Vol. 15, No. 4 (2026): 1559 - 1571 http://dx.doi.org/10.23960/jtepl.v15i4.1559-1571

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Phenolic Compounds of Guava Leaves (*Psidium guajava* L.): Their Antimicrobial Activity and Application in Pindang Eggs

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Article History:

Received : 5 December 2025
Revised : 3 February 2026
Accepted : 4 February 2026

Keywords:

Antibacterials,
Flavonoids,
Herbs,
Leaf extract,
Salmonella.

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ABSTRACT

Guava leaves (Psidium guajava L.) contain phenolic compounds with potential antimicrobial activity, but their application in pindang eggs has not been scientifically validated. This study identified phenolic compounds in guava leaf extracts and evaluated their antibacterial activity and effectiveness in extending the shelf life of pindang eggs. Guava leaves were extracted by maceration and infusion (sample-to-solvent ratio 1:5), while bioactive compounds in the ethanol extract were characterized using LC-MS/MS. Antibacterial activity against Salmonella, Escherichia coli, and Staphylococcus aureus was determined using the Kirby–Bauer disk diffusion method, and the preservative effect was evaluated through total plate count analysis during room-temperature storage. LC-MS/MS analysis revealed the presence of flavonoids, phenolic acids, coumarins, tannins, and stilbenoids in the ethanol extract. The strongest antibacterial activity of the ethanol extract was observed against E. coli at 50% concentration, producing an inhibition zone of 12.5 ± 8.8 mm, whereas the aqueous extract showed the highest activity against Salmonella with an inhibition zone of 11.0 ± 2.2 mm. Incorporating guava leaves during egg boiling delayed detectable microbial growth until the third day of storage, while control eggs exhibited microbial growth from day 0. These results demonstrate the potential of guava leaves as a natural antibacterial agent to improve microbial stability and shelf life of pindang eggs.

1. INTRODUCTION

Indonesia is a tropical country with high biodiversity, providing a wide range of plant species with potential applications in the food and healthcare sectors. One of these species is guava (*Psidium guajava* L.), a member of the Myrtaceae family that is widely distributed in tropical regions and is relatively easy to cultivate (Cahyaningsih *et al.*, 2021). Guava leaves are widely used in traditional medicine because they contain active compounds, mostly phenolic compounds, that exhibit antimicrobial properties, which can also be applied in food products.

Guava leaves contain various bioactive compounds, particularly tannins, saponins, and flavonoids, that exhibit strong antibacterial activity by disrupting bacterial cell structures and functions (Handarni *et al*., 2020). These phenolic-rich extracts have been shown to effectively inhibit pathogenic bacteria, such as Escherichia coli, which is supported by their high tannin content and demonstrated inhibition zones even at low concentrations (Fraga-Corral *et al*., 2021; Khanna *et al*., 2025; George, 2024). Their longstanding traditional use for treating gastrointestinal infections, as well as their approval as functional food products, such as guava leaf tea in Japan, further highlights their antimicrobial potential.

Aside from their medicinal applications, guava leaves are also employed in the processing of pindang eggs. Eggs are highly nutritious poultry products, containing approximately 13% protein, 12% fat, and essential vitamins and minerals (Yu *et al.*, 2024). However, this high nutritional content makes eggs susceptible to quality deterioration, spoilage, and microbial contamination. The loss of moisture and carbon dioxide through approximately 10,000 pores in the eggshell

facilitates easier microbial penetration, thereby accelerating quality degradation (Wengerska *et al*., 2023). To maintain quality and extend self-life, effective preservation methods are required. One proven method is the pindang process, which involves boiling whole eggs with their shells in a mixture of specific herbs. This method has been found to extend the shelf life of boiled eggs compared to conventional water boiling (Harlina *et al*., 2022; Hejazian *et al*., 2023). The pindang approach aligns with natural food preservation principles, where bioactive compounds from herbal ingredients inhibit microbial growth and slow product deterioration. Among these ingredients, guava leaves (*Psidium guajava* L.) have demonstrated significant antibacterial activity. Few studies have examined the relationship between the phenolic composition of *P. guajava* leaves and their preservation efficacy in eggs. Moreover, the underlying mechanisms, detailed bioactive profiles, and optimal application methods in food systems remain poorly understood. Therefore, this study aims to identify the phenolic compounds profile and antibacterial activity of *P. guajava* leaves and to evaluate their potential as natural ingredient for extending the shelf life of pindang eggs.

2. MATERIALS AND METHODS

2.1. Tools and Materials

The materials used were young and mature leaves of *Psidium guajava* (red-fleshed varieties) obtained from Botani Mart (Bogor, Indonesia) and microbial strains (*E. coli*, *Salmonella* spp., *S. aureus)* from Microbiology Laboratory, Department of Food Science and Technology, IPB University. Commercial broiler chicken eggs were purchased from market in Bogor area. Additional materials included ethanol (p.a., Merck), Mueller Hinton Agar (Oxoid), Plate Count Agar (Oxoid), Buffered Peptone Water (Oxoid), antimicrobial discs (Himedia), and chloramphenicol (Himedia). The guava leaves' phenolic compound were analyzed using the UHPLC Vanquish Tandem Q Exactive Plus Orbitrap MS/MS system (Thermo Scientific, USA).

2.2. Methods

The study employed a laboratory experimental design with treatments based on guava leaf age (young and mature), and type of extraction solvents (ethanol and water). The research procedures included leaf simplicia characterization, extract preparation, identification of phenolic compounds, antibacterial testing of various extract concentrations against *E. coli*, *Salmonella*, and *Staphylococcus aureus*, and application of the leaf in pindang eggs production. Data analysis was conducted using a univariate statistical approach with SPSS version 26. Statistical analysis was performed at a significance level of $\alpha = 0.05$, and the results were presented as mean ± standard deviation (SD).

2.2.1. Plant Material Preparation

Young leaves were defined as leaves positioned at nodes 1 to 4 from the shoot apex, whereas mature leaves were defined as those located at node 5 and beyond. A total of 1000 g of fresh leaves were washed, chopped, and dried (oven 40 °C, 24 h). The dried leaves were ground and sieved through an 80-mesh sieve. The leaf simplicia was weighed, packed in airtight container, and stored for further analysis. The moisture and ash content of guava leaf powder were analyzed based on gravimetry method (AOAC, 2012).

2.2.2. Leaf Extraction

The ethanol guava leaf extract preparation followed Nursanty *et al*. (2023) with minor modification. A total of 40 g of guava leaf powder was macerated in 200 mL of 96% ethanol (ratio 1:5) at room temperature for 24 h. The mixture was then filtered, and the filtrate was concentrated using a rotary evaporator (150 rpm, 40 °C) for 2–3 h.

The aqueous guava leaf extraction was adapted from Pereira *et al*. (2023) with slight modification. A 40 g of guava leaf powder was mixed with 200 mL of distilled water and heated at 60 °C for 30 min. The mixture was filtered and the filtrate was concentrated using a rotary evaporator (150 rpm, 40 °C). The yield and pH of the ethanolic and water leaf extract were subsequently determined.

