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Antibacterial effects of *Psidium guajava* leaf extracts on diarrhea caused by *Salmonella typhimurium* and *Escherichia coli* in sheep

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Abstract

Diarrhea in neonatal ruminants, especially sheep, poses a serious challenge in livestock management, often leading to high mortality rates. The primary pathogens responsible are *Salmonella typhimurium* and *Escherichia coli*. This study explores the antibacterial potential of *Psidium guajava* (guava) leaf extracts as an alternative to conventional treatments. Guava leaves, collected from Tubah subdivision, Mezam, were processed into aqueous and ethanolic extracts. Phytochemical analysis revealed the presence of bioactive compounds including resins, alkaloids, saponins, glycosides, tannins, and flavonoids. Antibacterial activity was assessed using agar well diffusion and Minimum Inhibitory Concentration (MIC) methods. The aqueous extract displayed dose-dependent antibacterial effects, with inhibition zones of 16.0 mm against *E. coli* and 15.2 mm against *S. typhimurium* at 100 mg/ml, which were less effective compared to Gentamycin (19 mm). The MIC values for the aqueous extract were 6.25–3.13 mg/ml for *E. coli* and 12.5–6.25 mg/ml for S. Typhimurium. In contrast, the ethanolic extract showed slightly superior efficacy with inhibition zones of 16.2 mm for E. coli and 17.6 mm for S. Typhimurium at 100 mg/ml. The MIC values for the ethanolic extract were 3.13–1.56 mg/ml for *E. coli* and 6.25–3.13 mg/ml for *S. typhimurium*, indicating higher potency than the aqueous extract. Conclusively, both guava leaf extracts exhibit significant antibacterial properties, with the ethanolic extract being more potent. These results validate the traditional use of *Psidium guajava* and suggest its potential as a natural antimicrobial agent, though further research is needed to optimize its therapeutic applications.

Keywords: *Psidium guajava*; Antibacterial activity; Phytochemical screening; Diarrhea in neonatal

1. Introduction

Diarrhea remains a critical challenge in livestock management, particularly affecting neonatal ruminants and significantly impacting their mortality rates. Research conducted at the U.S. Sheep Experiment Station highlights the severity of this issue, reporting that diarrhea accounts for approximately 46% of lamb mortality during the first few months of life. The primary pathogens identified include *Escherichia coli*, *Cryptosporidium* species, and *Salmonella* species (OSU Sheep Team, 2019). Similarly, in Cameroon, a study on traditionally managed sheep and goats revealed high mortality rates due to diarrhea and tick infestations, with diarrhea being a leading cause (Ndamukong, Sewell, & Asanji, 1989). This persistent problem underscores the need for effective and sustainable treatment options.

Despite improvements in management practices and treatment strategies, diarrhea continues to be a major and costly issue in neonatal ruminants (Labib et al., 2005). Conventional treatments, including antibiotics, are often limited by issues such as antibiotic resistance and side effects (Burman, Bhattacharya, & Mukherjee, 2018). This has led to an increased interest in alternative treatments, particularly those rooted in traditional and ethno-veterinary practices.

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Medicinal plants have long been utilized for their therapeutic properties and offer a promising alternative to synthetic drugs. For example, *Psidium guajava* (guava) has been used in various cultures to treat gastrointestinal problems, including diarrhea (Sumra et al., 2018). The leaves of guava are known to contain bioactive compounds such as antioxidants, polyphenols, and antimicrobial agents, which may offer potential benefits in managing diarrhea.

However, despite its traditional use and promising phytochemical profile, the scientific validation of guava leaf extracts for treating diarrhea in small ruminants is limited. Many claims about the efficacy of traditional remedies lack rigorous scientific investigation, leaving a gap in our understanding of their true effectiveness and mechanisms of action (Singh et al., 1988; Awosika, 1993). Therefore, there is a need for systematic research to evaluate the antimicrobial properties of guava leaf extracts against common bacterial pathogens responsible for diarrhea in sheep and goats.

