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Antimicrobial Activity of Ethanolic Extract of Loquat Leaves (*Eriobotrya japonica* (Lindl.)) Against *Salmonella* spp. and *Escherichia coli*

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Abstract

The use of chemical preservatives in animal-derived food products is still frequently found in traditional markets. The application of synthetic chemical preservatives poses significant health risks, as excessive concentrations may damage the structural quality of animal-based foods and adversely affect consumer health. Therefore, the exploration of safe and effective natural preservatives for animal-derived food products is essential. Loquat leaves *(Eriobotrya japonica (Lindl.)* contain polyphenolic compounds, particularly flavonoids and tannins, which exhibit antimicrobial properties and have potential applications as natural food preservatives and biopharmaceutical agents. This study aimed to evaluate the antimicrobial effectiveness of ethanolic extract of loquat leaves *(Eriobotrya japonica (Lindl.)* against common foodborne contaminant bacteria, namely *Salmonella* spp. and *Escherichia coli*. The leaf extract was tested at concentrations of 20%, 30%, 40%, 50%, and 60% using the disk diffusion method. The results showed that the inhibition zone increased with increasing extract concentration, with the strongest antibacterial activity observed at the 60% concentration against both bacterial species. The ethanolic extract exhibited greater inhibitory activity against *Salmonella* spp. than *Escherichia coli*. These findings indicate that ethanolic extract of loquat leaves has promising antibacterial potential and could be developed as a natural preservative for animal-derived food products.

Keywords: *Loquat leaves*; *Salmonella* spp; *Escherichia coli;*

Abstrak

Penggunaan bahan pengawet kimia pada produk pangan asal hewan masih sering dijumpai, terutama di pasar tradisional. Penggunaan bahan pengawet sintetis berpotensi menimbulkan risiko terhadap kesehatan karena pada konsentrasi yang berlebihan dapat merusak kualitas dan struktur pangan asal hewan serta memberikan dampak negatif bagi kesehatan konsumen. Oleh karena itu, eksplorasi bahan pengawet alami yang aman dan efektif untuk pangan asal hewan menjadi sangat penting. Daun biwa (*Eriobotrya japonica* (Lindl.)) mengandung senyawa polifenol, terutama flavonoid dan tanin, yang memiliki aktivitas antimikroba serta berpotensi dimanfaatkan sebagai bahan pengawet alami dan agen biofarmaka. Penelitian ini bertujuan untuk mengevaluasi efektivitas antimikroba ekstrak etanol daun biwa (*Eriobotrya japonica* (Lindl.)) terhadap bakteri kontaminan pangan, yaitu *Salmonella* spp. dan *Escherichia coli*. Ekstrak daun biwa diuji pada konsentrasi 20%, 30%, 40%, 50%, dan 60% menggunakan metode difusi cakram. Hasil penelitian ini diharapkan dapat memberikan bukti ilmiah yang mendukung pengembangan ekstrak daun biwa sebagai bahan pengawet alami untuk produk pangan asal hewan serta menjadi dasar bagi pemanfaatannya dalam teknologi pengawetan pangan dan pengembangan senyawa bioaktif.

Kata Kunci: Daun biwa; *Salmonella* spp; *Escherichia coli;*

1. INTRODUCTION

Loquat (*Eriobotrya japonica* (Lindl.)) is a fruit-bearing plant native to China and is widely distributed in subtropical and highland regions due to its adaptability and economic potential (Marianne et al., 2018). In Indonesia, E. japonica has been cultivated extensively, particularly in the Taman Simalem Resort area, Karo Regency, North Sumatra, which is recognized as the largest loquat cultivation area in Indonesia and one of the largest in Southeast Asia (Melsi et al., 2022; Henaulu & Kaihena, 2020). Loquat has attracted considerable attention because of its nutritional value and diverse biological activities. Various parts of this plant, especially the leaves and seeds, contain bioactive compounds with potential applications in pharmaceutical and food industries.