2.2.3. Identification of Phenolic Compound

The phenolic of ethanolic of guava leaf extracts was performed using UHPLC–HRMS, following the method described by Abu-Lafi *et al*. (2022). A total of 5 mg of ethanolic guava leaf extract was dissolved in 1 mL of methanol (MeOH) and filtered through a 0.2 µm nylon membrane filter. The sample was then injected into a UHPLC–HRMS system (Vanquish Tandem Q Exactive Plus Orbitrap HRMS) equipped with a C18 column (100 × 2.1 mm, 1.5 µm; Thermo Scientific) at a flow rate of 0.2 mL/min. The mobile phase consisted of water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B), with a gradient program of 0–1 min (5% B), 1–25 min (5–95% B), 25–28 min (95% B), and 28–33 min (5% B). The column temperature was set at 30°C, with an injection volume of 2.0 µL. Analysis was conducted over a mass range of 100–1500 m/z using negative ionization mode. Compound identification was carried out using the Compound Discoverer 3.2 database based on chromatographic profiles.

2.2.4. Antibacterial Activity Assay

Test solutions of guava leaf extracts (aqueous and ethanolic) from both young and mature leaves were prepared at a concentration of 100 mg/mL using sterile distilled water as the solvent and subsequently diluted into several concentrations. The microbial test suspensions, adjusted to the 0.5 McFarland standard (1×10^8 CFU/mL), were evenly spread onto the surface of Mueller-Hinton Agar (MHA) using a sterile cotton swab. Sterile discs pre-soaked in the extract solutions at various concentrations, along with positive control discs (chloramphenicol) and a negative control (sterile distilled water), were placed on the surface of the inoculated media. The plates were then incubated at 37 °C for 24 hours. Antibacterial activity was assessed by measuring the diameter (mm) of the clear inhibition zones formed around the discs.

2.2.5. Application of Guava Leaf in Pindang Eggs Processing

Guava leaves were prepared at 300 g and boiled in 1500 mL of water (1:5). Eggs were initially boiled together with the leaves for 15 min at < 80 °C until half-cooked, then cracked evenly across the surface. The cracked eggs were then subjected to a second boiling for 1 h 45 min at 90 °C until the eggs were fully cooked and the shells turned dark brown. The pindang eggs were drained, cooled to room temperature, and stored for up to 5 days. Microbiological analysis was conducted daily during storage.

2.2.6. Microbial Quality of Pindang Eggs

The microbial load was determined using the total plate count (TPC) method (BSN, 2008). Egg contents (albumen and yolk) were homogenized under aseptic conditions, and 10 g of homogenate was mixed with 90 mL of 0.1% Buffered Peptone Water (BPW) to obtain a 10^{-1} dilution. Serial dilutions were prepared up to 10^{-4}, and 1 mL of each dilution was plated in duplicate on sterile Petri dishes. Approximately 15–20 mL of molten Plate Count Agar (PCA) was poured into each plate, mixed by gentle swirling, and allowed to solidify. Plates were incubated at 37°C for 24–48 hours. Colony-forming units (CFU) were enumerated, and microbial counts were expressed as CFU/g.

3. RESULTS AND DISCUSSION

3.1. Characteristics of Guava Leaves and Leaf Extracts

The guava leaf (*Psidium guajava* L.) samples used in this study belong to the family Myrtaceae and were taxonomically identified (BRIN No. B-3845/II.6.2/IR.01.02/9/2025). The moisture content of the guava leaf simplicia complied with quality standards for herbal materials (moisture content ≤10%, (Kemenkes RI, 2017)). Excessively high moisture content in simplicia can compromise its stability by facilitating microbial growth and accelerating the degradation of bioactive compounds. Conversely, excessively low moisture content may result in the loss of volatile compounds, including essential oils (Nurhaslina *et al.,* 2022). The ash content of the guava leaf simplicia also met the quality standard established by the Indonesian Ministry of Health, which specifies a maximum permissible level of <6.1% (Kemenkes RI, 2020). The proximate composition of young and mature guava leaves, including moisture and ash contents, was presented in Table 1.

Table 1. Moisture and ash content of young and mature guava leaves

Parameter	Young Leaves	Mature Leaves
Moisture (%)	8.59 ± 0.30[a]	8.06 ± 0.43[a]
Ash (%)	5.64 ± 0.24[a]	5.40 ± 0.36[a]

Note: Values are expressed as mean ± standard deviation (n = 3). Means followed by the same superscript letter within the same row indicate no significant difference based on an independent t-test ($p < 0.05$).

Moisture and ash content in young and mature guava leaves did not differ significantly ($p > 0.05$). Moisture content was 8.59 ± 0.30% in young leaves and 8.06 ± 0.43% in mature leaves, while ash content was 5.64 ± 0.24% and 5.40 ± 0.36%, respectively. These results suggest that leaf maturity did not significantly affect the moisture or ash content of guava leaves raw materials.

The potential of the raw material to yield bioactive compounds was assessed through extraction using two different solvents. Extract yield was used to evaluate the efficiency of the extraction process in obtaining bioactive compounds from the plant material, while pH values reflected the acidity of the extracts, which could influence the stability and biological activity of the compounds. The results of the extract yield and pH for both ethanol and water extracts were presented in Table 2.

Table 2. Extract yield and pH of guava leaves

Extract Type	Leaf Type	Yield (%)	pH
Ethanol	Young leaves	15.99 ± 0.00[a]	4.30 ± 0.09[a]
	Mature leaves	15.51 ± 0.00[a]	4.52 ± 0.08[a]
Water	Young leaves	8.72 ± 0.00[b]	5.28 ± 0.05[b]
	Mature leaves	10.00 ± 0.00[b]	5.33 ± 0.09[b]

Note: Values are expressed as mean ± standard deviation ($n = 3$). The same superscript letters indicate no significant difference ($p < 0.05$) based on an independent t-test.

The highest extract yield was obtained using ethanol, reaching 15.99 ± 0.00 for young leaves and 15.51 ± 0.00 for mature leaves, whereas the lowest yield was observed in the water extract of young leaves (8.72 ± 0.00). Ethanol extracts exhibited lower pH values (4.30–4.52) compared to water extracts (5.28–5.33). These results indicate that the type of solvent significantly influenced extract yield and pH, while leaf maturity showed no significant effect within the same solvent.

The efficiency of solvent extraction is governed by the principle of "like dissolves like," which states that compounds tend to dissolve more readily in solvents with similar polarity (Handarni *et al*., 2020). Ethanol, having moderate polarity, can effectively extract both polar and semi-polar compounds, including phenolics and flavonoids, from both young and mature guava leaves. This explains the absence of significant differences in ethanol extract yield between leaf maturities. In contrast, water, as a highly polar solvent, primarily extracts polar compounds, resulting in lower extraction yields compared to ethanol. Although numerical differences were observed between young and mature leaves in water extracts, these differences were not statistically significant. These findings are in line with Chen *et al*. (2022), who reported that leaf maturity may influence the concentration and composition of bioactive compounds depending on the extraction solvent.