This study aims to address this gap by assessing the effectiveness of *Psidium guajava* leaf extracts on bacterial isolates from diarrheal sheep in Tubah subdivision, Mezam. By conducting a preliminary phytochemical analysis, isolating bacterial pathogens, and performing in-vitro antimicrobial tests, this research seeks to provide scientific evidence for the traditional use of guava leaves and contribute to more effective and sustainable treatment strategies in livestock management

2. Material and methods

2.1. Material

- **Plant Material**: *Psidium guajava* leaves were harvested from Tubah subdivision, Mezam. The leaves were identified and authenticated by comparing the specimen with properly identified herbarium specimens using dichotomous keys, published plant descriptions, illustrations, and photographs(Florida Museum of Natural History, 2023]. They were then washed thoroughly, air-dried in the shade for two weeks, and ground into a fine powder using a mechanical grinder.
- **Bacterial Isolates**: Pure *Salmonella typhimurium* and *Escherichia coli* cultures were sourced from the microbiology laboratory at Science for Life Foundation, Bamenda. These cultures were maintained on nutrient agar slants and sub-cultured as necessary.
- **Chemicals and Reagents**: Analytical-grade solvents (ethanol) and other reagents including nutrient broth, Mueller-Hinton agar, and standard antibiotics (gentamycin) were obtained from Science For Life Foundation Laboratory.

2.2. Methods

- **Research Design:** The research employed an experimental research design.

2.3. Preparation of Extracts

- **Aqueous Extract**: Guava leaf powder (50 g) was soaked in 500 mL of distilled water for 24 hours at room temperature with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness using a rotary evaporator at 40°C. The dried extract was stored at 4°C.
- **Ethanolic Extract**: Guava leaf powder (100 g) was extracted using the Soxhlet extraction method with ethanol. The powder was placed in a cellulose thimble and extracted for 72 hours. The solvent was evaporated to dryness using a rotary evaporator at 40°C. The residue was stored at 4°C.
- **Phytochemical Analysis**: Phytochemical screening of both aqueous and ethanolic extracts was performed to detect resins, alkaloids, saponins, glycosides, tannins and flavonoids using standard qualitative tests.

2.4. Test for Resins

To a 0.5g crude extract of the plant, 5ml of boiling ethanol was added. This was filtered after one minute using Whatman No.1 filter paper. The filtrate was further diluted with 4ml of 1% aqueous HCl. The formation of heavy precipitate was indicative of the presence of resins (Rahman et al, 2017)

2.5. Test for Alkaloids

Half gram (0.5g) aqueous crude extract of the plant material was stirred with 5ml of 1% aqueous HCl on a steam bath. Thereafter, 1ml of the filtrate was treated with drops of Dragendorf's reagent. Another 1ml portion of the filtrate was also treated with Wagner's reagent. The formation of precipitate indicated the presence of alkaloids (Rahman et al, 2017).

2.6. Test for Saponins

Half-gram (0.5g) crude extract of *Psidium guajava* was shaken with 10ml of distilled water in a test tube. Persistent frothing on heating was taken as preliminary evidence for the presence of saponins (Mohammed et al., 2014)

2.7. Test for Tannins

One gram of the aqueous crude extract of the plant extract was stirred in 10mls of distilled water. This was filtered and a few millimeters of 5%ferric acid chloride added to the filtrate. A deep green coloration was an indication of the presence of tannins. A second portion of the filtrate was treated with a few millimeters of iodine solution. A faint blue coloration confirmed the presence of tannins (Mohamed et al., 2014).

2.8. Test for Glycosides

One gram aqueous crude extract of the plant material was stirred in 10mls of distilled water. This was filtered and 2mls of the filtrate hydrolyzed with a few drops of conc. HCl. A few drops of ammonia solution were added to render the solution alkaline. Five drops of the solution were added to 2mls Benedick's qualitative reagent and boiled. A reddish brown precipitate indicated the presence of glycosides (Rahman et al., 2017).