The leaves and seeds of E. japonica contain several bioactive compounds, including amygdalin, phenolic compounds, flavonoids, tannins, triterpenoids, and other secondary metabolites. Amygdalin (laetrile or vitamin B17) found in loquat seeds has been reported to possess potential anticancer properties. Traditionally, loquat leaves have been used for the treatment of respiratory disorders, bronchitis, digestive problems, fever, nausea, vomiting, and prolonged belching (Sartika et al., 2013). Furthermore, previous studies have reported various biological activities of E. japonica, including antidiabetic, cytotoxic, hepatoprotective, antimutagenic, antitumor, antioxidant, and anti-inflammatory activities (Su'udi et al., 2025), (Wenas et al., 2020),. Recent phytochemical studies have demonstrated that loquat leaves contain diverse phenolic compounds and flavonoids that contribute to their biological activities, including antioxidant potential and therapeutic properties(Maliangkay et al., 2019).

The increasing use of synthetic chemical preservatives in animal-derived food products has become a major concern due to their potential adverse effects on consumer health and food quality(Pratiwi et al., 2024). Excessive application of synthetic preservatives may cause undesirable chemical residues and reduce consumer acceptance of processed food products (Irawan et al., 2025). Therefore, the exploration of natural preservatives derived from plant bioactive compounds is considered an important strategy for developing safer and environmentally friendly food preservation technologies (Syarifa, 2025;Handarini et al., 2025).

Foodborne contaminant bacteria, particularly *Salmonella* spp. and *Escherichia coli*, are among the major microorganisms associated with contamination of animal-derived food products(Pawesti, 2025). The presence of these bacteria can decrease food quality, shorten shelf life, and increase the risk of foodborne diseases (Suryoadji et al., 2025). Plant-derived antimicrobial compounds, especially polyphenols such as flavonoids and tannins, have demonstrated the ability to inhibit bacterial growth through several mechanisms, including disruption of bacterial cell membranes, inhibition of essential enzymes, and interference with microbial metabolism. Loquat leaves contain flavonoids, tannins, and phenolic compounds that have been reported to exhibit antioxidant and antimicrobial activities, indicating their potential as natural preservatives and bioactive agents.

Although previous studies have extensively investigated the pharmacological properties of E. japonica, research focusing on the antimicrobial effectiveness of ethanolic extract of loquat leaves against foodborne contaminant bacteria, particularly *Salmonella* spp. and *Escherichia coli*, remains limited. The relationship between antioxidant activity, antimicrobial compounds, and natural food preservation provides an important basis for developing plant-based preservatives as alternatives to synthetic chemicals.

Based on these considerations, this study aimed to evaluate the antimicrobial effectiveness of ethanolic extract of loquat leaves (*Eriobotrya japonica* (Lindl.)) against the growth of contaminant bacteria, namely *Salmonella spp*. and *Escherichia coli*. The antimicrobial activity was evaluated at different extract concentrations (20%, 30%, 40%, 50%, and 60%) using the disk diffusion method, while the Minimum Inhibitory Concentration (MIC) was determined to identify the lowest concentration capable of inhibiting bacterial growth. The results of this study are expected to provide scientific evidence supporting the utilization of loquat leaf extract as a natural antimicrobial agent and preservative for animal-derived food products and contribute to the development of sustainable food preservation technologies.

2. METHOD

2.1 Research Design

This study employed an experimental laboratory design to evaluate the antimicrobial effectiveness of ethanolic extract of loquat leaves (*Eriobotrya japonica* (Lindl.) against bacterial contaminants, namely *Salmonella spp.* and *Escherichia coli*. The research was conducted through antimicrobial activity testing using the disk diffusion method. The independent variable in this study was the concentration of loquat leaf ethanolic extract, while the dependent variable was the inhibition zone diameter produced against bacterial growth.

The study was conducted through several stages, including sample preparation, extraction of loquat leaves, preparation of bacterial cultures, antimicrobial activity testing, and data analysis. The research aimed to determine the potential of loquat leaf extract as a natural antimicrobial agent for controlling bacterial contamination.

