

Antibacterial Activity of *Psidium guajava* L. var. *pomifera* (Red Guava) and *Psidium guajava* L. var. *pyrifera* (White Guava) Bark Extracts on Gastroenteritis-causing Bacteria

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ABSTRACT

This study explores the antibacterial efficacy of *Psidium guajava* L. bark extracts against gastroenteritis-causing bacteria, addressing antibiotic resistance and acute gastroenteritis concerns. While prior research highlights *Psidium guajava*'s effectiveness against bacteria like *E. coli*, limited attention has been given to its bark extracts for gastroenteritis treatment. Through agar microdilution, the Minimum Inhibitory Concentrations (MIC) of *Psidium guajava* L. var. *pomifera* (red guava) and *Psidium guajava* L. var. *pyrifera* (white guava) bark extracts against *Escherichia coli* and *Salmonella typhimurium* were determined. Results revealed identical MIC values for both red and white guava bark extracts against the tested strains, indicating comparable antibacterial potency. Specifically, the MICs were 3.125 mg/mL for *Escherichia coli* and 1.563 mg/mL for *Salmonella typhimurium*. These findings highlight *Psidium guajava* L. bark extracts as promising antibacterial agents against gastroenteritis, regardless of guava variety. The significance of the results of this study will be of value to pharmaceutical and biotechnology companies along with medical professionals that can proceed with in vivo testing, agricultural practitioners who may use this information in agricultural activities, and future researchers who can improve upon the study.

Keywords: Antibacterial Efficacy, *Escherichia Coli*, *Salmonella Typhimurium*, *Psidium Guajava* L. Bark, Bark Extract

INTRODUCTION

As little is known about the antibacterial activity of different varieties *Psidium guajava* L., bark extracts against bacteria specifically identified as the causative agent of bacterial gastroenteritis, this study determined the antibacterial property of two varieties of *Psidium guajava* L. bark extracts against common bacteria causing gastroenteritis, *Escherichia coli* and *Salmonella typhimurium*. Bacterial gastroenteritis is a leading cause of illness and death

globally and antibiotic-resistant bacteria has been on the rise, which could make previously treatable diseases such as bacterial gastroenteritis fatal. A study showed that the medicinal compositions of *Psidium guajava L.* have proven effective against bacteria like *E. coli* by producing antibacterial activity (Aguiyi et al., 2019). Another study about *Psidium guajava L.* explained that its bark is proven to have more antibacterial activity than leaf extract, which could be beneficial in future studies that would be conducted (Adikwu et al., 2022).

Numerous studies demonstrate the antibacterial activity of the components of *Psidium guajava L.*, such as its leaves, against various bacteria. However, research on the antibacterial capabilities of *Psidium guajava L.* bark extract for diseases traditionally treated with *Psidium guajava L.* is limited as the focus of most studies concerning the plant is the phytochemical composition of *Psidium guajava L.* (Kumar et al., 2021).

Since little is known about the antibacterial activity of different varieties *Psidium guajava L.*, bark extracts against bacteria specifically identified as the causative agent of bacterial gastroenteritis, this study determined the antibacterial property of two varieties of *Psidium guajava L.* bark extracts against common bacteria that are known to cause gastroenteritis through agar microdilution. Preliminary findings indicate that both red and white guava bark extracts exhibit comparable antibacterial activities, with specific MIC values identified for *Escherichia coli* and *Salmonella typhimurium*. These results suggest that guava bark extracts, irrespective of variety, could offer a viable alternative for treating bacterial gastroenteritis, warranting further in vivo studies to confirm their therapeutic potential.