Differences in pH between solvents also reflect variations in the chemical composition of the extracts. Although the initial pH of both ethanol and water used as extraction solvents was neutral (approximately pH 7), the pH of the resulting extracts decreased due to the extraction of acidic bioactive compounds, particularly phenolic acids and tannins. Ethanol extracts showed lower pH values due to their higher content of organic acids and phenolic compounds, whereas water extracts exhibited higher pH values owing to their more selective solubility of polar constituents (Lee *et al*., 2024). According to Chen *et al*. (2022), such pH variations were indicative of differences in the types and concentrations of dissolved phytochemicals, which may ultimately influence antimicrobial performance. The pH environment further affects the stability and activity of bioactive compounds. Flavonoids, for instance, undergo structural changes at different pH levels, which can reduce their antimicrobial effectiveness (Wang *et al*., 2023; Xiang *et al*., 2017). According to Gama

et al. (2023) reported that antibacterial activity remains detectable under neutral to slightly basic conditions, but its potency declines as pH increases, suggesting that acidic environments enhance the functional performance of flavonoids and other phenolic compounds. Overall, ethanol proved to be a more efficient solvent than water for extracting bioactive compounds from guava leaves. Its ability to produce higher yields and more acidic extracts supports the enhanced solubility, stability, and potential antimicrobial activity of phenolic constituents, aligning with the observed chemical and biological characteristics of guava leaf extracts.

3.2. Phenolic Compound of Guava Leaves Extracts

Guava leaf extracts contain several metabolites, including flavonoids, triterpenoids, meroterpenoids, and carotenoids (Huang *et al*., 2021). A total of 18 groups of bioactive compounds were identified in the ethanolic extracts of young and mature guava leaves, including flavonoids, terpenoids, diterpenoids, triterpenoids, phenolic acids, tannins, stilbenoids, vitamins, carbohydrates, analgesic agents, coumarins, organophosphates, organic acids, carbonate esters, aromatics, steroids, fatty acids, and unknown compounds (Figure 1). The most abundant group was flavonoids.

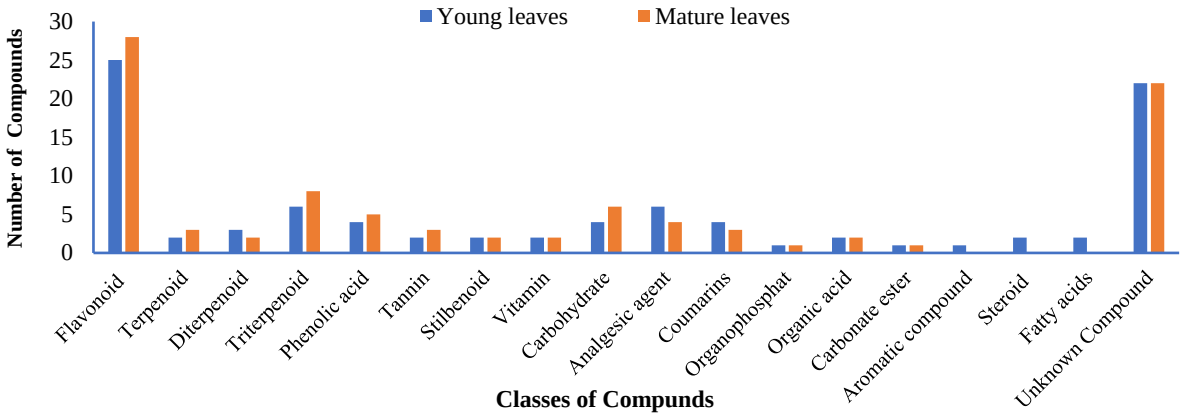

Figure 1. Phenolic compound components in ethanolic extracts of young and mature guava leaves

In addition to the phytochemical identification results, previous studies have reported the presence of several major bioactive compounds in guava leaves along with their biological activities. This information reinforces the current observations and highlights the potential biological functions of each detected compound. A summary of selected major bioactive compounds and their biological activities is presented in Table 3.

Phenolic analysis of ethanol extracts from guava leaves revealed the presence of diverse compounds representing several major chemical classes, including flavonoids, tannins, stilbenoids, and phenolic acids (Table 4). Flavonoids were the most abundant group, consisting of quercetin, quercitrin, and isoquercetin. These compounds demonstrate diverse biological activities, notably antibacterial, antioxidant, anti-inflammatory, and anticancer properties. Tannins, saponins, and flavonoids serve as the primary contributors to antibacterial activity, each functioning through different mechanisms. Flavonoids cause cell membrane damage, protein denaturation, and disruption of cellular metabolism (Zhang *et al*., 2020). In addition to flavonoids, tannin compounds such as gallic acid and ellagic acid were also detected, both of which are known for their strong antioxidant and antimicrobial activities. Tannins act by inducing cell membrane contraction, enzyme inactivation, and bacterial cell wall disruption (Hu *et al*., 2025). Other identified compounds, such as astringin (stilbenoid) and 5-caffeoyl shikimic acid (phenolic acid), also contribute to the extract's antioxidant capacity. This chemical diversity indicates that both young and mature guava leaves contain complex secondary metabolites that support their antimicrobial and antioxidant functions.

Previous studies similarly reported that guava leaves contain the highest total phenolic content (TPC) of 106.50 mg/g compared to other plant parts such as fruits, seeds, and bark stem (Keddy *et al*., 2023; Shabbir *et al*., 2020). Phenolic compounds, particularly polyphenols, are well known for their dual roles as antimicrobial and antioxidant agents (Beya *et al*., 2021). This was consistent with the present study, where most of the identified bioactive constituents were phenolic-derived flavonoids (Table 4). In this study, the high phenolic content (Table 4) in guava leaves was consistent

with the literature reported by Huynh *et al*. (2025), who stated that bioactive compounds were predominantly found in the roots, stem bark, and especially the leaves. They further noted that the total phenolic content in *Psidium guajava* leaves was higher than that in its stem bark.

Table 3. Biological activities of ethanol extracts from young and mature guava leaves

No	Compound Name	Formula	Biological Activity	Category	Reference
1	Quercetin	$C_{15}H_{10}O_7$	Antibacterial, Anticancer, Anti-inflammatory, Antiviral	Flavonoid	Bactiar & Fahami (2019)
2	Quercitrin	$C_{21}H_{20}O_{11}$	Antimicrobial	Flavonoid	Hamid *et al*. (2025)
3	Isoquercetin	$C_{21}H_{20}O_{12}$	Antimicrobial, Antioxidant	Flavonoid	Wang *et al*. (2013)
4	Gallic Acid	$C_7H_6O_5$	Antioxidant, Anticancer, Antimicrobial	Tannin	Kenmeni *et al*. (2024)
5	Naringenin	$C_{15}H_{12}O_5$	Anticancer, Antimicrobial, Antidiabetic, Antioxidant	Flavonoid	Duda-Madej *et al*. (2022)
6	Astringin	$C_{20}H_{22}O_9$	Antioxidant, Antimicrobial	Stilbenoid	Metsamuuronen & Siren (2019)
7	Ellagic Acid	$C_{14}H_6O_8$	Antimicrobial	Tannin	Aljassim *et al*. (2022)
8	Luteolin 7-Sulfate	$C_{15}H_{10}O_9S$	Antioxidant, Anti-inflammatory, Antibacterial	Flavonoid	Ren *et al*. (2024)
9	Cyanidanol	$C_{15}H_{14}O_6$	Analgesic, Anti-inflammatory, Antimicrobial, Antioxidant	Flavonoid	Barekat *et al*. (2022)
10	5-Caffeoyl Shikimic Acid	$C16H_{16}O_8$	Antimicrobial, Antioxidant	Phenolic Acid	Thongphicai *et al*. (2023)

Tabel 4. Phenolic compound groups in ethanol extracts of young and mature guava leaves

