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Antibacterial Activity of Pasak Bumi Stem (*Eurycoma longifolia* J.) Extract against *Salmonella typhi*

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ABSTRACT

Typhoid fever is caused by consuming food or water contaminated with *Salmonella typhi*. The disease develops from bacterial infection through the consumption of contaminated sustenance and drink. The bacterium can cause bacteremia, which is bacteria living in the blood, penetrating the mucosal epithelium of the small intestine and entering the lymphatic flow. Therefore, this study aimed to assess the potential inhibitory effect of ethanol extracts derived from stems of *Eurycoma longifolia* (pasak bumi) on the growth of *Salmonella typhi* bacteria. Compounds such as alkaloids, saponins, flavonoids, terpenoids, and tannins in pasak bumi stems possessed antibacterial properties. Extracts were made using 96% ethanol at varying concentrations (10%, 20%, 30%, 40%, 50%) with distilled water and chloramphenicol as negative and positive control. The result showed that pasak bumi stem extracts inhibited *Salmonella typhi*, with increasing efficacy at higher concentrations and statistical analysis reported significant differences between all treatment groups ($p<0.001$). Average zone diameter was 0 mm and 23.10 mm for negative and positive control, as well as 2.75 mm, 4.10 mm, 5.24 mm, 6.98 mm, and 8.55 mm for 10%, 20%, 30%, 40% and 50% extracts, respectively. This study provided verification of antibacterial effects of pasak bumi stem ethanol extracts against *Salmonella typhi*.

Keywords: antibacterial; disc diffusion; *Eurycoma longifolia* J.; extract; *Salmonella typhi*.

INTRODUCTION

Typhoid fever is a significant global health issue, particularly in developing regions such as Africa, America, Southeast Asia, and Western Pacific. In the context of this fever, WHO (2018) estimated 11-20 million cases and 128,000-161,000 annual deaths worldwide. Indonesia experiences a prevalence of typhoid fever, ranging from 350 to 810 cases per 100,000 individuals. (Afifah, , 2019) According to the Central Kalimantan Provincial Health Office, the period between January and December 2018 experienced 896 documented cases of clinical typhoid fever and 1,644 individuals with positive Widal test (Samputri et al., 2020). This disease stems from infection by *Salmonella typhi* bacterium (Afifah, , 2019), a rod-shaped, Gram-negative pathogen with peritrichous flagella that cannot form spores (Syahrurachman et al.,, 2019). People typically contract *S. typhi* by ingesting food or water contaminated with the bacteria, which breach the small intestinal mucosa to access the lymphatic system and bloodstream (Sharma et al., 2022) resulting in systemic infection.

The main treatment for typhoid fever is antibiotics but the first-line chloramphenicol drug is no longer used due to the high recurrence rate and dangerous side effects in the form of bone marrow depression, causing inhibition of the formation of red blood cells and aplastic anemia (Gunawan et al. , 2020). Based on previous study, there is an increase in antibiotic resistance to *Salmonella typhi* bacteria. A result of a previous study showed that 3 out of 30 samples tested were resistant to chloramphenicol due to chromosomal mutations or the exchange of genetic material through transformation, transduction, and plasmid conjugation (Sandika& Suwandi2017). The use of drugs from natural ingredients was developed to overcome these problems. Pasak Bumi is one of the plants in the forests of Southeast Asia that traditional communities have widely used to heal various diseases. The plant can grow up to 10 m tall with a monopodial branching pattern of one main stem, as shown in Figure 1. Pasak bumi is a shrub with a hard and strong woody stem used to protect the body from free radicals. The roots are adopted

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Figure 1. Pasak Bumi plant (*Eurycoma longifolia* Jack.)

as antimalarial, heat-lowering, dysentery, ulcer, antitumor, increased stamina, and increased male vitality due to aphrodisiac properties (Nurani et al., 2017). Meanwhile, the flowers are commonly used to treat stomachaches, headaches, and bone pain. The bark and stem also treat fever, mouth ulcers, bone pain, and stomach worms and as a tonic after childbirth. Stem is commonly used as an itch remedy and has antimalarial activity. The leaves are adopted as a traditional medicine to cure malaria, stomach ulcers, infections of the gums, and sexual diseases such as gonorrhea and syphilis. Pasak bumi plants are consumed as a postpartum tonic, antihypertensive, antitumor, antibacterial, anti-inflammatory, antipyretic, treat stomach pain, ulcers, malaria, and dysentery (Siburian &Marlinza, 2009).