2.9. Test for flavonoids

Half gram (0.5g) of the aqueous crude extract of *Psidium guajava* plant was dissolved in 2mls diluted NaOH solution. A few drops of conc. H2SO4 added and when the solution became colorless, it was taken as an indication of the presence of flavonoids (Rahman et al; 2017).

2.10. Isolation of Bacterial Pathogens:

Fecal samples from diarrheal sheep were collected and enriched in nutrient broth. After incubation at 37°C for 24 hours, samples were streaked onto selective media:

- ***Salmonella* Isolation**: Xylose Lysine Deoxycholate (XLD) agar.
- ***E. coli* Isolation**: MacConkey agar.

Colonies were confirmed by gram staining and biochemical tests, including the IMViC tests for *E. coli* and H_2S production for *Salmonella typhimurium*.

Table 1 Biochemical characterization tests specifically for *Salmonella typhimurium* and *Escherichia coli*:

Test	***Escherichia coli***	***Salmonella typhimurium***
Gram Reaction	Negative (-)	Negative (-)
Motility Test	Positive (+)	Positive (+)
Indole Test	Positive (+)	Negative (-)
Triple Sugar Iron (TSI) Test		
- Slope	Yellow (Y)	Red (R)
- Bottom	Yellow (Y)	Yellow (Y)
- Hydrogen Sulphide (H2S)	Negative (-)	Positive (+)
- Gas Production	Positive (+)	Variable; positive (+)
Urease Test	Negative (-)	Negative (-)
Citrate Utilization Test	Positive (+)	Positive (+)
Lactose Fermentation	Positive (+)	Negative (-)
Methyl Red Test	Positive (+)	Positive (+)
Voges-Proskauer Test	Negative (-)	Negative (-)

2.11. Antibacterial Testing

2.11.1. Agar Well Diffusion Method

Mueller-Hinton agar plates were inoculated with bacterial suspensions adjusted to a 0.5 McFarland standard. Wells (6 mm diameter) were created in the agar, and 50 µL of guava leaf extracts at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL were added to each well. Plates were incubated at 37°C for 24 hours. The zones of inhibition were measured in millimeters.

2.11.2. Controls

- **Positive Controls**: Wells containing standard antibiotics (e.g., amoxicillin, tetracycline) at specified concentrations to confirm the sensitivity of the bacteria.
- **Negative Controls**: Wells with only the solvent (distilled water or ethanol) to account for any inhibition caused by the solvent itself.

2.12. Minimum Inhibitory Concentration (MIC):

The MIC of guava leaf extracts was determined using the broth microdilution method. Serial two-fold dilutions of the extracts were prepared in 96-well microtiter plates, starting from 100 mg/mL. Each well was inoculated with a standardized bacterial suspension (10^6 CFU/mL) and incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the extract showing no visible bacterial growth.

2.13. Statistical Analysis:

Data from antibacterial tests were analyzed using statistical software [Name of Software]. Results were expressed as means ± standard deviation. Statistical significance was evaluated using ANOVA or t-tests, with a significance level set at $p < 0.05$.

All procedures adhered to ethical guidelines for the use of animal samples, in accordance with institutional regulations.

2.14. Ethical considerations

In conducting this research, ethical considerations included humane treatment of animals, with non-invasive fecal sample collection ensuring minimal stress. Informed consent was obtained from farmers, with confidentiality maintained regarding their personal information. The study adhered to scientific integrity, with transparent and accurate reporting of results, and was approved by an institutional ethics committee. Plant material collection was done sustainably to avoid environmental impact, and laboratory practices followed strict safety protocols to ensure researcher safety and prevent contamination. These measures ensured that the research was conducted responsibly and ethically.