Type of Phenolic Compounds	Number of Compounds (Young Leaves)	% Area relative	Number of Compounds (Mature Leaves)	% Area relative
Flavonoids	25	44.06	28	37.47
Phenolic acids	4	3.10	5	6.21
Stilbenoids	2	0.35	2	0.76
Tannin	2	0.40	3	1.32
Coumarins	4	2.64	3	1.63
Total	37	50.55	41	47.39

The main phenolic compounds detected in guava leaf extracts included gallic acid, catechin, caffeic acid, epicatechin, rutin, quercetin, kaempferol, and luteolin (Amaral *et al*., 2021). Similarly, Ruksiriwanich *et al*. (2022) identified eleven phenolic compounds, such as quercetin, rutin, naringenin, and gallic acid, which demonstrated strong antimicrobial potential. Dominant phenolic compounds, such as gallic acid, catechin, quercetin, and myricetin, play critical roles in antimicrobial activity through mechanisms including metabolic inhibition, cytoplasmic membrane disruption, and interference with protein synthesis and cell division (Mowes *et al*., 2025; Pereira *et al*., 2023). The antimicrobial activity of guava leaf extracts has been well-documented. Kumar *et al*. (2021) demonstrated notable inhibition of *S. aureus* by ethanolic extracts (13.6 ± 0.01 mm). Similarly, Kaware & Ismail (2021) reported that both aqueous and ethanolic extracts effectively inhibited *S. aureus* and *Salmonella*. These findings indicate that the antimicrobial efficacy of guava leaf extracts is largely driven by the synergistic actions of their phenolic constituents.

3.3. Antimicrobial Activity of Guava Leaf Extracts

The antimicrobial activity of ethanol and aqueous leaf extracts from young and mature leaves against *E. coli*, *Salmonella,* and *S. aureus* was summarized in Table 5. The results of this study indicate that both aqueous and ethanolic extracts of young and mature guava leaves exhibit antibacterial activity against *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus* at specific concentrations. The highest antibacterial activity of the ethanolic extract was observed against *E. coli* at a 50% concentration, producing an inhibition zone of 12.5 ± 8.8 mm from the young leaf extract. In contrast, the aqueous extract exhibited the highest activity against *Salmonella* at a 50% concentration, with an inhibition zone of 11.0 ± 2.2 mm from the mature leaf extract. These findings suggest that the antibacterial efficacy of guava leaf extracts is influenced by the type of solvent, extract concentration, and leaf maturity.

Table 5. Antimicrobial activity of young and mature guava leaf extracts

Leaf Type	Extract Type	Extract Concentration (%)	Inhibition Zone (mm)		
			E. coli	*Salmonella*	*S. aureus*
Young leaves	Ethanol	50	12.5 ± 8.8	-	10.0 ± 7.0
		25	-	-	-
		Positive Control	26.5 ± 1.8	18.3 ± 5.6	20.7 ± 2.9
		Negative Control	-	-	-
Mature leaves		50	10.1 ± 1.2	9.0 ± 0.7	9.2 ± 0.7
		25	-	-	7.8 ±5.5
		Positive Control	26.9 ± 0.3	18.0 ± 2.5	18.3 ± 0.6
		Negative Control	-	-	-
Young leaves	Water	50	8.6 ± 6.0	9.3 ± 0.6	8.6 ± 6.0
		25	8.1 ± 5.7	9.6 ± 6.7	9.0 ± 6.3
		Positive Control	26.6 ± 0.1	17.4 ± 4.5	16.9 ± 1.6
		Negative Control	-	-	-
Mature leaves		50	-	11.0 ± 2.2	10.4 ± 0.4
		25	-	7.9 ± 1.2	8.2 ± 0.2
		Kontrol positif	28. 0 ± 0.2	16.8 ± 0.5	20.2 ± 0.3
		Kontrol negatif	-	-	-

Note: Values are presented as mean ± standard deviation (SD) from two independent experiments (n = 2).

The observed antimicrobial activity can be attributed to the presence of bioactive compounds, particularly phenolic compounds, including flavonoids and tannins. Flavonoids are known to inhibit bacterial growth through multiple mechanisms, such as disrupting cellular metabolism, inhibiting nucleic acid synthesis, altering membrane permeability, and suppressing biofilm formation (Pramusita *et al*., 2023; Mowes *et al*., 2025). Tannins can interact with bacterial proteins, especially proline-rich proteins, thereby interfering with protein synthesis and bacterial metabolism (Kaware & Ismail, 2020). Phenolic compounds also exert their antibacterial effects by damaging cell membranes, inhibiting virulence factors such as enzymes and toxins, and preventing biofilm formation. Visual representations of the antibacterial activity of ethanolic extracts against the three test bacteria are shown in 4, while the activity of water extracts is presented in Figure 5.

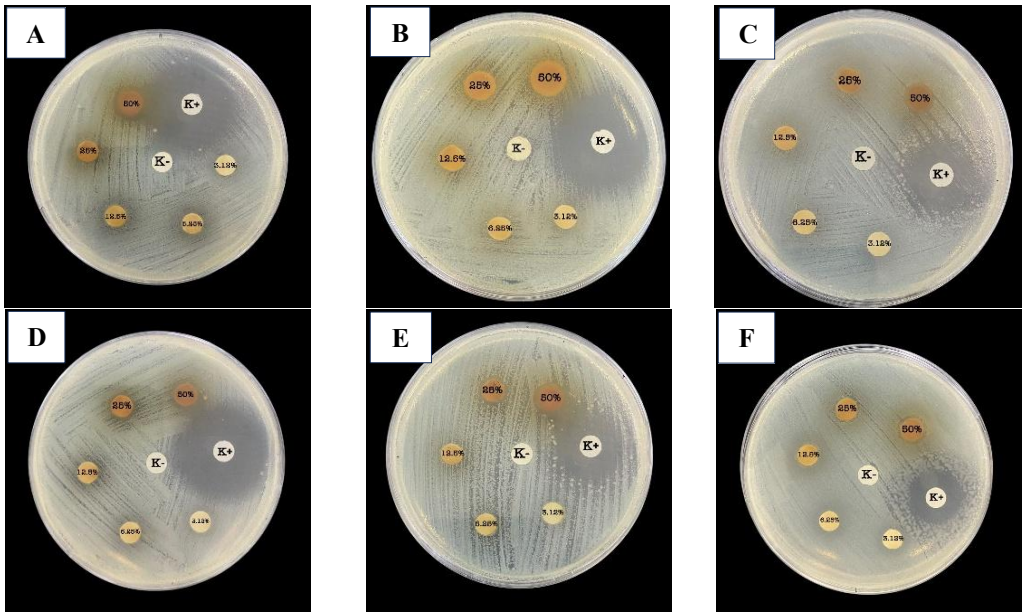

Figure 4. Antimicrobial activity of ethanol extracts from young and mature guava leaves (*Psidium guajava* L.) against three test bacteria: *Escherichia coli*, *Salmonella*., and *Staphylococcus aureus*. A = *Escherichia coli* (young leaves); B = *Salmonella* (young leaves); C = *Staphylococcus aureus* (young leaves); D = *Escherichia coli* (mature leaves); E = *Salmonella* (mature leaves); F = *Staphylococcus aureus* (mature leaves).