2.2 Research Tools and Materials

The main material used in this study was loquat leaves (*Eriobotrya japonica* (Thunb.) Lindl.). Loquat leaves were selected because they contain bioactive compounds, including flavonoids, tannins, phenolic compounds, and amygdalin, which have been reported to possess antioxidant and antimicrobial activities.

The bacterial test materials used were *Salmonella* spp. and *Escherichia coli* as foodborne contaminant bacteria. The tools used included analytical balance, oven/drying equipment, blender or grinder, incubator, autoclave, petri dishes, sterile paper discs, micropipettes, measuring instruments, and other microbiological laboratory equipment required for antimicrobial testing. The materials used included ethanol as an extraction solvent, bacterial growth media, sterile distilled water, and other supporting materials for microbial analysis.

2.3 Research Procedure

The research procedure consisted of several stages, starting with sample collection and preparation. Loquat leaves were collected, washed thoroughly using clean water to remove impurities and surface contaminants, and drained to eliminate excess water. The leaves were then dried to reduce moisture content while maintaining the stability of bioactive compounds. The dried leaves were subsequently processed into powder and prepared for extraction using ethanol.

The ethanolic extract of loquat leaves was prepared and tested against *Salmonella* spp. and *Escherichia coli*. Antimicrobial activity testing was performed using the disk diffusion method. Different concentrations of loquat leaf extract (20%, 30%, 40%, 50%, and 60%) were applied to sterile paper discs and placed on bacterial culture media. The plates were incubated under appropriate conditions, and the inhibition zones formed around the discs were measured. The research workflow is presented in Figure 1.

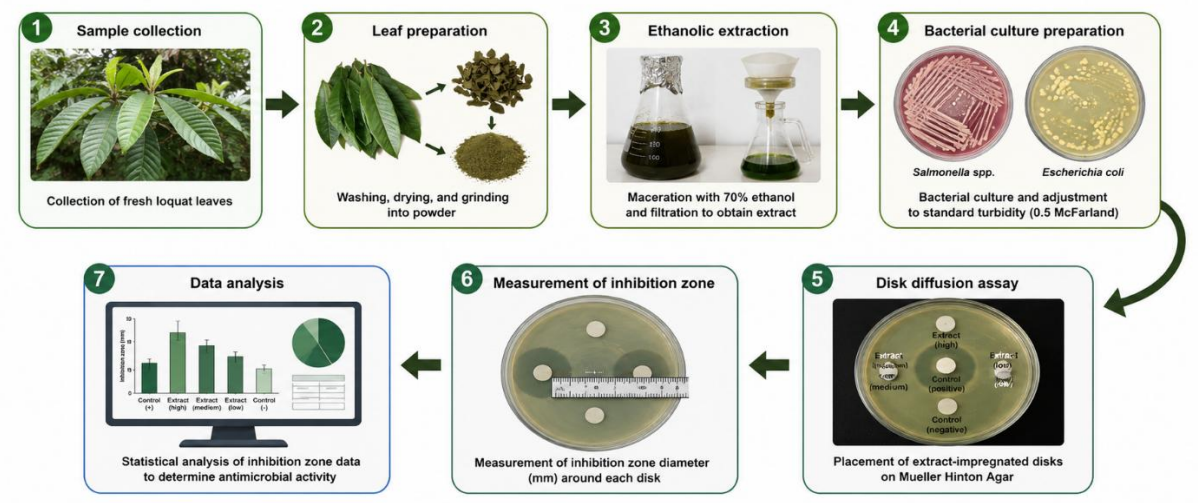

Figure 1. Research workflow of antimicrobial activity testing of ethanolic loquat leaf extract against *Salmonella* spp. and *Escherichia coli*.

2.4 Research Instrument

The research instruments used in this study included microbiological laboratory equipment and measurement instruments. The disk diffusion assay was used as the main instrument to evaluate antimicrobial activity. The antimicrobial effectiveness of loquat leaf extract was measured based on the diameter of the inhibition zone (mm) formed around the paper disc.

A measuring instrument such as a digital caliper or ruler was used to determine the diameter of the inhibition zone accurately. The measurement results represented the ability of loquat leaf extract to inhibit the growth of *Salmonella* spp. and *Escherichia coli*.