LITERATURE REVIEW

Escherichia coli

Escherichia coli (*E. coli*) is a common gram-negative bacterium responsible for gastrointestinal and nosocomial infections like urinary tract infections (UTIs) and pneumonia. It's found in soil, water, and undercooked meats, causing intestinal illness when ingested. Pathogenic strains are classified into subtypes based on O and H antigens (Mueller & Tainter, 2023). Notable subtypes include ETEC, EPEC, EAEC, EHEC, and EIEC. EHEC causes significant diarrheal outbreaks from contaminated products and undercooked beef O157: H7 and O104: H4 are concerning serotypes (Babak et al., 2021). According to Pakbin, Bruck, & Rossen (2018), EHEC's primary virulence factor is shiga-like toxin (SLT), linked to symptoms like hemolytic uremic syndrome (HUS) and renal failure. Multidrug-resistant strains of *E. coli* are a growing global issue (Wu et al., 2018).

Salmonella typhimurium

Salmonella, a genus of gram-negative bacteria in the *Enterobacteriaceae* family, is a significant cause of foodborne illnesses globally, particularly diarrheal diseases. Serovars like *typhimurium* and *enteritidis* are common culprits (Won and Lee, 2017). Its virulence is attributed to chromosomal and plasmid-based factors, including genes like *inv*, *spv*, *fimA*, and *stn*, with *Salmonella* Pathogenicity Islands (SPIs) playing a crucial role (Chaudhary et. al., 2015). Enterotoxin production, facilitated by the *stn* gene, contributes to gastroenteritis. Antibiotic resistance in *Salmonella typhimurium* is a growing concern, with patterns like

ASSuT and ACSSuT being prevalent, indicating diversified multi-drug resistant strains (Wang et al., 2019).

Varieties of *Psidium guajava* L.

Psidium guajava L., commonly known as guava, is a tropical tree valued for its fruit. Belonging to the Magnoliophyta class Magnoliopsida and Myrtaceae family, it grows up to 6 to 25 feet tall with green leaves and small flowers that develop into fruits (Naseer et al., 2018). It is rich in vitamins A, C, phosphorus, and calcium, along with antioxidants and phytochemicals, contributing to its therapeutic potential.

Psidium guajava L. encompasses two main varieties: *Psidium guajava* L. var. *pomifera* (red guava) and *Psidium guajava* L. var. *pyrifera* (white guava), differing in color and nutritional composition. Red guava typically has reddish-pink skin and higher concentrations of fiber, vitamin C, antioxidants, and lycopene compared to white guava, which usually has greenish-white or pale-yellow skin. Both types are rich in natural compounds like tannins, flavonoids, and phenolic compounds, contributing to their antimicrobial properties. Research suggests that red guava may exhibit stronger antibacterial effects due to its higher content of flavonoids and phenolic compounds, as demonstrated in a study by Maysarah et al. (2016) on red and white guava leaf ethanolic extracts against *Staphylococcus aureus* and *Escherichia coli*.

Medicinal use of *Psidium guajava* L. bark

Herbal plants play a major role in traditional Filipino medicine, which has been practiced for millennia and is still widely used in contemporary society (Maramba-Lazarte, 2020). In the Philippines, traditional healers use over 1,500 medicinal herbs, of which 120 have been proven to be safe and effective (Dapar et al., 2020).

Despite the vast diversity within the genus, *Psidium guajava* L. is the most grown species, prized for its pharmacological activities. Its leaves, consumed as both food and folk medicine across various regions, exhibit a range of biological effects, including antibacterial, antioxidant, antifungal, and anti-cancer properties (Naseer et al., 2018).

Phytochemical compounds of *Psidium guajava* L. bark

Plants rich in tannins have been traditionally used to treat diarrhea due to their astringent properties. *Psidium guajava* L. containing tannins, alkaloids, flavonoids, terpenoids, quercetin, and guaijaverin, showcases antimicrobial, antioxidant, and anti-inflammatory effects. Research indicates that compounds like flavonoids, saponins, alkaloids, tannins, and phenols found in guava leaves and bark extracts possess antibacterial properties (Sampath et al., 2021).