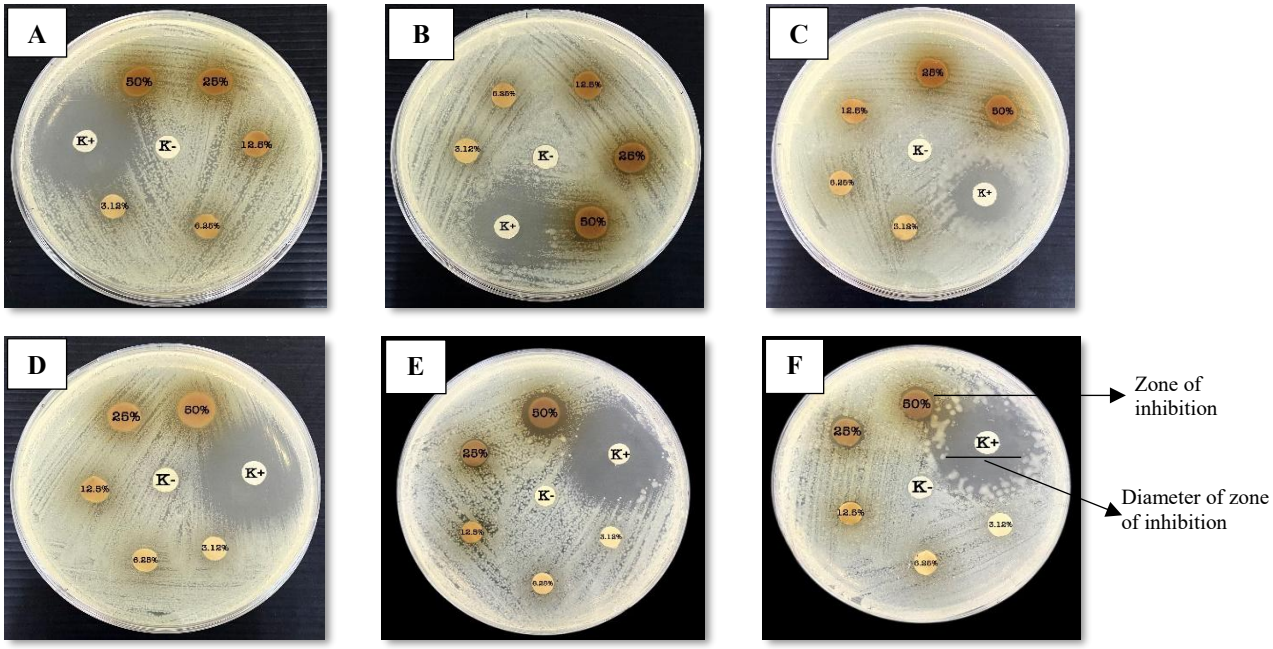

Figure 5. Antimicrobial activity of water extracts from young and mature guava leaves (*Psidium guajava* L.) against three test bacteria: *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*. A = *Escherichia coli* (young leaves); B = *Salmonella* (young leaves); C = *Staphylococcus aureus* (young leaves); D = *Escherichia coli* (mature leaves); E = *Salmonella* (mature leaves); F = *Staphylococcus aureus* (mature leaves).

Differences in bacterial susceptibility were observed between Gram-negative and Gram-positive bacteria. Gram-negative bacteria, such as *E. coli* and *Salmonella s*, possess an outer membrane containing lipopolysaccharides (LPS), which limits the penetration of bioactive compounds and contributes to their relative resistance (Paracini *et al*., 2022). In contrast, Gram-positive *S. aureus* has a single layer of peptidoglycan that was more easily penetrated, making it more susceptible to plant-derived antibacterial compounds (Nguyen & Bhattacharya, 2022). Interestingly, the present study demonstrated that guava leaf extracts exhibited higher antibacterial activity against Gram-negative bacteria (*E. coli* and *Salmonella*) compared to the Gram-positive *S. aureus*. Although Gram-negative bacteria are generally regarded as more resistant due to the presence of an outer membrane containing lipopolysaccharides (LPS), which restricts the penetration of many bioactive compounds, specific phytochemicals in guava leaves may interact selectively with Gram-negative cellular structures. This can be explained based on the literature regarding plant flavonoids. Flavonoids possess multiple mechanisms to inhibit bacterial growth, not only by disrupting the cell membrane but also by targeting intracellular components, such as DNA gyrase. In addition, some flavonoids are more effective against Gram-negative bacteria due to their chemical properties, which facilitate interactions with bacterial cells. The combination of these mechanisms allows flavonoids to penetrate or interfere with Gram-negative bacteria despite their complex outer membrane, resulting in higher antibacterial activity against this group of bacteria (Yan *et al*., 2024).

Leaf maturity further influenced antimicrobial activity due to variations in the content of secondary metabolites. Young leaves are richer in flavonoids and phenolic acids (Table 4), which contribute to stronger inhibition against *E. coli* and *S. aureus*, whereas mature leaves undergo lignification and metabolite transformations that reduce some bioactive compounds (Díaz-De-Cerio *et al*., 2016). However, mature leaves contain higher levels of tannins and triterpenoids, which may enhance their inhibitory effect against *Salmonella* spp. (Bhardwaj & Naruka, 2023; Buah *et al*., 2023).

Overall, these results demonstrate that guava leaves contain bioactive compounds with significant antibacterial potential, and their effectiveness depends on the solvent type, extract concentration, and physical characteristics of the leaves. These findings highlight the promising potential of guava leaf extracts as natural antibacterial agents.

3.4. Microbial Quality of Pindang Eggs

The antibacterial effectiveness of guava leaves was applied in the production of pindang eggs. This study employed fresh guava leaves directly during the egg-boiling process. The use of whole leaves was chosen to simulate common household practices and to enable the natural release of antimicrobial compounds during heating without the need for an additional extraction step. Young and mature guava leaves were added during the boiling process, and the microbial quality of the eggs was monitored daily throughout a 5-day storage period at room temperature. The TPC results revealed differences in bacterial growth among treatments (Table 5). From day 0 to day 2, only the control samples exhibited bacterial growth, increasing from 1.65×10^2 CFU/g (log 2.22) on day 0 to 1.115×10^3 CFU/g (log 3.05) on day 2. In contrast, eggs with intact shells and those treated with either young or mature guava leaves showed no detectable microbial growth during this early storage period. It was indicating that the physical barrier of the eggshell combined with from guava leaves effectively inhibited microbial proliferation. Notably, microbial growth in eggs treated with guava leaves was first detected only on day 3, demonstrating the efficacy of the leaves in delaying spoilage. These findings are consistent with Dumpala *et al*. (2023), who reported that treating fresh pangasius fish with 80% guava leaf extract extended its shelf life by three days, whereas the control group experienced spoilage on the first day. Similarly, Eregama *et al*. (2024) highlighted that boiled eggs stored at room temperature provide a favorable environment for microbial growth, including *Salmonella*, emphasizing that post-boiling storage at ambient conditions was not recommended.