2.5 Data Analysis

The antimicrobial activity data were analyzed quantitatively based on the diameter of the inhibition zone produced by each extract concentration. The inhibition zone diameter was measured in millimeters (mm). The effectiveness of loquat leaf extract was evaluated by comparing the inhibition zones produced at different extract concentrations. Statistical analysis was performed to determine significant differences in antibacterial activity among the tested extract concentrations. The lowest extract concentration that produced a visible inhibition zone was recorded as the lowest effective concentration under the disk diffusion assay. The results are presented as mean ± standard deviation, and statistical significance was determined at $p < 0.05$.

3. RESULT AND DISCUSSION

The antibacterial activity of ethanolic extract of loquat leaves (*Eriobotrya japonica* (Lindl.)) against contaminant bacteria, namely *Salmonella spp.* and *Escherichia coli*, was evaluated using the agar diffusion method. The antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the paper discs on Nutrient Agar (NA) medium.

Figure 2. Morphological characteristics of *Eriobotrya japonica* (Lindl.) used in this study.

Figure 2 illustrates the morphological characteristics of *Eriobotrya japonica* (Lindl.), the plant species used as the source of ethanolic leaf extract in this study. The plant belongs to the family Rosaceae and is characterized by a woody stem, dark green leathery leaves with serrated margins, fragrant white flowers, and yellow–orange pome fruits. Mature and healthy leaves were selected as the plant material because they contain bioactive compounds, including flavonoids, tannins, and phenolic compounds, which have been reported to exhibit antibacterial activity.

The experiment consisted of seven treatments, including P0 (negative control), P1 (20% extract concentration), P2 (30% extract concentration), P3 (40% extract concentration), P4 (50% extract concentration), P5 (60% extract concentration), and P6 (positive control using erythromycin 0.25 mg). Each treatment was performed in three replications. The average inhibition zone diameter obtained from each treatment was measured and subsequently classified based on the bacterial growth inhibition sensitivity category.

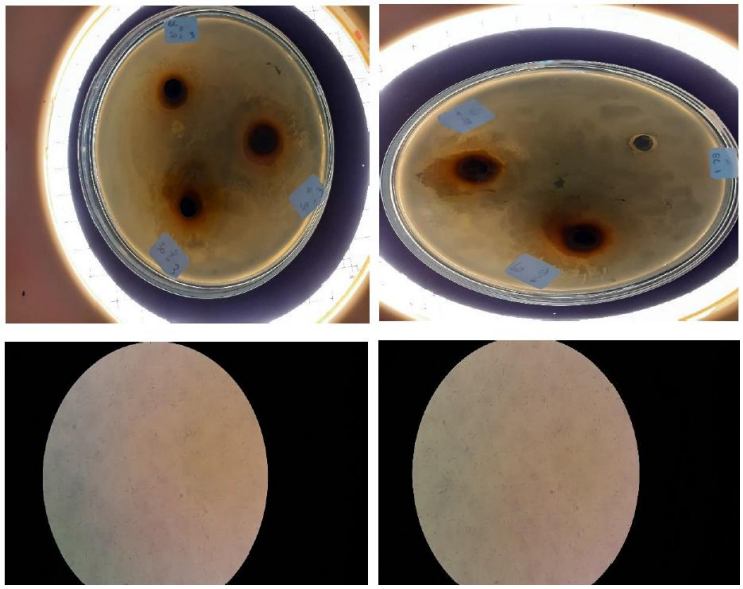

Figure 3. Representative inhibition zones of ethanolic *Eriobotrya japonica* leaf extract against *Escherichia coli*.

