5.8	5.1	4.8	5.2
48 hours	3	0	9.1	4	5.4	3	4.8	6.8	5.6
	averange	0	9,06	4,13	5,46	5	4,7	5,56	5,83
	1	0	8.6	3.4	4.8	4.4	4	4.2	4.7
	2	0	8.3	3.8	4.2	5.1	4.8	3.5	5
72 hours	3	0	8.8	3.6	2.8	2	3.4	4.5	5.2

Table 2 shows that the adaptive capacity of Yersinia enterocolitica was most affected at the 80% concentration of Planchonia valida leaf extract. Concentrations of 30%, 40%, 50%, 60%, and 70% fall into the moderate category, where inhibition zones were observed. A notably large inhibition zone was recorded in the positive control group, with an average diameter of 10.53 mm at 24 hours, 9.00 mm at 48 hours, and 8.56 mm at 72 hours of incubation. In contrast, no inhibition zone was found in the negative control group. Based on the data

presented in Table 1, it can be inferred that Planchonia valida leaf extract is capable of inhibiting the growth of Yersinia enterocolitica.

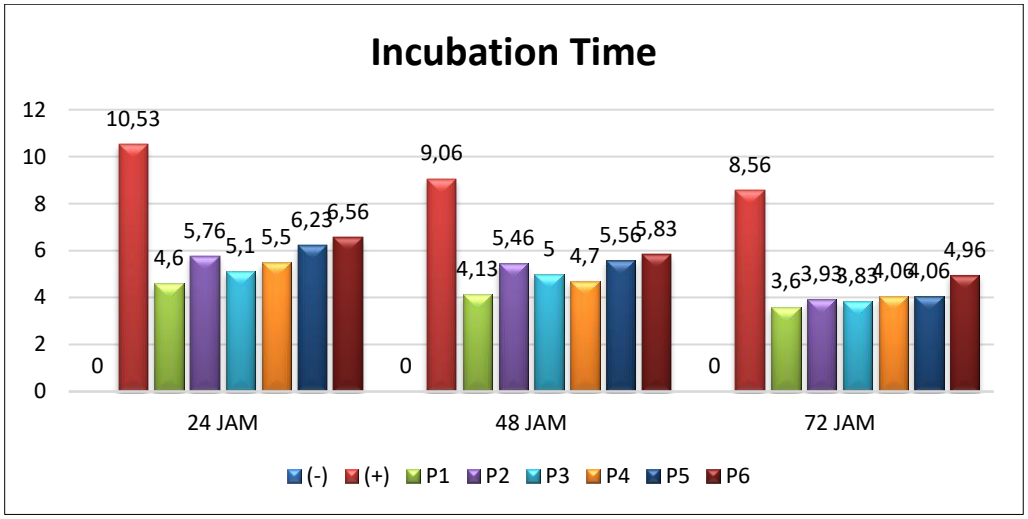

Figure 2. Adaptive Capacity of *Yersinia enterocolitica*

(1: Negative Control, 2: Positive Control, 3: Mean Inhibition Zone at 30%, 4: Mean Inhibition Zone at 40%, 5: Mean Inhibition Zone at 50%, 6: Mean Inhibition Zone at 60%, 7: Mean Inhibition Zone at 70%, 8: Mean Inhibition Zone at 80%)

The treatment with Planchonia valida leaf extract showed the greatest inhibitory effect at the 80% concentration, with a mean inhibition zone of 6.56 mm. Based on the one-way ANOVA statistical test, the effect of Planchonia valida leaf extract on the growth of Yersinia enterocolitica is presented in Table 2.