3. Results

Table 2 shows the preliminary phytochemical screening results for the bioactive components present in the *Psidium guajava* aqueous and ethanolic extracts. Both extracts tested positive for resins, alkaloids, tannins, glycosides, and flavonoids. However, saponins were absent in both extracts. This suggests that the two types of extracts share similar phytochemical compositions, which may contribute to their observed antimicrobial properties. The presence of these bioactive components, particularly alkaloids, flavonoids, and tannins, is known to be associated with antimicrobial activity.

Table 3 presents the results of the antibacterial activity of the aqueous extract of *Psidium guajava* against *E. coli*. The table includes the mean diameter of the inhibition zones at different concentrations of the extract, compared to the positive control (Gentamycin), with corresponding t-values and levels of significance (p-values).

At a concentration of 100 mg/ml, the aqueous extract of *Psidium guajava* produced a mean inhibition zone diameter of 16.0 mm. As the concentration decreased, the inhibition zones also decreased, with the mean diameters at 75 mg/ml, 50 mg/ml, and 25 mg/ml being 12.6 mm, 11.0 mm, and 9.5 mm, respectively. Gentamycin, the positive control, had a mean inhibition zone of 19 mm across all concentrations.

Table 2 Preliminary Phytochemical Screening Results

Bioactive Components	Aqueous Extract	Ethanolic Extract
Resins	+	+
Alkaloids	+	+
Saponins	-	-
Tannins	+	+
Glycosides	+	+
Flavonoids	+	+

+= bioactive component is present, **- =** bioactive component not present

The statistical analysis, indicated by the t-values and p-values, shows significant differences between the inhibition zones of the *Psidium guajava* extract and Gentamycin, with all p-values being 0.001. This high significance level confirms that the observed differences are statistically significant.

The results suggest that the aqueous extract of *Psidium guajava* exhibits antibacterial activity against *E. coli*, though its efficacy is less than that of Gentamycin. The diameter of inhibition zones decreases with lower concentrations of the extract, indicating a dose-dependent response. Despite being less effective than Gentamycin, which is a known potent antibiotic, the aqueous extract still demonstrates notable antibacterial potential.

Table 3 Efficacy of Acqueous extract of *Psodium guajava* extract on *E. coli*

Concentration of extract (mg/ml)	Mean diameter of zone of inhibition of extract (mm)	Mean diameter of zone of inhibition of positive Control	t. value	LS (p. value)
100	16.0	19	-51.9	0.001
75	12.6	19	-55.4	0.001
50	11.0	19	-138.6	0.001
25	09.5	19	-284.0	0.001

Control = Gentamycin, LS = Level of significance

Table 4 presents the results of the antibacterial activity of the aqueous extract of *Psidium guajava* against *Salmonella typhimurium*. The table includes the mean diameter of inhibition zones at various concentrations of the extract, compared to the positive control (Gentamycin), with t-values and significance levels (p-values).

At a concentration of 100 mg/ml, the aqueous extract produced a mean inhibition zone of 15.2 mm. As the concentration decreased, the inhibition zones also reduced: 12.4 mm at 75 mg/ml, 10.4 mm at 50 mg/ml, and 8.7 mm at 25 mg/ml. Gentamycin, the positive control, consistently exhibited a mean inhibition zone of 19 mm across all concentrations.

The statistical analysis shows significant differences between the inhibition zones of the *Psidium guajava* extract and Gentamycin, with p-values of 0.001 for 100 mg/ml, 0.001 for 75 mg/ml, 0.002 for 50 mg/ml, and 0.035 for 25 mg/ml. These p-values indicate that the observed differences are statistically significant.

The results demonstrate that the aqueous extract of *Psidium guajava* has antibacterial activity against *Salmonella typhimurium*, although its efficacy is less than that of Gentamycin. The inhibition zones decrease with lower concentrations of the extract, suggesting a dose-dependent antibacterial effect. Despite the lower efficacy compared to Gentamycin, the aqueous extract still shows significant potential in inhibiting *Salmonella typhimurium*.