Flavonoids, for instance, create complexes with bacterial cell walls and proteins, exhibiting antimicrobial action (Ekeleme et al., 2017). Terpenoids, typically known for their fragrance, can also act as antibacterial agents (Kumar et al., 2021). Alkaloids damage bacterial cell membranes, causing leakage and cell death (Yan et al., 2018). Similarly, phenolic substances derived from natural sources demonstrate strong antibacterial action by damaging bacterial cell membranes (Ecevit et al., 2022). Phytochemical analysis of *Psidium guajava* L.

bark extracts reveal chemical components with antibacterial properties, offering potential treatment for infectious diseases, especially amidst rising antimicrobial resistance trends.

Antibacterial activity of *Psidium guajava L.* on Selected Bacteria

Adikwu et al. (2022) conducted a study on *Psidium guajava L.*, finding that its stem and leaf bark contain chemicals with significant antibacterial and antimicrobial properties. They tested extracts on *Staphylococcus aureus*, *Escherichia coli*, *Proteus sp.*, and *Salmonella typhi*, noting inhibitory effects from both aqueous and methanol extracts. The methanol bark extract showed strong potency, particularly against *Staphylococcus aureus*. Minimum inhibitory concentrations (MICs) revealed effective inhibition against all tested bacteria, with *Psidium guajava L.* extract showing promise in preventing their growth.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) is a measure of a plant extract's effectiveness against a specific microbe. In bacteriology, it's the lowest extract concentration that prevents visible microbe growth. Using the agar microdilution method, a series of plates with the same extract concentration are made, then diluted in increments. After adding microbes and incubating, the MIC is determined as the lowest extract concentration inhibiting microbe growth (Kowalska-Krochmal & Dudek-Wicher, 2021).

METHODS

This study utilized an experimental research design to determine the antibacterial activity of *Psidium guajava L.* (red and white guava) bark extract on *Escherichia coli* and *Salmonella typhimurium*. In this study, the red and white guava bark extract were the independent variables, while the antibacterial activity it produced was the dependent variable. The population of this study were *Psidium guajava L.* (red and white guava) methanolic bark extracts, which are the two common guava varieties in the Philippines and the following bacteria: *Escherichia coli* and *Salmonella typhimurium*, which are commonly known to cause gastroenteritis among humans. This study used the purposive sampling technique. Fresh bark of *Psidium guajava L.* (red and white guava) were harvested at the Hacienda Malagar resort in San Juan, Batangas, and Santa Rosa, Laguna. They were then processed, beginning with methanol extraction, followed by freeze drying, and then prepared to be an extract working solution. Once the serial dilutions and inoculum were prepared, triplicates of agar microdilution were done on the serial dilutions. The plate was then observed for bacterial growth after 24 hours to determine the MIC which was the lowest concentration that did not show bacterial growth on the agar's surface. Positive and negative controls were included in every triplicate to ensure the reliability of the tests performed.

The statistical data gathered in this study were the mean and deviation. A T-test was to be used to compare the data between *Psidium guajava L.* (red and white guava) barks extract used in agar microdilution for MIC.

RESULTS AND DISCUSSION

The triplicates run for *Psidium guajava L.* (red and white guava) bark extracts against *Escherichia coli* showed that growth was last observed in the seventh well for *Escherichia coli* for both extracts means that both varieties of extracts were able to achieve an MIC of 3.125 mg/mL. (Figure 1). On the other hand there was a lack of growth in the eighth wells of the triplicates for the extracts against *Salmonella typhimurium*. This means that an MIC of 1.563 mg/mL was attained by both extracts. The results indicated that a minimum of 3.125 mg/mL of *Psidium guajava L.* (red and white guava) bark extract is needed to achieve inhibition against *Escherichia coli*, and 1.563 mg/mL is needed to inhibit *Salmonella typhimurium*. Due to results being consistent across the triplicates, the standard deviation is zero, which indicated no need for a T-test. The antibacterial activity of the extracts on the bacterial isolates showed no significant difference whether *Psidium guajava L. var. pomifera* (red guava) bark extract or *Psidium guajava L. var. pyrifer*a (white guava) bark extract was used. Both methanol bark extracts produced an MIC of 3.125 mg/mL against *Escherichia coli*, which is supported by the study of Adikwu et al. (2022) which demonstrated the same antibacterial activity for an unidentified specie of *Psidium guava L.* methanol bark extract while using the broth dilution technique.