Previous studies have shown that ethanol extracts of pasak bumi can restrict the growth of certain Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus* and *Escherichia coli* (Farouk & Benafri, 2007). Further analyses verified that these extracts, with antibacterial effects, contain bioactive alkaloids, phenols, flavonoids, saponins, tannins, and terpenoids (Mahardini, 2018). Considering the properties, this work examined activity of pasak bumi stem ethanol extracts, against *Salmonella typhi* bacteria.

MATERIALS AND METHODS

This study received ethical permission (No. 32/UN24.9/LL/2023) from the Health Research Ethics Committee (KEPK) of the Faculty of Medicine, Palangka Raya University. The type of study used was true experimental with a posttest-only control group design.

Materials

The material used are pasak bumi stems (*Eurycoma longifolia* J.), 96% ethanol, distilled water, *Salmonella typhi* bacterial culture, Salmonella Shigella Agar (SSA) media, Mueller Hinton Agar (MHA) media, and 0.9% NaCl, vortex, rotary evaporator, incubator, autoclave, object glass, cover glass, microscope, crystal violet, lugol, safranin, 95% alcohol, petri dish, ose needle, paper disc, bunsen flame, test tube, and caliper.

Methods

Working Procedure

Preparation of 96% ethanol extract of pasak bumi stem

Pasak bumi stem plants were obtained from Bawan village, Banama Tingang sub-district, Pulang Pisau district, Central Kalimantan province. Subsequently, a determination test was carried out at the Lambung Mangkurat University FMIPA Laboratory with certificate No. 107/LB.LABDASAR/III/2023.

Approximately 2 kg of Pasak bumi stems with a height of 150-200 cm were taken, washed, cleaned and dried in the sun for 48 hours, before blending until smooth. Furthermore, 1007 grams of pasak bumi stems were macerated using 96% ethanol. The filtered extracts were concentrated using a rotary evaporator and evaporated in a water bath to achieve a thick extract. This was diluted with distilled water to obtain 10%, 20%, 30%, 40%, and 50% concentrations as shown in Table I. The yield of extracted material was determined by dividing the final extract weight (g) by the initial simplisia weight (g) and multiplying it by 100% (Susanty et al., 2019).

$$\%\ \text{Yield of extract} = \frac{\text{the weight of exctract (g)}}{\text{the weight of crude (g)}} \times 100\ \%$$

Phytochemical screening of 96% ethanol extract of pasak bumi stem

Qualitative and quantitative phytochemical tests of 96% ethanol extract were carried out at the Lambung Mangkurat University FMIPA Laboratory with certificate No. 89/UN8.1.17.2.2/PP/2023.

Antibacterial Test

Bacterial Identification Test

A bacterial identification test was carried out using gram staining, and test bacteria were *Salmonella typhi* with a reddish color. Therefore, the bacteria were Gram-negative and had a bacillus or rod-shaped form, as shown in Figure 2.

Preparation of Test Bacteria

Salmonella typhi ATCC 14028 bacteria used was provided by a lab in Samarinda with a Certificate of Test Results (SHU) from Thermo Fisher Scientific. Bacteria were cultured on Salmonella-Shigella Agar (SSA) media, and a Gram stain identification test was performed. In addition,

Table I. Extract Concentration Formulation

Ingredients	Formulation				
	Concentration 10%	Concentration 20%	Concentration 30%	Concentration 40%	Concentration 50%
Pasak Bumi Stem Extract	1 g	2 g	3 g	4 g	5 g
Aquades	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL	ad 10 mL