Table 2. Analysis of Variance (ANOVA) Results on the Inhibition Zone of Yersinia enterocolitica Growth

	Df	Incubation Time											
		1 x 24 Jam				2 x 24 Hours				3 x 24 Hours			
		Sum of Squares	Mean Square	F	Sig.	Sum of Squares	Mean Square	F	Sig.	Sum of Squares	Mean Square	F	Sig.
Between Groups	7	174.870	24.981	32.852	.000	130.816	18.688	27.601	.000	113.570	16.224	28.094	.000
Within Grups	16	12.167	.760			10.833	677			9.240	578		
Total	23	187.036				141.650				122.810			

Table 2 illustrates the adaptive capacity of *Yersinia enterocolitica* during incubation periods of 24, 48, and 72 hours. The one-way ANOVA test was conducted to determine whether the data followed a normal distribution. The result showed an F-value of 32.852 with a p-value = 0.000. Since the p-value < α (α = 0.05), the data indicate that the ethanolic extract of *Planchonia valida* leaves had a highly significant effect on *Yersinia enterocolitica*. At 48 hours of incubation, the F-value was 27.601 with a p-value = 0.000. Given that p-value < α (α = 0.05), the alternative hypothesis (H_1) is accepted while the null hypothesis (H_0) is rejected at a 1% level of significance. This confirms that the extract has a highly significant effect. Similarly, at 72 hours of incubation, the F-value was 28.094 with a p-value = 0.000, again supporting the acceptance of H_1 and the rejection of H_0 at the 1% level of significance. The ethanolic bioherbal extract of *Planchonia valida* significantly affected *Yersinia enterocolitica* across all incubation periods studied.

This finding reinforces the effectiveness of the extract in inhibiting bacterial growth and highlights its potential application as an antimicrobial agent against *Yersinia enterocolitica.*[11] The inhibitory interaction may be mediated by metabolic mechanisms. Although the specific chemical composition of the extract is not detailed, it is presumed that active compounds such as alkaloids, flavonoids, tannins, and terpenoids in *Planchonia valida* leaves contribute to the antimicrobial effect. Tannins are known to form complexes with proteins and bacterial cell membranes, potentially inhibiting growth and reproduction.[12] Flavonoids may interact with cell membranes and key enzymes, disrupt bacterial membrane integrity, and inhibit vital biological activities.

There is also the possibility of interference with major bacterial metabolic mechanisms, including protein synthesis, energy metabolism, and cell wall biosynthesis, all of which contribute to the inhibition of *Yersinia enterocolitica* proliferation. Additionally, immunomodulatory responses may enhance the host immune system's activity against the bacteria. [13]

The mechanism by which the ethanolic bioherbal extract of *Planchonia valida* inhibits *Yersinia enterocolitica* proliferation likely involves complex interactions between its active compounds and the pathogen. Tannins, as one of the principal components, have the potential to form complexes with bacterial proteins and membranes. This may inhibit enzymatic activities and damage membrane integrity, thereby suppressing vital bacterial functions.[14] Flavonoids, commonly found in plants, can also compromise membrane integrity and inhibit essential enzymes, resulting in growth suppression. [15]

Terpenoids, which also possess antimicrobial properties, may contribute to growth inhibition by damaging cell membrane structures or interfering with bacterial biosynthetic pathways. Additionally, the plant extract's potential immunomodulatory effects may enhance immune responses, creating an unfavorable environment for bacterial growth. Overall, the molecular interaction between the extract's active compounds and *Yersinia enterocolitica* components presents a potential mechanism for bacterial growth inhibition, [16] These findings indicate that the ethanolic bioherbal extract of *Planchonia valida* has a significant inhibitory effect on *Yersinia enterocolitica*. The effective concentration for inhibiting *Yersinia enterocolitica*—a causative agent of yersiniosis—was further analyzed using Duncan's post-hoc test, as presented in Table 3.

Table 3. Duncan's 1% Post-Hoc Test Results on the Inhibition Zone of *Yersinia enterocolitica* Growth

Treatment	N	Notasi Subset for alpha = 0.05										
		1x24 hours				2x24 hours				3x24 hours		
		1	2	3	4	1	2	3	4	1	2	3
P1 (-)	3	000[a]				000[a]				000[a]		
P3 (30%)	3		4.600[b]				4.133[b]				3.600[b]	
P5(50%)	3		5.100[b]	5.100[c]			4.700[b]	4.700[c]			3.833[b]	
P6(60%)	3		5.500[b]	5.500[c]			5.000[b]	5.000[c]			3.933[b]	
P4(40%)	3		5.767[b]	5.767[c]			5.467[b]	5.467[c]			4.067[b]	
P7(70%)	3		6.233[b]	6.233[c]			5.567[b]	5.567[c]			4.067[b]	
P8(80%)	3			6.567[c]				5.833c			4.967[b]	
P2(+)	3				10.533[d]				9.067[d]			8.567[c]
Sig.		1.000	053	080	1.00	1.000	071	146	1.000	1.000	066	1.000

