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Phytochemical Screening and Antibacterial Activity of Jackfruit Seed Extract (*Artocarpus heterophyllus* Lamk.) Against *Salmonella typhi* In Vitro

Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Biji Nangka (*Artocarpus heterophyllus* Lamk.) terhadap *Salmonella typhi* secara In Vitro

Ami Febriza[1]*, Salwa Amalia Kartika[2], Asdar Tajuddin[3]

[1]*Department of Physiology, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Makassar, South Sulawesi, 90221, Indonesia*
[2]*Bachelor of Medicine Study Program, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Makassar, South Sulawesi, 90221, Indonesia*
[3]*Department of Surgery, Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar, Makassar, South Sulawesi, 90221, Indonesia*

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ABSTRACT

Salmonella typhi infection remains a public health concern, necessitating natural treatment alternatives. This study aimed to evaluate the antibacterial activity and identify the active compounds of jackfruit seed (*Artocarpus heterophyllus* Lamk) ethanol extract against *S. typhi* in vitro. Antibacterial activity was tested using the well diffusion method at extract concentrations of 25%, 50%, 75%, and 100%, with chloramphenicol and 10% DMSO as positive and negative controls. All extract concentrations produced significant inhibition zones (mean: 19.83 mm to 31.42 mm; $p < 0.05$). Phytochemical screening revealed flavonoids, alkaloids, steroids, and saponins as the active compounds. Antibacterial efficacy increased with concentration and was comparable to chloramphenicol (mean: 33.49 mm). These results indicate that jackfruit seed extract holds promise as a natural antibacterial agent against *S. typhi*.

ABSTRAK

Infeksi *Salmonella typhi* masih menjadi masalah kesehatan masyarakat, sehingga diperlukan alternatif pengobatan alami. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri dan kandungan senyawa aktif dari ekstrak etanol biji nangka (*Artocarpus heterophyllus* Lamk.) terhadap *S. typhi* secara in vitro. Uji antibakteri dilakukan dengan metode difusi sumur pada konsentrasi ekstrak 25%, 50%, 75%, dan 100%, serta dibandingkan dengan kloramfenikol (kontrol positif) dan DMSO 10% (kontrol negatif). Hasil menunjukkan zona hambat signifikan pada semua konsentrasi ekstrak (rata-rata: 19,83 mm hingga 31,42 mm; $p < 0,05$). Skrining fitokimia mengidentifikasi keberadaan flavonoid, alkaloid, steroid, dan saponin sebagai senyawa aktif. Efektivitas antibakteri meningkat seiring kenaikan konsentrasi, dan mendekati aktivitas kloramfenikol (rata-rata 33,49 mm). Hasil ini menunjukkan bahwa ekstrak biji nangka berpotensi sebagai agen antibakteri alami terhadap *S. typhi*.

**Corresponding author:*
Ami Febriza (amifebriza@med.unismuh.ac.id)

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1. INTRODUCTION

Typhoid fever is primarily caused by the bacterium *Salmonella typhi* and remains a major public health challenge, particularly in developing regions such as Southeast Asia and Africa**.** According to World Health Organization data, approximately 9 million people were reported to have typhoid fever in 2019. This contributed significantly to increased morbidity and mortality, resulting in an estimated 110,000 deaths annually (Crump, 2019).

The bacterium *S. typhi* belongs to the Enterobacteriaceae family, which includes *S. bongori* and *S. enterica* as its two primary species classifications. Each species comprises approximately 2,500 serovars or serotypes (Thiery et al., 2023). Transmission in humans primarily occurs through ingestion of contaminated food, water, or fecal matter. This pathogen may invade the intestinal mucosa by targeting dendritic cells and enterocytes of the epithelial barrier, leading to widespread systemic dissemination (Lamichhane et al., 2024).

Typhoid fever is typically treated with antibiotic therapy. When antibiotics are properly administered at therapeutic doses, antibiotic resistance may be mitigated, treatment side effects minimized, and patient recovery can be enhanced (Chaudhari et al., 2023; Qin et al., 2022). Conversely, misuse or improper use of antibiotics can increase side effects, risk of recurrence, and contribute to the emergence of antibiotic resistance—a growing global concern (Castro-Vargas et al., 2020). By 2050, antibiotic resistance is projected to cause 10 million deaths annually worldwide, highlighting its status as a persistent global health crisis (Tang et al., 2023).

Due to the rise in bacterial resistance, researchers have increasingly sought antibiotic-free alternatives to inhibit the growth of *S. typhi*. Addressing resistance in clinically important pathogens necessitates alternative treatment strategies, with plant-based compounds offering promising antimicrobial potential (Gootz, 1990). These alternatives may involve antibacterial components found in plants, such as seeds, fruits, or leaves (Ramli et al., 2021). Studies have demonstrated that plants contain antimicrobial compounds capable of modulating antibiotic effectiveness. Natural products like herbs possess unique molecular diversity and bioactivity, making them valuable sources for novel drug development (Gootz, 1990).