Figure 3 presents the inhibition zones produced by the ethanolic extract against *Salmonella spp*. Similar to the results obtained for *Escherichia coli*, larger inhibition zones were observed at higher extract concentrations, demonstrating the antibacterial potential of loquat leaf extract against *Salmonella spp*. The average inhibition zone diameter of ethanolic loquat leaf extract against the growth of *Salmonella spp. and Escherichia coli* is presented in Table 1 and Table 2

Table 1. Average inhibition zone diameter of ethanolic loquat leaf extract (*Eriobotrya japonica* (Lindl.)) against the growth of *Escherichia coli*

Treatment	Concentration (%)	Inhibition Zone Diameter (mm)	Average Inhibition Zone Diameter (mm)	Growth Inhibition Response
P0	Negative control	0	0	No inhibition
P1	20	0	0	No inhibition
P2	30	0	0	No inhibition
P3	40	0	0	No inhibition
P4	50	13.32 9.25 8.52	10.36	Strong
P5	60	15.48 13.33; 9.37	12.73	Strong
P6	Positive control	24.17 21.90 27.70	24.59	Very strong

Based on Table 1, the application of ethanolic loquat leaf extract (*Eriobotrya japonica* (Lindl.) showed differences in antibacterial activity against the growth of *Escherichia coli* at various extract concentrations. The extract concentrations of 20%, 30%, and 40% did not show any inhibition zone formation, indicated by an inhibition zone diameter of 0 mm. Meanwhile, extract concentrations of 50% and 60% were able to inhibit the growth of E. coli, with average inhibition zone diameters of 10.36 mm and 24.59 mm, respectively. Based on the bacterial inhibition classification, the 50% extract concentration was categorized as strong inhibition, while the 60% extract concentration was categorized as very strong inhibition.

The positive control treatment using erythromycin 0.25 mg produced an average inhibition zone diameter of 24.59 mm and was categorized as very strong inhibition against *E. coli* growth. In contrast, the negative control treatment using distilled water did not show any inhibitory activity, with an inhibition zone diameter of 0 mm.

Table 2. Average inhibition zone diameter of ethanolic loquat leaf extract (*Eriobotrya japonica* (Lindl.) against the growth of *Salmonella spp*.

Treatment	Concentration (%)	Inhibition Zone Diameter (mm)	Mean Inhibition Zone Diameter (mm)	Growth Inhibition Response
P0	Control (-)	0	0.00	No inhibition
P1	20	13.08 9.17 17.28	13.18	Strong
P2	30	10.72 10.50 11.32	10.84	Strong
P3	40	14.23 13.90 9.10	12.41	Strong
P4	50	13.62 24.67 15.35	17.88	Strong
P5	60	10.13 13.93 11.90	11.99	Strong
P6	Control (+)	37.72 21.03 26.67	28.47	Strong

The data presented in Table 2 showed that ethanolic loquat leaf extract (*Eriobotrya japonica* (Lindl.)) exhibited antibacterial activity against *Salmonella* spp. at all tested concentrations. The extract concentrations of 20%, 30%, 40%, 50%, and 60% produced average inhibition zone diameters of 13.18, 10.84, 12.41, 17.88, and 11.99 mm, respectively. Based on the inhibition zone classification, all extract concentrations demonstrated strong antibacterial activity against *Salmonella* spp. The positive control treatment with ceftazidime produced an inhibition zone diameter of 28.47 mm, categorized as very strong inhibition.

3.1. Discussion

The results of antibacterial evaluation demonstrated that ethanolic extract of loquat leaves (*Eriobotrya japonica* (Lindl.)) was able to inhibit the growth of contaminant bacteria, namely *Escherichia coli* and *Salmonella spp* using the agar diffusion method. The inhibition activity against E. coli was observed at extract concentrations of 50% and 60%, producing inhibition zones of 10.36 mm and 24.59 mm, respectively. Meanwhile, the ethanolic extract showed stronger inhibitory activity against *Salmonella spp* where all tested concentrations (20–60%) produced inhibition zones ranging from 10.84 to 17.88 mm, which were classified as strong inhibition.