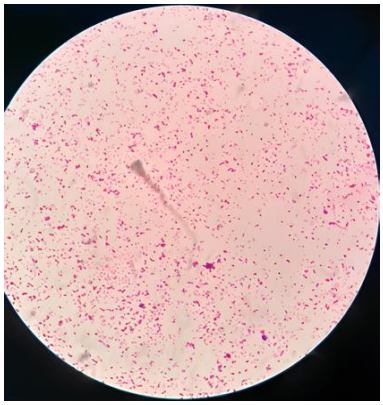

Figure 2. Results of Bacterial Identification Test with Gram Stain

the samples were taken as one ose isolate and mixed into a tube containing 3 mL of 0.9% NaCl solution to make a suspension of test bacterial culture. A vortex was used to homogenize the bacterial suspension for 15 seconds to turbidity matching the 0.5 McFarland standard, equivalent to 1.5 x 10^8 CFU/mL (Julianti, 2022)) ((Tazkia, 2020; Vebiola et al., 2020).

Antibacterial Test

Antibacterial testing used the disc diffusion method on Mueller Hinton Agar (MHA) medium inoculated with *Salmonella typhi*. Discs containing pasak bumi stem extracts were inserted into the inoculated agar and incubated at 37°C for 24 hours. Meanwhile, each of the seven treatment groups was tested in quadruplicate. After incubation, the clear zone was observed and measured by calipers, and the disc diameter was subtracted to determine the growth inhibition area.

Diameter of zone of inhibition = Total diameter - Diameter of disc paper

Data Analysis

Data was obtained in the form of inhibition zone diameter and were statistically tested using SPSS software. Furthermore, the samples were first tested using the normality, Saphiro-Wilk test, homogeneity, and Levene test. Analysis of Variance (ANOVA) test was conducted with a significant level of $p<0.05$ and continued with the Post Hoc test (LSD).

RESULT

Extraction of Pasak Bumi Stem

The yield of pasak bumi stem extract was 2.58% with a viscous weight of 26 grams as shown in Table II. The different extract yield values are influenced by the method and the type of solvent used. This study used the maceration extraction method due to its simplicity and widespread usage. The selected method and equipment offer advantages such as simplicity, cost-effectiveness, and the absence of heat treatment.

Phytochemical Screening Results

Phytochemical screening quantitatively detected 144.68 mg/ml of alkaloids (orange with Dragendorf, white precipitate with Meyer, brownish-orange with Wagner), 87.30 mg/ml saponins (foaming when shaken), 21.500 mg/ml flavonoids (orange coloration), 244.300 mg/ml terpenoids (two layers formed), and 2.329 mg/ml tannins (greenish-black color). Positive test for alkaloids include color changes with different reagents. Meanwhile, saponins produce foam, and flavonoids and steroids yield orange and green hues. Terpenoids separate into two phases, and tannins show a characteristic greenish-black pigment.

Antibacterial activity

Antibacterial activity of Eurycoma longifolia (pasak bumi) stem extracts at varying concentrations was tested against Salmonella typhi, compared to distilled water negative and chloramphenicol positive controls as shown in Figure 3. Calipers measured growth inhibition zones and the average diameters were 2.75 mm (10% extract), 4.10 mm (20%), 5.24 mm (30%), 6.98 mm (40%), 8.55 mm (50%), 23.10 mm for positive control chloramphenicol, and no zone in the negative control.

Results of Data Analysis

The data was first assessed for normality by the Shapiro-Wilk test and for homogeneity by Levene's test. The normality test provided a p-value <0.05, showing that the data followed a normal distribution and were homogeneous. Considering the results, the parametric Analysis of