Jackfruit (*Artocarpus heterophyllus* Lamk.) is a tropical fruit tree native to South and Southeast Asia. It thrives in environments with sufficient rainfall and grows optimally at temperatures ranging from 24°C to 29°C. All plant parts, including leaves, bark, and fruit, have medicinal or nutritional value**.** Jackfruit leaves exhibit antibacterial activity, while the roots and bark are traditionally used to treat infections and skin conditions. An extract from the seeds has also been shown to possess antibacterial effects. Jackfruit is rich in vitamin C, B vitamins, and minerals, making it a valuable nutritional source (Gupta et al., 2020).

Various parts of the jackfruit plant—including the stem, fruit, peel, leaves, and seeds—have been studied as potential remedies for bacterial infections. According to findings from previous studies, a 12.5% inhibitory effect of ethanol extract from jackfruit leaves was observed on *S. typhi* at concentrations of 20%, 40%, and 60% (Mawardika et al., 2023). The bark of the jackfruit tree has demonstrated inhibitory effects on *S. typhi*, with reported inhibition zones ranging from 18.31 mm to 19.02 mm at extract concentrations between 25% and 100% (Sari et al., 2018). Furthermore, a previous study examined methanol extract of jackfruit seed powder against methicillin-resistant *Staphylococcus aureus* (MRSA). The findings revealed a 6.1% inhibition rate at a concentration of 1,000 mg/mL (Yulianto, 2018). The present study investigates the antimicrobial activity of *A. heterophyllus* seed extract against *S. typhi* and identifies the active phytochemical compounds involved.

2. METHODS

2.1. Study Design and Ethical Consideration

This study applied a true experimental design with a post-test-only control group to evaluate the antibacterial activity of ethanol extract from jackfruit seeds against *S. typhi* in vitro. The research protocol received ethical approval from the Ethics Committee of the Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Makassar (Approval No. 588/UM.PKE/VIII/46/2024).

2.2. Preparation of Jackfruit Seed Extract

Fresh jackfruit seeds were cleaned, cut into small pieces, and air-dried for three days to obtain simplicia. A total of 200 g of the dried material was macerated in 96% ethanol using a solvent-to-sample ratio of 10:1 (w/v). The mixture was stirred every two hours and covered with aluminum foil to prevent evaporation. After 24 hours, the mixture was filtered, and the maceration process was repeated twice. The collected filtrates were then evaporated using a water bath at 50˚C to obtain a thick extract.

2.3. Bacterial Preparation and Antibacterial Assay

The *S. typhi* strain (ATCC 6539) was cultured on Nutrient Agar (NA) medium and incubated at 37˚C for 24 hours to obtain fresh bacterial colonies. Sterile metal cylinders were used to create uniform wells (6 mm in diameter) on NA plates previously inoculated with the bacterial suspension. Each well was filled with 50 µL of the jackfruit seed extract at concentrations of 25%, 50%, 75%, or 100%**.** Chloramphenicol (30 µg/mL) was used as the positive control based on its minimum inhibitory concentration (Robertson et al., 1968), and 10% dimethyl sulfoxide (DMSO) served as the negative control to validate the solvent's neutrality. All plates were incubated at 37˚C for 24 hours to allow for inhibition zone development.

2.4. Measurement of Antibacterial Activity

Following incubation, the diameter of inhibition zones was measured using a digital caliper. Measurements were taken from the edge of the well to the outer boundary of the clear zone and

recorded in millimeters. The results were categorized according to Greenwood's (2000) standard: >20 mm (strong inhibition), 16–20 mm (moderate), 10–15 mm (weak), and <10 mm (no inhibition).

2.5. Phytochemical Screening

Phytochemical analysis of the jackfruit seed extract was performed using thin-layer chromatography (TLC) to identify the presence of secondary metabolites. The extract was spotted on TLC plates and observed under ultraviolet light at 254 nm and 366 nm. Fluorescence changes and color variations were used to indicate the presence of flavonoids, alkaloids, steroids, and saponins (Kowalska & Sajewicz, 2022).

2.6. Statistical Analysis

Data were analyzed using SPSS statistical software. Normality of data distribution was evaluated using the Shapiro–Wilk test, and homogeneity of variances was tested with Levene's test. One-way analysis of variance (ANOVA) was applied to assess differences in inhibition zone diameters among groups (significance level set at $p < 0.05$), followed by a least significant difference (LSD) post-hoc test.

