Dr. Samson L. Mangin, 2026, 14:2
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology
An Open Access Journal

Ethanolic Leaf Extract of Guava Leaves (Psidium guajava) Against Staphylococcus aureus and Escherichia coli

Dr. Samson L. Mangin

Abstract- Guava (Psidium guajava) is a fruit-bearing tree known for its numerous health benefits. Its leaves are widely recognized for their medicinal properties and have long been used in traditional medicine. In emergency situations, guava leaf extracts have been commonly utilized as antibacterial agents and have been applied in the treatment of common wound infections. This study aimed to determine which concentration of guava leaf extract is most effective as an antimicrobial agent against Staphylococcus aureus and Escherichia coli, which are common causes of wound infections. The experiment began with the extraction of dried guava leaves at concentrations of 50%, 75%, and 95%. Mueller–Hinton agar was used to culture the two pathogenic bacteria along with the extracted guava leaf solutions. Each bacterium was exposed to the three different concentrations of guava leaf extract, while penicillin was used as the control. The cultures were then incubated at 37°C for 24 hours to observe the zones of inhibition. After 24 hours, the results showed that guava leaf extract produced measurable zones of inhibition, indicating antimicrobial activity. The 50% guava leaf extract showed the lowest zone of inhibition against Staphylococcus aureus, with an average of 15.67 mm. In contrast, the 95% guava leaf extract exhibited the highest zone of inhibition against Escherichia coli, with an average of 22.33 mm. Based on these findings, the study conclude that guava leaf extract is effective as an antimicrobial agent against Staphylococcus aureus and Escherichia coli.

Keywords: guava leaf extract, antimicrobial activity, Staphylococcus aureus, Escherichia coli.

I. INTRODUCTION

Psidium guajava (guava) is a fruit-bearing tree known for its numerous medicinal and nutritional benefits. Various parts of the plant—from its roots to its fruits—have traditionally been used in folk medicine to treat a wide range of conditions, including skin rashes and complications related to childbirth. Among these parts, guava leaves are considered particularly valuable due to their diverse therapeutic properties and potential health benefits (Sanda et al., 2011). In many developing regions, guava leaf extracts have been widely utilized as an alternative antibacterial agent, particularly in emergency situations or in communities with limited access to conventional medical treatments. These extracts have been traditionally applied in the treatment of common wound infections due to their presumed antimicrobial properties. Staphylococcus aureus is a Gram-positive, coccal bacterium that is commonly found in the human respiratory tract and on the skin. It is catalase-positive and capable of nitrate reduction. Although not always pathogenic, S. aureus is a well-known cause of various infections, including skin infections such as boils, respiratory diseases such as sinusitis, and cases of food poisoning (Cole, 2001). Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium belonging to the genus Escherichia. It is commonly found in the lower intestine of warm-blooded organisms. While most E. coli strains are harmless and form part of the normal gut microbiota, certain pathogenic serotypes can cause severe foodborne illnesses. These strains are also responsible for many cases of food contamination and product recalls (Singleton, 1999). Despite the traditional use of guava leaf extracts as antimicrobial agents, limited contemporary studies have examined

their effectiveness at varying concentrations, particularly against commonly isolated bacterial pathogens such as Staphylococcus aureus and Escherichia coli. As antimicrobial resistance continues to rise globally, the exploration of plant-derived antimicrobial agents has become increasingly important. Therefore, this study aims to determine the effectiveness of different concentrations of guava (Psidium guajava) leaf extract as a potential antimicrobial agent against Staphylococcus aureus and Escherichia coli. This study aims to determine the antimicrobial effectiveness of guava (Psidium guajava) leaf extract against Staphylococcus aureus and Escherichia coli. Specifically, it seeks to evaluate the average zone of inhibition produced by varying concentrations of guava leaf extract when applied to these microorganisms. Furthermore, the study intends to determine whether different concentrations of the extract exhibit antimicrobial activity against Staphylococcus aureus and Escherichia coli. Lastly, the study aims to assess whether significant differences exist in the antimicrobial effects of the various concentrations of guava leaf extract against the two tested microorganisms.

II. MATERIALS AND METHODS

The study employed an experimental research design to investigate the antimicrobial activity of guava (Psidium guajava) leaf extract against Staphylococcus aureus and Escherichia coli. Different concentrations of the guava leaf extract were prepared and tested to determine their effectiveness in inhibiting the growth of the selected bacterial microorganisms.

III. MATERIALS USED IN THE STUDY

The materials used in this study included fresh guava leaves, ethanol as the extraction solvent, sterile containers, beakers, and laboratory weighing equipment. The guava leaves were processed and weighed according to the required amounts for the preparation of different extract concentrations. Ethanol was used as the solvent to facilitate the extraction of the bioactive compounds present in the guava leaves.