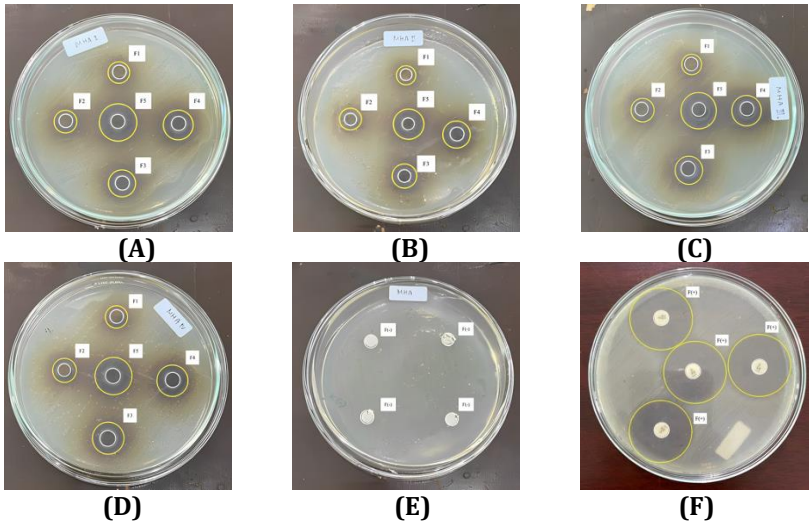

Figure 3. Results of antibacterial test of pasak bumi stem extract (*Eurycoma longifolia* J.) against Salmonella typhi bacteria; (A)Repetition 1; (B) Repetition 2; (C) Repetition 3; (D) Repetition 4; (E) Negative control (F) = Positive control

Description: F(-) = Paper disc + distilled water; F(+) = Chloramphenicol; F1 = 10% concentration of pasak bumi stem extract; F2 = 20% concentration of pasak bumi stem extract; F3 = 30% concentration of pasak bumi stem extract; F4 = 40% concentration of pasak bumi stem extract; F5 = 50% concentration of pasak bumi stem extract (White circles show diameter of paper disc; yellow circles show diameter of inhibition zone)

Table II. Stem Extraction Results

Initial Weight	Dry Weight	Simplisia Powder	Filtration Result	Thick Extract	Extract Yield
2 kg	1050 gr	1007 gr	6760 ml	26 gr	2,58%

Variance (ANOVA) test was applied for further analysis. This yielded a significant p-value of <0.001, less than 0.05, showing a statistical difference in the mean values between the six treatment groups. Further test was carried out in the form of Post Hoc using Least Significant Difference (LSD) to obtain significant values between concentration groups smaller than $p < 0.05$. Therefore, each concentration has a significant difference against the positive and negative controls of chloramphenicol and distilled water as shown in Table III.

DISCUSSION

Extraction of Pasak Bumi Stem

The yield of pasak bumi stem extract in this research was 2.58%. In a previous study using the same extraction method and different solvents, namely maceration and 70% ethanol, the yield was 4.420% (Rahima, 2020). Another study with 96% ethanol and a digestion method, obtained a yield of 0.957% of extract (Supartini & Cahyono, 2020).

This study used the maceration extraction method due to its simplicity and widespread usage. Consequently, it proves to be a suitable choice for extracting compounds that are not resistant to heat (Rahima, 2020). The remaceration process, or the repeated extraction process using a relatively consistent amount of solvent, ensures the proper dissolution of compounds within the sample (Badaring et al., 2020). The selection of ethanol as the solvent is grounded in a previous study, where 96% ethanol showed superior extraction of active compounds from pasak bumi stem compared to water and ethyl acetate (Supartini & Cahyono, 2020) Additionally, the preference for 96% ethanol is based on its low water content since the solvents with high water tend to be less selective, susceptible to microbial contamination, and result in quicker damage (Nugroho, 2017).

Antibacterial activity

Calipers measured growth inhibition zones and the average diameters in this research were 2.75 mm (10% extract), 4.10 mm (20%), 5.24 mm (30%), 6.98 mm (40%), 8.55 mm (50%), 23.10 mm for positive control chloramphenicol, and no zone in the negative control. The negative control of

Table III. Result of Antibacterial Test of Pasak Bumi

No	Formulation	Concentration (%)	Mean (mm) ± SD
1.	F(-)	Aquades	0±0
2.	F(+)	Chloramphenicol	23,10±0,632[a,c,d,e,f,g]
3.	F1	10%	2,75±0,37[a,b,d,e,f,g]
4.	F2	20%	4,10±0,365[a,b,c,e,f,g]
5.	F3	30%	5,24±0,347[a,b,c,d,f,g]
6.	F4	40%	6,98±0,685[a,b,c,d,e,g]
7.	F5	50%	8,55± 0,929[a,b,c,d,e,f]