The antibacterial activity classification was determined based on the diameter of the inhibition zone, where inhibition zones below 5 mm are categorized as weak, 5–10 mm as moderate, 10–20 mm as strong, and ≥20 mm as very strong inhibition (Daud et al., 2023). Based on this classification, ethanolic loquat leaf extract demonstrated potential antibacterial activity against both bacterial species tested. The differences in inhibition zone diameters among extract concentrations were influenced by variations in the amount of antimicrobial compounds present in the extract (La Bassy et al., 2023). Increasing extract concentrations generally resulted in greater diffusion of antimicrobial compounds into the agar medium, leading to enhanced inhibition of bacterial growth. However, the highest concentration does not always produce the strongest antibacterial activity because the effectiveness of antimicrobial compounds may depend on the optimal concentration (Wulandari & Umam, 2023). Excessive concentrations of certain antimicrobial compounds may not proportionally increase antibacterial effectiveness after reaching a specific threshold concentration. The formation of inhibition zones around the discs indicates the presence of antibacterial compounds in loquat leaves that are capable of inhibiting bacterial growth. These antibacterial compounds are secondary metabolites produced by plants and stored in various plant organs, including leaves, stems, fruits, and seeds. The composition and

concentration of secondary metabolites may vary depending on plant species and environmental conditions.

Loquat leaves contain various bioactive compounds, including amygdalin, flavonoids, tannins, and other phenolic compounds. Flavonoids in loquat leaves have been reported to contribute to antioxidant and antimicrobial activities. Previous studies have shown that flavonoids, tannins, and saponins possess antibacterial properties by interfering with bacterial growth mechanisms. Saponins are secondary metabolites produced by plants that exhibit antimicrobial activity. These compounds act as antibacterial agents by disrupting bacterial cell membrane stability, increasing membrane permeability, and causing leakage of essential intracellular components, including proteins, nucleic acids, and nucleotides. This mechanism results in membrane damage and ultimately leads to bacterial cell lysis(Auza et al., 2025).

The antibacterial activity of ethanolic loquat leaf extract was observed to be more effective against *Salmonella spp*. compared with E. coli. This finding indicates that *Salmonella spp*. has higher sensitivity toward loquat leaf extract than E. coli. Differences in bacterial sensitivity may be associated with variations in bacterial cell wall structure and composition, which influence the ability of bacteria to resist antimicrobial compounds. The structural differences between bacterial cell walls may affect the penetration and activity of bioactive compounds contained in the extract (Khoirotunnisa et al., 2025).

Based on the inhibition zone measurements, the Minimum Inhibitory Concentration (MIC) of ethanolic loquat leaf extract against *E. coli* was observed at a concentration of 50%, with an average inhibition zone diameter of 10.36 mm. Meanwhile, the MIC against *Salmonella spp*. was observed at a concentration of 60%, with an average inhibition zone diameter of 11.99 mm. These results indicate that loquat leaf extract possesses antibacterial potential because relatively low concentrations were able to inhibit bacterial growth. The presence of antibacterial activity at low extract concentrations highlights the potential utilization of loquat leaves as a natural antibacterial agent. The bioactive compounds contained in loquat leaves provide scientific evidence for the development of plant-based antimicrobial agents as an alternative to synthetic preservatives for controlling bacterial contamination in food products.

4. CONCLUSION

Based on the measurement of the inhibition zones formed against the growth of *Escherichia coli* and *Salmonella* spp., the Minimum Inhibitory Concentration (MIC) of the ethanolic loquat (*Eriobotrya japonica*) leaf extract was determined. The MIC of the ethanolic loquat leaf extract against *Escherichia coli* was observed at a concentration of 50%, producing a mean inhibition zone of 10.36 mm, whereas the MIC against *Salmonella* spp. was observed at a concentration of **60%**, with a mean inhibition zone of 11.99 mm. The difference in the MIC values between the two bacterial species indicates that bacterial growth could be inhibited at these concentrations, although the resulting inhibition zones were relatively small and classified as weak. This finding is consistent with previous studies, which define the Minimum Inhibitory Concentration (MIC) as the lowest concentration that produces a statistically significant difference in bacterial growth compared with the positive control. The relatively low MIC values observed for both bacterial species highlight the potential of loquat (*Eriobotrya japonica*) leaves as a natural source of traditional antibacterial agents.

5. ACKNOWLEDGMENT

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