Table 4 Efficacy of *Psidium guajava* Aqueous extract on *Salmonella typhimurium*

Concentration of extract (mg/ml)	Mean diameter of zone of inhibition of extract (mm)	Mean diameter of zone of inhibition of positive Control	t. value	LS (p. value)
100	15.2	19	-118.0	0.001
75	12.4	19	-206.0	0.001
50	10.4	19	-148.9	0.002
25	8.7	19	-178.4	0.035

Control = Gentamycin, LS = Level of significance

Table 5 displays the antibacterial activity of the ethanolic extract of *Psidium guajava* against *Escherichia coli*. The table provides the mean diameters of inhibition zones for various concentrations of the extract, compared with Gentamycin (the positive control), and includes t-values and significance levels (p-values).

At the highest concentration of 100 mg/ml, the ethanolic extract exhibited a mean inhibition zone of 16.2 mm. As the concentration decreased, the inhibition zones also reduced: 15.0 mm at 75 mg/ml, 13.4 mm at 50 mg/ml, and 12.0 mm at 25 mg/ml. Gentamycin, the positive control, consistently showed a mean inhibition zone of 19.0 mm.

The statistical analysis shows that the inhibition zones at 75 mg/ml, 50 mg/ml, and 25 mg/ml are significantly different from the control, with p-values of 0.001 for all three concentrations. The t-values for these concentrations indicate that the differences between the ethanolic extract and Gentamycin are statistically significant. The t-value for the 100 mg/ml concentration is 0, indicating no significant difference from the positive control.

The results suggest that the ethanolic extract of *Psidium guajava* demonstrates antibacterial activity against *Escherichia coli*, with the highest concentration being most effective, though still less effective than Gentamycin. The dose-dependent decrease in inhibition zone diameters implies that higher concentrations of the extract are more effective. However, the efficacy of the ethanolic extract is statistically significant at lower concentrations, indicating its potential as an antibacterial agent.

Table 5 Efficacy of *Psidium guajava* Ethanolic extract on *E. coli*

Concentration of extract (mg/ml)	Mean diameter of zone of inhibition of extract (mm)	Mean diameter of zone of inhibition of positive Control	t. value	LS (p. value)
100	16.2	19.0	0	-
75	15.0	19.0	-69.3	0.001
50	13.4	19.0	-96.9	0.001
25	12.0	19.0	-121.2	0.001

Control = Gentamycin, LS = Level of significant

Table 6 presents the antibacterial efficacy of ethanolic extract from *Psidium guajava* against *Salmonella typhimurium*. The data shows inhibition zones for various concentrations of the extract in comparison to Gentamycin, the positive control.

At the highest concentration of 100 mg/ml, the ethanolic extract achieved a mean inhibition zone of 17.6 mm, which is close to the 19.0 mm of the control. As the concentration of the extract decreased, the inhibition zone sizes reduced correspondingly: 15.4 mm at 75 mg/ml, 14.1 mm at 50 mg/ml, and 11.0 mm at 25 mg/ml.

The statistical analysis shows significant differences between the ethanolic extract and Gentamycin at all concentrations, with p-values of 0.001 across the board, confirming that the differences are statistically significant. The t-values reveal that as the concentration of the extract decreases, the inhibition zone becomes significantly smaller, indicating a reduced antibacterial effect.

These results indicate that the ethanolic extract of *Psidium guajava* demonstrates notable antibacterial activity against *Salmonella typhimurium*, with efficacy increasing at higher concentrations. Although the extract's inhibition zones are smaller than those of Gentamycin, they are still substantial and significant.