Large inhibition zones, indicating strong antibacterial activity against Yersinia enterocolitica, were observed at concentrations of 40%, 50%, 60%, 70%, and 80%. Among these, the 80% concentration exhibited the largest inhibition zone. However, statistical analysis revealed that the bactericidal effect of the 80% concentration did not significantly differ from those of the 40%, 50%, 60%, and 70% concentrations, as evidenced by identical notations in the Duncan post-hoc test results presented in Table 3. This similarity in notation was consistent across the 24-hour, 48-hour, and 72-hour incubation periods, indicating that the 40% concentration possesses a comparable ability to inhibit Yersinia enterocolitica. It can

therefore be concluded that the 40% concentration of Planchonia valida leaf extract is the most effective for inhibiting the growth of Yersinia enterocolitica.

The metabolic analysis that underlies the effective concentration in inhibiting Yersinia enterocolitica involves a detailed understanding of the active compounds contained within the Planchonia valida leaf extract. Identifying these active compounds is essential for elucidating the antibacterial mechanisms involved. [17] Furthermore, the ability of these compounds to penetrate bacterial cell membranes plays a key role, as it determines the extent to which they can interact with cellular components and inhibit growth. The analysis must also consider interactions between active compounds and critical metabolic pathways in Yersinia enterocolitica, such as disruption of protein or nucleic acid synthesis.

Understanding the reactivity of these compounds under various environmental conditions, such as pH and temperature, is also vital to ensure their stability and optimal activity. [18] Of course, the optimal concentration of active compounds must be established to achieve antimicrobial effects without inducing harmful toxicity. Additionally, the analysis should take into account the potential for bacterial resistance development and the interaction of the compounds with normal gut microbiota to prevent detrimental imbalances. Through this comprehensive approach, a deeper understanding of the antibacterial mechanisms of Planchonia valida leaf extract at its effective concentration can be achieved, optimizing its use in inhibiting the growth of Yersinia enterocolitica.[19-23]

CONCLUSIONS AND RECOMMENDATIONS

Based on the experimental data, it can be concluded that the ethanolic leaf extract of Planchonia valida significantly affects Yersinia enterocolitica across various incubation periods (24 hours, 48 hours, and 72 hours). Statistical analysis using ANOVA indicated that the p-values at each incubation period were lower than the significance level ($\alpha = 0.05$), suggesting significant differences in the bacterial adaptive response to the treatment. The research hypothesis (H_1) was accepted, while the null hypothesis (H_0) was rejected at all incubation stages.

Furthermore, the inhibition zone test results showed that the 40% concentration of Planchonia valida extract exhibited comparable effectiveness to higher concentrations (50%, 60%, 70%, and 80%). Although the 80% concentration produced the largest inhibition zone, its antibacterial activity was not statistically different from the lower concentrations, as demonstrated by statistical analyses across all time intervals. Therefore, the 40% concentration is considered the most effective in inhibiting Yersinia enterocolitica growth.

Metabolic analysis revealed that the active compounds in Planchonia valida leaf extract play a critical role in bacterial growth inhibition through their influence on metabolic pathways and cell penetration mechanisms. Understanding the extract's environmental reactivity and the potential for bacterial resistance provides a strong basis for elucidating its antibacterial mode of action.

As a follow-up, it is recommended that further research be conducted, including toxicity testing of the extract on human cells to evaluate its safety, the development of topical formulations such as gels for clinical application, and the isolation of dominant active compounds to optimize dosage and further elucidate specific mechanisms of action. This approach is essential to ensure that the antimicrobial potential of Planchonia valida can be translated into effective and safe therapeutic applications..

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