Table 6. Total Plate Count (TPC) of pindang eggs during room temperature storage

Storage Day	Sample	CFU/g	Log CFU/g
0	Control	1.65×10^2	2.22
	Control intact shell	TSUD	<1
	Egg + Young leaves	TSUD	<1
	Egg + Mature leaves	TSUD	<1
1	Control	4.3×10^2	2.63
	Control intact shell	TSUD	<1
	Egg + Young leaves	TSUD	<1
	Egg + Mature leaves	TSUD	<1
2	Control	1.115×10^3	3.05
	Control intact shell	TSUD	<1
	Egg + Young leaves	TSUD	<1
	Egg + Mature leaves	TSUD	<1
3	Control	3×10^5	5.48
	Control intact shell	TSUD	<1
	Egg + Young leaves	4.1×10^3	3.61
	Egg + Mature leaves	3.45×10^3	3.54
4	Control	3×10^5	5.48
	Control intact shell	TSUD	<1
	Egg + Young leaves	3×10^5	5.48
	Egg + Mature leaves	3×10^5	5.48
5	Control	Egg spoiled	
	Control intact shell	TSUD	<1
	Egg + Young leaves	Egg spoiled	
	Egg + Mature leaves	Egg spoiled	

Note: TSBC = too small to be counted

The antimicrobial properties of guava leaves were largely attributed to bioactive compounds such as flavonoids, tannins, and stilbenoids, which exhibit antibacterial, antioxidant, and anti-inflammatory activities (Ugbogu *et al*., 2022; Barekat *et al*., 2022; Metsamuuronen & Siren, 2019). The lower microbial count in boiled eggs cooked with guava leaves compared to boiled eggs without guava leaves is likely due to the bioactive compounds present in the guava leaves. The use of guava leaf extracts has traditionally been applied in folk medicine worldwide because they contain

the highest levels of phenolic compounds, particularly flavonols, compared to other plants, thereby exhibiting antimicrobial properties (Diaz-de-Cerio *et al*. 2016).

The use of natural antibacterial agents has great potential because they utilize bioactive compounds that offer various benefits, such as antimicrobial and antioxidant activities, are safe for health, and can help maintain the nutritional value of food. One of the most common bacteria causing foodborne illness worldwide is *Salmonella*. The presence of *Salmonella* is often used as an indicator in microbiological analyses to assess microbial contamination in stored eggs. Furthermore, egg production methods, storage, handling, and food processing all have the potential to influence contamination levels, making natural preservation an important strategy for ensuring the safety and quality of eggs (FDA, 2009).

The novelty of this study lies in the integration of phenolic compound characterization of guava leaves (*Psidium guajava* L.) at different maturity stages using UHPLC-HRMS, evaluation of the antibacterial activity of ethanol and aqueous extracts, and their application to pindang eggs. This study provides a scientific contribution by expanding the understanding of the relationship between phenolic compound composition and antibacterial activity, while supporting the utilization of guava leaves as an alternative natural preservative in food products.

4. CONCLUSIONS

Guava leaves (*Psidium guajava* L.) used in this study were identified as members of the Myrtaceae family and met quality standards for both crude material and extracts, with moisture, ash, yield, and pH values within acceptable ranges. Bioactive compound analysis of ethanol extracts from young and mature leaves revealed a predominance of phenolic compounds, particularly flavonoids, which are widely recognized for their antibacterial activity. Both water and ethanol extracts effectively inhibited the growth of *Escherichia coli*, *Salmonella*, and *Staphylococcus aureus*. Application of guava leaves in pindang egg processing successfully suppressed microbial growth during room-temperature storage, maintaining the Total Plate Count (TPC) below the maximum limit for edible eggs established by the Indonesian National Standard ($\leq 1 \times 10^5$ cfu/g; SNI 3926:2008). These findings indicate that guava leaves are a promising source of phenolic compounds with antibacterial properties and have potential as a natural antimicrobial agent to extend shelf life and enhance food safety of pindang eggs.

DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE MANUSCRIPT PREPARATION

Not applicable. The authors did not use any generative AI tools or services in the preparation of this manuscript. All content is entirely the intellectual work of the authors.

AUTHOR CONTRIBUTION

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
RJN	✓	✓	✓		✓	✓	✓		✓	✓	✓		✓	✓
DI		✓		✓				✓		✓		✓	✓	
HDK		✓		✓				✓		✓		✓	✓	

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

REFERENCES

Abu-Lafi S, Akkawi M, Abu-Remeleh Q, & Lutgen P. (2021). Screening of guava (*Psidium guajava*) leaves extracts against β-hematin formation. *Pharm Pharmacol Int J.*, ***9***(1), 11–15.

Aljassim, Z.G., Kadhim, H.M., & Al-Mizraqchi, A.S. (2022). Evaluation of antimicrobial activity of ellagic acid on Methicillin-resistant Staphylococcus aureus (MRSA), *Pseudomonas aeruginosa*, and *Escherichia coli*. *International Journal of Health Sciences*, ***6***(S3), 11891–11899. https://doi.org/10.53730/ijhs.v6nS3.8956

Amaral, V.A., Alves, T.F.R., Souza, J.F., Batain, F., Crescencio, K.M.M., Soeiro, V.S., Barros, C.T., & Chaud, M.V. (2021). Phenolic compounds from *Psidium guajava* (Linn.) leaves: Effect of the extraction-assisted method upon total phenolics content and antioxidant activity. Biointerface *Research in Applied Chemistry*, ***11***(2), 9346–9357. https://doi.org/10.33263/BRIAC112.93469357

Association of Official Analytical Chemists [AOAC]. (2012). *Official Methods of Analysis of AOAC International*. USA: AOAC.

Bactiar, C.F., & Fahami, N.A.M. (2019). LC-MS analysis of phytocomponents in the methanol extract of *Piper sarmentosum* leaves. *Pharmacognosy Journal*, ***11***(5), 1071–1076. https://doi.org/10.5530/pj.2019.11.167

Badan Standardisasi Nasional [BSN]. (2008). *SNI 2987:2008: Metode pengujian cemaran mikroba dalam daging, telur, dan susu serta hasil olahannya*. Badan Standardisasi Nasional.

Barekat, S., Nasirpour, A., Keramat, J., Dinari, M., Meziane-Kaci, M., Paris, C., & Desobry, S. (2022). Phytochemical composition, antimicrobial, anticancer properties, and antioxidant potential of green husk from several walnut varieties (*Juglans regia* L.). *Antioxidants*, ***12***(1), 52. https://doi.org/10.3390/antiox12010052

Beya, M.M., Netzel, M.E., Sultanbawa, Y., Smyth, H., & Hoffman, L.C. (2021). Plant-based phenolic molecules as natural preservatives in comminuted meats: A review. *Antioxidants*, ***10***(2), 263. https://doi.org/10.3390/antiox10020263

Bhardwaj, R., & Naruka, D. (2023). Phytochemical screening and antibacterial activity of leaf and fruit extracts of guava (*Psidium guajava*). *The Indian Journal of Agricultural Sciences*, ***93***(11), 1220–1224. https://doi.org/10.56093/ijas.v93i11.141132

Buah, J.D., Musa, D.D., & Eberemu, N.C. (2023). Antibacterial activities of guava (*Psidium guajava*) and orange (*Citrus sinensis*) leaves extracts on *Staphylococcus aureus* and *Escherichia coli*. *Sahel Journal of Life Sciences FUDMA*, ***1***(1), 130–136. https://doi.org/10.33003/sajols-2023-0101-014

Cahyaningsih, R., Magos Brehm, J., & Maxted, N. (2021). Setting the priority medicinal plants for conservation in Indonesia. *Genetic Resources and Crop Evolution*, ***68***(5), 2019–2050. https://doi.org/10.1007/s10722-021-01115-6