3. RESULTS AND DISCUSSION

Phytochemical analysis of the ethanol extract of jackfruit seeds was conducted using thin-layer chromatography (TLC). The presence of secondary metabolites was identified based on spot characteristics and fluorescence changes under UV light at 254 nm and 366 nm. The results are presented in **Table 1** and **Figure 1**. These findings confirmed the presence of flavonoids, alkaloids, steroids, and saponins—compounds that have been widely associated with antimicrobial properties. Tannins were not detected in this extract.

Table 1. TLC Observation Phytochemical Screening Results of Jackfruit Seeds

Phytochemical	TLC Observation	Result	UV254	UV366
Flavonoid	Red/yellow fluorescence and dark spots	Positive	Yes	Yes
Alkaloid	Dark stains	Positive	Yes	Yes
Tannin	No fluorescence changes or spots	Negative	No	No
Steroid	Green/yellow fluorescence and vivid stains	Positive	Yes	Yes
Saponin	Bright stains and markings	Positive	Yes	Yes

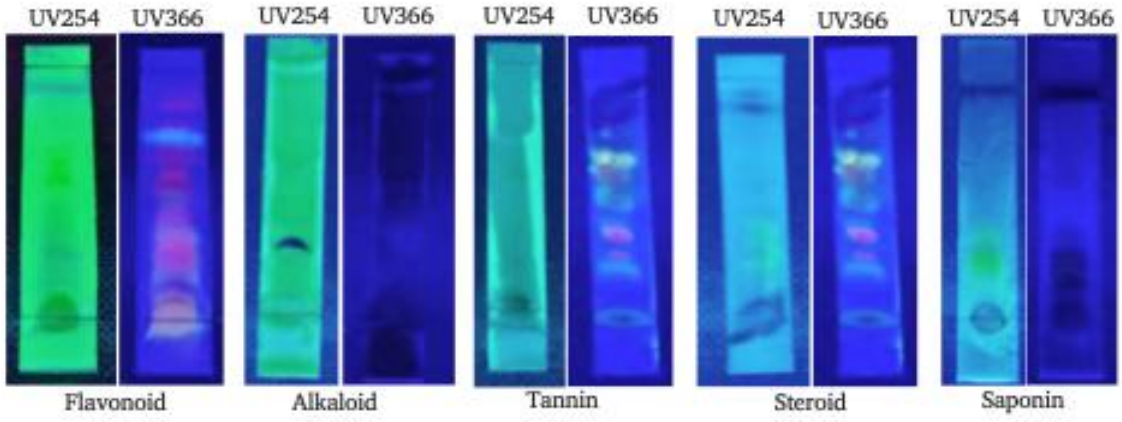

Figure 1. Phytochemical Screening of Jackfruit Seed Extract Using Thin-Layer Chromatography (TLC) Under UV Light at 254 nm and 366 nm. Visual observation of fluorescent patterns indicating the presence of flavonoids, alkaloids, steroids, and saponins. No spot was detected for tannins.

The antibacterial potential of the jackfruit seed extract against *Salmonella typhi* was evaluated using the agar well diffusion method (**Figure 2**). Various extract concentrations were tested (25%, 50%, 75%, and 100%), along with chloramphenicol (positive control) and DMSO 10% (negative control). The mean inhibition zone diameters are shown in **Table 2**.

Table 2. Mean Inhibition Zone Diameters of Jackfruit Seed Extract Against *S. typhi*

Treatment Group	Mean ± SD (mm)	Inhibition Classification
25% Extract	19.83 ± 0.85	Moderate
50% Extract	21.64 ± 0.36	Strong
75% Extract	24.16 ± 1.09	Strong
100% Extract	31.42 ± 0.32	Strong
Positive Control (Chloramphenicol)	33.49 ± 0.74	Strong
Negative Control (DMSO)	0.00 ± 0.00	No Inhibition

Statistical analysis using one-way ANOVA demonstrated significant differences among the groups ($p < 0.05$). The LSD post-hoc test confirmed that all differences between concentrations were statistically significant. The antibacterial activity increased in a dose-dependent manner, with the 100% concentration showing inhibition nearly comparable to chloramphenicol (33.49 mm). The absence of inhibition in the negative control validated the activity was due to the extract itself.