[a] = Significantly different to F(-) with p<0.05;[b] = Significantly different to F(+) with p p<0.05 [c] = Significantly different to F(1) with p<0.05;[d] = Significantly different to F(2) with p<0.05; [e] = Significantly different to F(3) with p<0.05;[f] = Significantly different to F(4) with p<0.05; [g] = Significantly different to F(5) with p<0.05.
Description: F(-) = Disc paper + distilled water; F(+) = Chloramphenicol F1 = Pasak bumi stem extract 10% concentration; F2 = Pasak bumi stem extract 20% concentration; F3 = Pasak bumi stem extract 30% concentration; F4 = Pasak bumi stem extract 40% concentration; F5 = Pasak bumi stem extract 50% concentration.

distilled water showed no inhibition zone as a neutral compound that does not affect bacterial growth (Henaulu & Kaihena, 2020). The positive control chloramphenicol is able to inhibit bacterial growth due to its ability to reduce protein synthesis by binding to the 50S ribosomal subunit which is an important process in peptide bonding (Trisharyanti & Febriani, 2017). The diameter of the inhibition zone produced by E. longifolia stem extracts is smaller than the positive control inhibition zone of chloramphenicol caused by several factors, such as the concentration or reduced dose. The extracts also inhibited *Escherichia coli* (Halim, 2018) and *Staphylococcus aureus* (Mahardini, 2018), attributed to flavonoids, terpenoids, and alkaloids. Therefore, pasak bumi stem extracts have antibacterial effects against multiple pathogens, including *Salmonella typhi*.

Pasak bumi (*Eurycoma longifolia*) stem extract was able to inhibit *Salmonella typhi* due to its content of alkaloids, saponins, flavonoids, terpenoids, and tannins. Specifically, the alkaloids act as antibacterials by disrupting the formation of the peptidoglycan cell wall and inhibiting bacterial DNA topoisomerase (Anggraini et al., 2019). The saponins increase the permeability of the bacterial cell membrane, allowing leakage of proteins and enzymes (Suharto et al., 2012). Flavonoids act as antibacterials by binding to proteins through hydrogen bonds, which inhibits cell membrane/wall function. In this context, energy metabolism is also disrupted by limiting bacterial oxygen usage. Additionally, flavonoids inhibit the topoisomerase II (DNA gyrase) enzyme needed for DNA replication and transcription (Nomer, et al., 2019)Terpenoids reduce cell wall permeability by forming strong polymer bonds with porins (transmembrane proteins), preventing the acquisition of adequate nutrients. (Balafif, et al., 2013) Finally, tannins are proposed to wrinkle the cell membrane/wall, disrupting permeability (Dwicahmi, 2015).

Results of Data Analysis

The data was first assessed for normality using the Shapiro-Wilk test and homogeneity using Levene's test. The normality test provided a p-value <0.05, showing that the data followed a normal distribution and were homogeneous. Considering the results, the parametric Analysis of Variance (ANOVA) test was applied for further analysis. This yielded a significant p-value of <0.001, less than 0.05, showing a statistical difference in the mean values between the six treatment groups. A further test was carried out in Post Hoc using the Least Significant Difference (LSD) to obtain significant values between concentration groups smaller than p < 0.05. Therefore, each concentration has a significant difference against the positive and negative controls of chloramphenicol and distilled water. Antibacterial tests reported that higher concentrations of pasak bumi stem extract correlated with larger *Salmonella typhi* growth inhibition zone diameters, and the maximum inhibition was observed at 50%. According to this concentration-dependent effect, pasak bumi stem extract possesses antibacterial properties and can effectively suppress *Salmonella typhi* growth.

CONCLUSION

In conclusion, extracts from stems of *Eurycoma longifolia (*pasak bumi) were reported to possess antibacterial activity against *Salmonella typhi* based on the inhibition zone*.* Higher extract concentrations corresponded to larger inhibition

zone diameters, showing concentration-dependent antibacterial activity of pasak bumi stem extracts against *Salmonella typhi*.

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