Table 1

Antibacterial Activity of Psidium guajava L. var. pomifera (red guava) Bark Extract

Bacteria	Type of Guava Bark Extract	Mean	SD
<i>Escherichia coli</i>	<i>Psidium guajava L.</i> (red and white guava) bark methanolic extract	3.125 mg/mL	0
<i>Salmonella typhimurium</i>	<i>Psidium guajava L.</i> (red and white guava) bark methanolic extract	1.563 mg/mL	0

The MICs produced by *Psidium guajava L. var. pomifera* (red guava) bark extract and *Psidium guajava L. var. pyrifer*a (white guava) against *Escherichia coli* and *Salmonella typhimurium* were shown to have no significant difference as they achieved identical strengths of antibacterial activity across all triplicates using the agar microdilution technique, resulting in a standard deviation of zero. Both *Psidium guajava L. var. pomifera* (red guava) bark extract and *Psidium guajava L. var. pyrifer*a (white guava) produced an MIC of 3.125 mg/mL against *Escherichia coli* and 1.563 mg/mL against *Salmonella typhimurium*. The methanol bark extracts produced an MIC of 3.125 mg/mL against *Escherichia coli*, which is supported by the study of Adikwu et al. (2022) that demonstrated the same antibacterial activity for an unidentified specie of *Psidium guava L.* methanol bark extract while using the broth dilution technique.

Although no other research has been done on *Psidium guava L.* methanol bark against other species of *Salmonella typhimurium*, Saleh and Al-Mariri (2020) found that the methanol extracts of the leaves and twigs of *Psidium guava L.* were also capable of generating a significant amount of antibacterial activity against *Salmonella typhimurium* with both having an MIC of 5.0 mg/mL. This corroborates with the findings of both Adikwu et al. (2022) and Elekwa et al. (2009), with which they were able to demonstrate that the bark extract of *Psidium*

guava L. had a greater antibacterial effect on certain bacterial isolates in comparison to the leaf extract.

Furthermore, collecting medicinal bark extract presents a viable option as an alternative antimicrobial source. When harvested using proper techniques, the tree can sustain its natural bark regrowth process, resulting in minimal to no damage, unlike the high-volume requirement for leaf harvesting, which may potentially harm the tree. Patel et al. (2023) demonstrated that bark harvesting methods are the only ones that pose a risk to the tree, and this risk can be mitigated by adopting non-destructive and sustainable practices. Sustainable harvesting methods should offer livelihood opportunities, particularly benefiting rural communities, while ensuring the regenerative capacity of the forest and ongoing contribution to human well-being remain intact.

CONCLUSION, IMPLICATION, SUGGESTION, AND LIMITATIONS

In consideration of the results of the study, it can be concluded that both *Psidium guajava L. var. pomifera* (red guava) bark extract and *Psidium guajava L. var. pyriferum* (white guava) have antibacterial activity against certain strains of bacteria that are known to be causative agents of bacterial gastroenteritis. This means that regardless of the origin of the guava bark, whether it be from a variety that produces red or white guava, the *Psidium guajava L.* bark extract can be used as an antibacterial tool against gastroenteritis-causing bacteria without the need to compromise depending on the species of *Psidium guajava L.*

This study focused on comparing the antibacterial activity of *Psidium guajava L. var. pomifera* (red guava) and *Psidium guajava L. var. pyriferum* (white guava) bark extracts against *Escherichia coli* and *Salmonella typhimurium*, both of which are common causes of gastroenteritis. The research was conducted *in vitro*, using minimum inhibitory concentration (MIC) to assess the extracts' effectiveness. The study was limited to the bark of these two guava varieties, which are abundant in Southeast Asia, to explore their potential applications in medicine and agriculture, while acknowledging that the findings may not fully represent the extracts' effects *in vivo*. Further research, including *in vivo* studies, is needed to determine the therapeutic value of these extracts.

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