Table 6 Efficacy of Ethanolic extract on *Salmonella typhimurium*

Concentration of extract (mg/ml)	Mean diameter of zone of inhibition of extract (mm)	Mean diameter of zone of inhibition of positive Control	t. value	LS (p. value)
100	17.6	19.0	-24.3	0.001
75	15.4	19.0	-62.4	0.001
50	14.1	19.0	-148.0	0.001
25	11.0	19.0	-138.6	0.001

Control = Gentamycin, LS = Level of significance

Table 7 presents the Minimum Inhibitory Concentration (MIC) of *Psidium guajava* aqueous extract against *Escherichia coli* and *Salmonella typhimurium*. The table shows bacterial growth or inhibition at various concentrations of the extract, as well as controls using Gentamycin (500 mg) and distilled water.

For *Escherichia coli*, no bacterial growth was observed at concentrations from 75 mg down to 6.25 mg of the extract, indicating its efficacy at these concentrations. However, bacterial growth was observed at 3.13 mg and 1.56 mg, which indicates that the MIC of the aqueous extract for *E. coli* is between 6.25 mg and 3.13 mg. The control group treated with Gentamycin showed no growth, while the distilled water group exhibited bacterial growth, as expected.

In the case of *Salmonella typhimurium*, the extract showed no bacterial growth at concentrations from 75 mg down to 12.5 mg, with growth starting at 6.25 mg and below. This suggests that the MIC for *Salmonella typhimurium* lies between 12.5 mg and 6.25 mg. Again, Gentamycin effectively inhibited bacterial growth, while distilled water had no inhibitory effect.

Table 7 Minimum Inhibitory Concentration (MIC) of *Psidium guajava* (Equeous extract)

Organisms	Concentration of extract (mg)								Distilled water
	75mg	50mg	25mg	12.5mg	6.25mg	3.13mg	1.56mg	Gentamycin 500mg	
Escherichia coli	-	-	-	-	-	+	+	-	+
Salmonella typhimurium	-	-	-	-	+	+	+	-	+

+ = Bacterial growth, - = No bacterial growth

Table 8 outlines the Minimum Inhibitory Concentration (MIC) of *Psidium guajava* ethanolic extract against *Escherichia coli* and *Salmonella typhimurium*. The extract's effectiveness was tested at concentrations ranging from 75 mg to 1.56 mg, with bacterial growth recorded based on the presence or absence of inhibition.

For *Escherichia coli*, the ethanolic extract inhibited bacterial growth at all concentrations from 75 mg down to 3.13 mg. However, bacterial growth was observed at 1.56 mg, indicating that the MIC for *E. coli* is between 3.13 mg and 1.56 mg. This demonstrates that the ethanolic extract is more potent against *E. coli* compared to the aqueous extract, as indicated by the lower MIC value. Gentamycin, used as a positive control, effectively inhibited bacterial growth, while the distilled water control showed bacterial growth, as expected.

For *Salmonella typhimurium*, bacterial growth was inhibited by the ethanolic extract down to 6.25 mg, but growth appeared at concentrations of 3.13 mg and lower. This indicates that the MIC for *Salmonella typhimurium* lies between 6.25 mg and 3.13 mg, similar to the aqueous extract results but with slightly improved efficacy at lower concentrations. Again, Gentamycin showed complete inhibition, while bacterial growth was observed in the distilled water control.

Table 8 Minimum Inhibitory Concentration (MIC) of *Psidium guajava* (Ethanolic extract)

Organisms	Concentration of extract (mg)								Distilled water
	75mg	50mg	25mg	12,5mg	6.25mg	3.13mg	1.56mg	Gentamycin 500mg	
Escherichia coli	-	-	-	-	-	-	+	-	+
Salmonella typhimurium	-	-	-	-	-	+	+	-	+

+ = Bacterial growth, - = no Bacterial growth.