Chen, C., Mokhtar, R.A.M., Sani, M.S.A., & Noor, N.Q.I.M. (2022). The effect of maturity and extraction solvents on bioactive compounds and antioxidant activity of mulberry (*Morus alba*) fruits and leaves. *Molecules*, ***27***(8), 2406. https://doi.org/10.3390/molecules27082406

Díaz-De-Cerio, E., Verardo, V., Gómez-Caravaca, A. M., Fernández-Gutiérrez, A., & Segura-Carretero, A. (2016). Exploratory characterization of phenolic compounds with demonstrated anti-diabetic activity in guava leaves at different oxidation states. *International Journal of Molecular Sciences*, ***17***(5), 699. https://doi.org/10.3390/ijms17050699

Duda-Madej, A., Stecko, J., Sobieraj, J., Szymańska, N., & Kozłowska, J. (2022). Naringenin and its derivatives—Health-promoting phytobiotic against resistant bacteria and fungi in humans. *Antibiotics*, ***11***(11), 1628. https://doi.org/10.3390/antibiotics11111628

Dumpala, S., Chintada, V., Prasad, S.H., Raju, C.S., Bhasha, S., Prasanna, L.P., Veeraiah, K., & Ramaneswari, K. (2023). Exploring the natural preservation potential of aqueous guava leaf extracts on Pangasius pangasius: An experimental study. *Journal of Advanced Zoology*, ***44***(S5), 124–137. https://doi.org/10.17762/jaz.v44iS-5.1195

Eregama, G.S.R., Aung, S.H., Pitawala, H.M.J.C., Ali, M., Lee, S.-Y., Park, J.-Y., Abeyrathne, E.D.N.S., & Nam, K.-C. (2024). Evaluation of physicochemical changes in hard-boiled eggs stored at different temperatures. Food Science of Animal Resources, 44(1), 74–86. https://doi.org/10.5851/kosfa.2023.e57

Food and Drug Administration [FDA]. (2009). *Prevention of Salmonella enteritidis in shell eggs during production, storage, and transportation*. Food and Drug Administration.

Fraga-Corral, M., Otero, P., Cassani, L., Echave, J., Garcia-Oliveira, P., Carpena, M., Chamorro, F., Lourenço-Lopes, C., Prieto, M. A., & Simal-Gandara, J. (2021). Traditional applications of tannin-rich extracts supported by scientific data: Chemical composition, bioavailability and bioaccessibility. *Foods*, ***10***(2), 251. https://doi.org/10.3390/foods10020251

Gama, G.S.P., Pimenta, A.S., Feijó, F.M.C., Santos, C.S., Castro, R.V.O., Azevedo, T.K.B., & Medeiros, L.C.D. (2023). Effect of pH on the antibacterial and antifungal activity of wood vinegar (pyroligneous extract) from Eucalyptus. *Revista Árvore*, ***47***, e4711. https://doi.org/10.1590/1806-908820230000011

George, A. (2024). Phytochemical screening and antimicrobial efficacy of leaf extract of guava (*Psidium guajava*) on selected bacteria. *International Journal of Contemporary Microbiology*, ***20***(4), e0321190. https://doi.org/10.37506/rqcmd547

Hamid, H.K., Safi, I.N., Hussein, F.H., & Yousif, A. (2025). Evaluation of the anti-bacterial effect of quercitrin in comparison with

chlorhexidine in dental implants: An in vitro study. *Journal of Baghdad College of Dentistry*, ***37***(2), 37–49. https://doi.org/10.26477/jbcd.v37i2.3966

Handarni, D., Putri, S. H., & Tensiska, T. (2020). Skrining kualitatif fitokimia senyawa antibakteri pada ekstrak daun jambu biji (*Psidium guajava* L.). *Jurnal Keteknikan Pertanian Tropis dan Biosistem*, ***8***(2), 182–188. https://doi.org/10.21776/ub.jkptb.2020.008.02.08

Harlina, P.W., Ma, M., Shahzad, R., & Khalifa, I. (2022). Effect of rosemary extract on lipid oxidation, fatty acid composition, antioxidant capacity, and volatile compounds of salted duck eggs. *Food Science of Animal Resources*, ***42***, 689–711. https://doi.org/10.5851/kosfa.2022.e30

Hejazian, S., Ghorbani-Hasansaraei, A., Ahmadi, M., & Shahidi, S.A. (2023). The effect of chia seed mucilage shell coating in combination with turmeric essential oil on egg shelf life and quality properties. *Journal of Food Measurement and Characterization*, ***17***, 4175–4190. https://doi.org/10.1007/s11694-023-01923-7

Hu, Z., Hu, Z., Zhang, H., Yu, Z., & Xie, Y. (2025). Antimicrobial effects of tannic acid combined with plasma activated water and their application in strawberry preservation. *Foods*, ***14***(13), 2216. https://doi.org/10.3390/foods14132216

Huang, J., Li, C., Ma, J., Xu, K., Chen, X., Jiang, J., & Zhang, D. (2021). Chemical constituents of *Psidium guajava* leaves and their antibacterial activity. *Phytochemistry*, ***186***, 1–7. https://doi.org/10.1016/j.phytochem.2021.112746

Huynh, H.D., Nargotra, P., Wang, H.M.D., Shieh, C.J., Liu, Y.C., & Kuo, C.H. (2025). Bioactive compounds from guava leaves (*Psidium guajava* L.): Characterization, biological activity, synergistic effects, and technological applications. *Molecules*, ***30***(6), 1278. https://doi.org/10.3390/molecules30061278

Kaware, M.S., & Ismail, M.S. (2021). Antimicrobial activity of guava leaf (*Psidium guajava*) extracts against some clinical bacteria isolates. *Dutse Journal of Pure and Applied Sciences (DUJOPAS)*, *7*(2b), 245–252.

Keddy, G.G., Kareru, P.G., Wanakai, S.I., Karenju, M.W., Kisoi, G.K., & Njenga, P.L. (2023). Antioxidant and antimicrobial activity of *Psidium guajava* (pomifera and pyrifera) aqueous leaf extract varieties. *Advances in Research*, ***24***(5), 124–137. https://doi.org/10.9734/air/2023/v24i5964

Kementrian Kesehatan Republik Indonesia [Kemenkes RI]. (2020). Farmakope Herbal Indonesia Edisi IV. Jakarta: Kementerian Kesehatan RI.