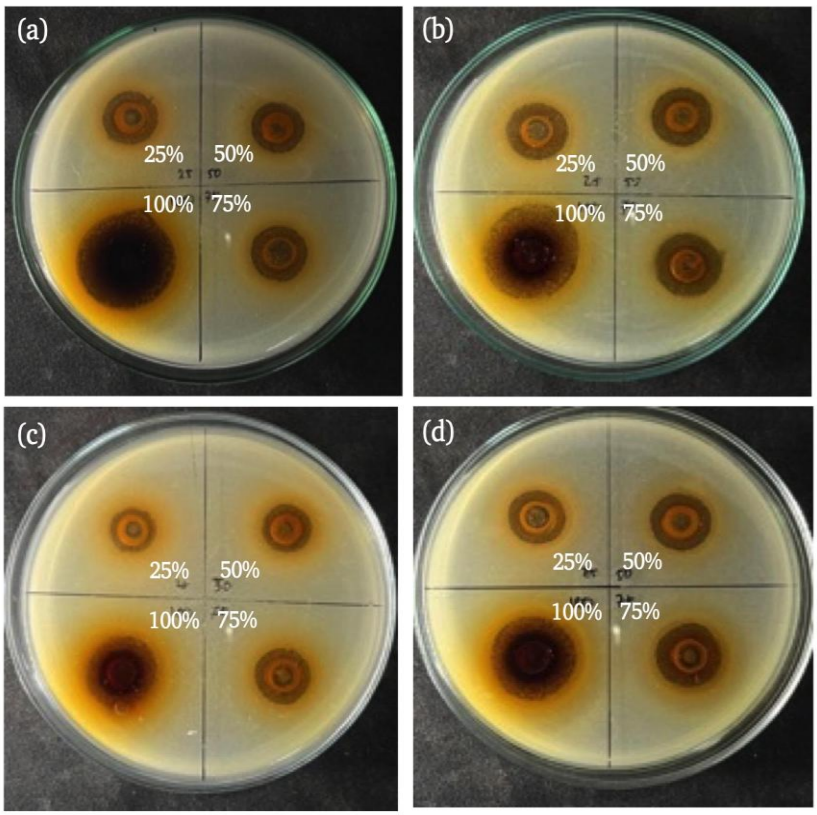

Figure 2. Antibacterial Activity of Jackfruit Seed Extract Against *S. typhi* in Petri Dishes (Replicates 1–4; a–d). Visible inhibition zones around the wells indicate antimicrobial activity of the extract, with clearer and wider zones at higher concentrations.

The antibacterial activity observed in the jackfruit seed extract is closely associated with the presence of key phytochemical compounds, including flavonoids, alkaloids, saponins, and steroids, as identified in the TLC analysis. Flavonoids contribute to bacterial inhibition by generating reactive oxygen species (ROS), disrupting microbial membranes, and interfering with nucleic acid synthesis (Robak & Gryglewski, 1988; Fathima & Rao, 2016). Alkaloids interact with bacterial DNA and enzymes, impeding essential cellular processes such as replication and protein synthesis (Cowan, 1999). Saponins act as natural surfactants that compromise membrane integrity, while steroids may enhance membrane permeability, promoting cell lysis (Böttger et al., 2012). Compared to previous studies using jackfruit bark and leaves—which showed inhibition zones of 18.31–19.02 mm and ~12.5 mm, respectively (Sari et al., 2018; Mawardika et al., 2023)—the seed extract demonstrated notably higher activity, especially at 100% concentration (31.42 mm), approaching the efficacy of chloramphenicol (33.49 mm). The findings also indicate a clear dose-dependent pattern, with statistically significant increases in inhibition across all tested concentrations. The absence of inhibitory effect in the negative control (10% DMSO) validates the extract as the sole source of antibacterial activity. These data strongly support the contribution of bioactive compounds in jackfruit seed extract to the inhibition of *S. typhi* growth and affirm the reproducibility of its antimicrobial effect across experimental replicates.

4. CONCLUSION

The ethanol extract of jackfruit seeds (*Artocarpus heterophyllus* Lamk) demonstrated significant antibacterial activity against *Salmonella typhi*, with a clear dose-dependent increase in inhibition zone diameter. The strongest effect was observed at 100% extract concentration, which approached the efficacy of the standard antibiotic chloramphenicol. Phytochemical screening confirmed the presence of flavonoids, alkaloids, saponins, and steroids—bioactive compounds likely responsible for the antibacterial properties. These findings suggest that jackfruit seed extract holds promise as a natural antibacterial agent. However, further in vivo studies are necessary to evaluate its safety, pharmacodynamics, and clinical applicability.

AUTHOR CONTRIBUTIONS

Conceptualization, Ami Febriza; methodology, Ami Febriza and Salwa Amalia Kartika; validation, Ami Febriza and Asdar Tajuddin; formal analysis, Ami Febriza; investigation, Salwa Amalia Kartika; writing—original draft preparation, Ami Febriza; writing—review and editing, Ami Febriza and Asdar Tajuddin;

visualization, Salwa Amalia Kartika; supervision, Ami Febriza. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

This study was approved by the Faculty of Medicine and Health Sciences, Makassar, Indonesia, with number 588/UM.PKE/VIII/46/2024.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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CONFLICTS OF INTEREST

The authors declare there are no competing interests.

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