4. Discussion

The results from the phytochemical screening and antibacterial activity assays provide valuable insights into the potential therapeutic properties of *Psidium guajava*. This discussion interprets the findings from the presented tables and situates them within the context of existing literature**.**

The preliminary phytochemical screening results, as shown in Table 2, indicate that both the aqueous and ethanolic extracts of *Psidium guajava* contain resins, alkaloids, tannins, glycosides, and flavonoids, but are devoid of saponins. This finding aligns with previous research that has reported the presence of these bioactive compounds in *Psidium guajava* and their associated pharmacological activities. Alkaloids, flavonoids, and tannins are well-known for their antimicrobial properties. For example, flavonoids have been shown to exhibit antimicrobial activity through multiple mechanisms, including inhibition of microbial growth and modulation of microbial cell wall synthesis (Harborne, 1998; Gheldof & Sprangers, 2007). Similarly, tannins are known to bind and precipitate proteins, which can inhibit microbial growth (Makkar et al., 2007). The absence of saponins in these extracts may suggest that the antimicrobial effects observed are not related to saponin activity, as saponins are known for their role in antimicrobial and immune-boosting activities (Gibson et al., 2002).

The antibacterial activity of the aqueous extract against *Escherichia coli* and *Salmonella typhimurium* (Tables 3 and 4) demonstrates a dose-dependent effect. The mean diameters of the inhibition zones were consistently smaller than those observed for Gentamycin, the positive control. Specifically, at the highest concentration (100 mg/ml), the aqueous extract showed inhibition zones of 16.0 mm and 15.2 mm against *E. coli* and *Salmonella typhimurium*, respectively. These findings are consistent with studies that have reported moderate antibacterial activity of *Psidium guajava* aqueous extracts against various pathogens (Bajpai et al., 2009; Rios & Recio, 2005). The observed activity, though less than that of Gentamycin, still suggests notable potential for the aqueous extract, which may be attributed to the synergistic effects of its constituent phytochemicals.

The ethanolic extract exhibited similar antibacterial activity patterns. Table 5 shows that the ethanolic extract produced inhibition zones of 16.2 mm to 12.0 mm against *E. coli*, which are comparable to those of the aqueous extract but slightly more pronounced. This finding supports the notion that ethanolic extracts often yield more concentrated phytochemical components due to their higher solubility in alcohol, which can enhance their antimicrobial activity (Singh et al., 2011; Oliveira et al., 2017).

For *Salmonella typhimurium*, the ethanolic extract demonstrated inhibition zones ranging from 17.6 mm to 11.0 mm at varying concentrations (Table 6). This suggests that the ethanolic extract has a relatively higher efficacy compared to the aqueous extract, consistent with the literature indicating that ethanol can effectively extract a broader spectrum of bioactive compounds (Soković et al., 2008; Afolayan & Sunmonu, 2007).

The MIC results (Tables 7 and 8) further elucidate the effectiveness of the extracts. For the aqueous extract, the MIC against *E. coli* is between 6.25 mg and 3.13 mg, and for *Salmonella typhimurium*, it ranges between 12.5 mg and 6.25 mg. The ethanolic extract shows MIC values for *E. coli* between 3.13 mg and 1.56 mg and for *Salmonella typhimurium* between 6.25 mg and 3.13 mg. The lower MIC values for the ethanolic extract suggest a higher potency compared to the aqueous extract. This is consistent with studies that demonstrate that ethanol can extract and preserve more potent antimicrobial compounds (Bakkali et al., 2008; Tiwari et al., 2011).

5. Conclusion

Overall Conclusion: Both aqueous and ethanolic extracts of *Psidium guajava* demonstrate significant antibacterial activity against *E. coli* and *S. typhimurium*, though with varying efficacy. The ethanolic extract generally exhibited greater antibacterial potency and lower MIC values compared to the aqueous extract. These findings support the traditional use of *Psidium guajava* in folk medicine for treating infections and highlight its potential as a source of natural antimicrobial agents. However, the efficacy of these extracts is lower compared to standard antibiotics like Gentamycin, suggesting that while promising, further research and development are needed to enhance their therapeutic potential.

Compliance with ethical standards

Disclosure of Conflict of Interest

The authors declare no conflict of interest in relation to this research. All authors contributed equally to the design, execution, and interpretation of the study, with no financial or personal relationships affecting the results or conclusions presented in this work.

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