Kenmeni, J.F., Sifi, I., Bisso, B.N., Kayoka-Kabongo, P.N., Tsopmene, U.J., & Dzoyem, J.P. (2024). Exploring medicinal plants for antimicrobial activity and synergistic effects with doxycycline against bacterial species. *The Scientific World Journal*, ***2024***, 6238852. https://doi.org/10.1155/2024/6238852

Khanna, S., Singh, P., & Chauhan, E.S. (2025). Comparative analysis of bioactive compounds in guava (*Psidium guajava* L.) fruit and leaves. *Asian Journal of Research in Biochemistry*, ***15***(2), 366. https://doi.org/10.9734/ajrb/2025/v15i2366

Kumar, N.S.S., Sarbon, N.M., Rana, S.S., Chintagunta, A.D., Prathibha, S., Ingilala, S.K., Jeevan Kumar, S.P., Sai Anvesh, B., & Dirisala, V.R. (2021). Extraction of bioactive compounds from *Psidium guajava* leaves and its utilization in preparation of jellies. *AMB Express*, ***11***(1). https://doi.org/10.1186/s13568-021-01194-9

Lee, J.E., Jayakody, J.T.M., Kim, J.I., Jeong, J.W., Choi, K.M., Kim, T.S., Seo, C., Azimi, I., Hyun, J.M., & Ryu, B.M. (2024). The influence of solvent choice on the extraction of bioactive compounds from Asteraceae: A comparative review. *Foods*, ***13***, 3151. https://doi.org/10.3390/foods13193151

Metsamuuronen, S., & Sirén, H. (2019). Bioactive phenolic compounds, metabolism and properties: A review on valuable chemical compounds in Scots pine and Norway spruce. *Phytochemistry Reviews*, ***18***(3), 623–664. https://doi.org/10.1007/s11101-019-09630-2

Mowes, M., Kandanda, G.K., Nangolo, L.N., Shafodino, F.S., & Mwapagha, L.M. (2025). Qualitative phytochemical profiling and in vitro antimicrobial and antioxidant activity of *Psidium guajava* (guava). *PLOS ONE*, ***20***(4), e0321190. https://doi.org/10.1371/journal.pone.0321190

Nguyen, T.L.A., & Bhattacharya, D. (2022). Antimicrobial activity of quercetin: An approach to its mechanistic principle. *Molecules*, ***27***(8), 2494. https://doi.org/10.3390/molecules27082494

Nurhaslina, C.R., Bacho, S.A., & Mustapa, A.N. (2022). Review on drying methods for herbal plants. *Materials Today: Proceedings*, ***63***, S122–S139. https://doi.org/10.1016/j.matpr.2022.02.052

Nursanty, R., Padzil, K.N.B., Ramli, N.I.B., Mahyudin, N.A., Jaafar, A.B., & Rukayadi, Y. (2023). Phytochemical analysis of ethanolic *Psidium guajava* leaves extract using GC-MS and LC-MS. *Biodiversitas*, ***24***(5), 2723–2732. https://doi.org/10.13057/biodiv/d240526

Paracini, N., Schneck, E., Imberty, A., & Micciulla, S. (2022). Lipopolysaccharides at solid and liquid interfaces: Models for

biophysical studies of the Gram-negative bacterial outer membrane. *Advances in Colloid and Interface Science*, ***301***, 102603. https://doi.org/10.1016/j.cis.2022.102603

Pereira, G.A., Chaves, D.S.d.A., Silva, T.M.e., Motta, R.E.d.A., Silva, A.B.R.d., Patricio, T.C.d.C., Fernandes, A.J.B., Coelho, S.d.M.d.O., Ożarowski, M., Cid, Y.P., & Karpiński, T.M. (2023). Antimicrobial Activity of *Psidium guajava* aqueous extract against sensitive and resistant bacterial strains. *Microorganisms*, ***11***(7), 1784. https://doi.org/10.3390/microorganisms11071784

Pramusita, A., Yunus, R.M., Mukharromah, L., & Andriana, A. (2023). Antibacterial efficacy of *Mangifera indica* L. leaves extract against Streptococcus mutants. *Indonesian Journal of Dental Medicine*, ***6***(2), 52–55. https://doi.org/10.20473/ijdm.v6i2.2023.52-55

Ren, F., Li, Y., Luo, H., Gao, S., Jiang, S., Yang, J., Rao, C., Chen, Y., & Peng, C. (2024). Extraction, detection, bioactivity, and product development of luteolin: A review. *Heliyon*, ***10***(24), e41068. https://doi.org/10.1016/j.heliyon.2024.e41068

Ruksiriwanich, W., Khantham, C., Muangsanguan, A., Phimolsiripol, Y., Barba, F.J., Sringarm, K., Rachtanapun, P., Jantanasakulwong, K., Jantrawut, P., Chittasupho, C., Chutoprapat, R., Boonpisuttinant, K., & Sommano, S.R. (2022). Guava (*Psidium guajava* L.) leaf extract as bioactive substances for anti-androgen and antioxidant activities. *Plants*, ***11***(24), 3514. https://doi.org/10.3390/plants11243514

Shabbir, H., Kausar, T., Noreen, S., Rehman, H. u., Hussain, A., Huang, Q., Gani, A., Su, S., & Nawaz, A. (2020). In vivo screening and antidiabetic potential of polyphenol extracts from guava pulp, seeds and leaves. *Animals*, ***10***(9), 1714. https://doi.org/10.3390/ani10091714

Thongphichai, W., Pongkittiphan, V., Laorpaksa, A., Wiwatcharakornkul, W., & Sukrong, S. (2023). Antimicrobial activity against foodborne pathogens and antioxidant activity of plant leaves traditionally used as food packaging. *Foods*, ***12***(12), 2409. https://doi.org/10.3390/foods12122409

Ugbogu, E.A., Emmanuel, O., Uche, M.E., Dike, E.D., Okoro, B.C., Ibe, C., Ude, V.C., Ekweogu, C.N., & Ugbogu, O.C. (2022). The ethnobotanical, phytochemistry and pharmacological activities of *Psidium guajava* L. *Arabian Journal of Chemistry*, ***15***, 103759. https://doi.org/10.1016/j.arabjc.2022.103759

Wang, Z., Yang, Q., Zhang, H., He, Y., Wang, R., & Lu, X. (2023). Isolation, identification, and antibacterial activities of flavonoids from jujube (*Ziziphus jujuba* Mill.) fruit. *International Journal of Fruit Science*, ***23***, 51–61. https://doi.org/10.1080/15538362.2023.2186149

Wengerska, K., Batkowska, J., & Drabik, K. (2023). The eggshell defect as a factor affecting the egg quality after storage. *Poultry Science*, ***102***(7), 102749. https://doi.org/10.1016/j.psj.2023.102749

Xiang, D., Wang, C.G., Wang, W.Q., Shi, C.Y., Xiong, W., Wang, M.D., & Fang, J.G. (2017). Gastrointestinal stability of dihydromyricetin, myricetin, and myricitrin: An in vitro investigation. *International Journal of Food Sciences and Nutrition*, ***68***(6), 704–711. https://doi.org/10.1080/09637486.2016.1276518

Yan, Y., Xia, X., Fatima, A., Zhang, L., Yuan, G., Lian, F., & Wang, Y. (2024). Antibacterial activity and mechanisms of plant flavonoids against gram-negative bacteria based on the antibacterial statistical model. *Pharmaceuticals*, ***17***(3), 292. https://doi.org/10.3390/ph17030292

Yu, A.-C., Deng, Y.-H., Long, C., Sheng, X.-H., Wang, X.-G., Xiao, L.-F., Lv, X.-Z., Chen, X.-N., Chen, L., & Qi, X.-L. (2024). High dietary folic acid supplementation reduced the composition of fatty acids and amino acids in fortified eggs. *Foods*, ***13***(7), 1048. https://doi.org/10.3390/foods13071048

Zhang, W., Wang, J., Chen, Y., Zheng, H., Xie, B., & Sun, Z. (2020). Flavonoid compounds and antibacterial mechanisms of different parts of white guava (*Psidium guajava* L. cv. Pearl). *Natural Product Research*, ***34***(11), 1621–1625. https://doi.org/10.1080/14786419.2018.1522313