A rotary evaporator was utilized to efficiently remove the solvent from the extracted samples through controlled evaporation. This process allowed the researchers to obtain a concentrated guava leaf extract while preserving the active compounds responsible for its antimicrobial activity.

IV. EXTRACTION (PREPARATION OF PLANT EXTRACT)

Leaf samples were collected from guava trees located in randomly selected areas. The collected leaves were placed in plastic zip-lock bags, properly labeled, and stored in an ice cooler during transport to the laboratory for extraction.

In the laboratory, the leaf samples were washed thoroughly with tap water and then air-dried at room temperature. The dried leaves were subsequently ground using a mechanical grinder until a fine powder was obtained. Ethanol (95%) was used as the extraction solvent. The powdered leaves were mixed with ethanol to prepare three different extract concentrations: 50%, 75%, and 95%. Each mixture was prepared in sterile 125-mL Erlenmeyer flasks, which were wrapped in aluminum foil to prevent evaporation and exposure to light. The mixtures were allowed to soak for three days at room temperature.

After the soaking period, the mixtures were transferred into 50-mL tubes and subjected to solvent removal using a rotary evaporator, allowing efficient and gentle evaporation of the solvent. The resulting supernatant was collected and stored at 4°C until further use.

V. CULTURE AND SENSITIVITY OF BACTERIA

To evaluate antibacterial activity, Gram-positive bacteria (Staphylococcus aureus) and Gram-negative bacteria (Escherichia coli), both commonly found in hospital environments, were used in the experiment. These bacteria were untreated.

Four treatments were prepared for each bacterial species, requiring a total of eight agar plates. The treatments were labeled as follows:

T0: Control group (penicillin) T1: 50% guava leaf extract, T2: 75% guava leaf extract, T3: 95% guava leaf extract.

VI. STEPS IN CULTURE AND SENSITIVITY TESTING

Prepare the agar medium and pour it into sterile Petri dishes. Store the Petri dishes in a refrigerator until they are ready for use. Once the agar has solidified and the plates reach room temperature, introduce the bacterial cultures into the Petri dishes. Label each Petri dish accordingly and seal the lid with laboratory tape to prevent contamination.

Incubate the plates in a warm, dark environment for 24 hours to allow bacterial growth. The plates should be stored in an inverted position to prevent water condensation from disturbing bacterial colonies. After the incubation period, record the observed bacterial growth in each Petri dish. Evaluate the effectiveness of the antibacterial agents based on the observed results.

VII. MEASUREMENT OF BACTERIAL GROWTH

As bacterial colonies develop in the Petri dishes, a clear circular region, often referred to as a "halo", appears around the location where the antibacterial agent was applied. This region is known as the zone of inhibition.

The effectiveness of antimicrobial agents can be determined by measuring the diameter of the zone of inhibition in each Petri dish. In this study, antibacterial effectiveness was evaluated based on the measured zone diameter, expressed in millimeters (mm). Generally, a larger zone of inhibition indicates stronger antibacterial activity.

VIII. STATISTICAL TREATMENT

The collected data were analyzed to compare the antibacterial activity of the guava leaf extracts and to determine the susceptibility of the tested microorganisms to the treatments. The mean and standard deviation of the zone of inhibition were calculated for each treatment. Hypothesis testing was conducted using a significance level (α) of 0.05. The probability values were obtained through Analysis of Variance (ANOVA) to determine whether significant differences existed among the treatment groups.

The statistical analysis specifically examined the antimicrobial effects of different concentrations of guava leaf extract on Staphylococcus aureus and Escherichia coli. When significant differences were detected in the ANOVA results, a Duncan Multiple Range Test (DMRT) was applied as a post-hoc analysis to identify which treatment groups differed significantly from one another.

IX. RESULTS AND DISCUSSION

The results and the discussion of the data gathered after the experiments are conducted.

Average of zone of inhibition of varying percentage or concentrations of Guava Leaves extract against Staphylococcus aureus and Escherichia coli

Table 1

Descriptive Summary of the Zone of Inhibition

Guava Leaves Extracts Mean (mm) SD (mm) Minimum (mm) Maximum (mm)

Guava Leaves Extracts	**Mean (mm)**	**SD (mm)**	**Minimum (mm)**	**Maximum (mm)**
(Against S. aureus)				

Control	16.00	1.00	15	17
50%	15.67	1.53	14	17
75%	18.33	1.16	17	19
95%	21.33	1.53	20	23
Total	**17.83**	**2.62**	**14**	**23**
(Against E. coli)				
Control	18.67	1.16	18	20
50%	16.33	1.53	15	18
75%	19.67	2.52	17	22
95%	22.33	3.06	19	25
Total	**19.25**	**2.93**	**15**	**25**

Note: In every % concentration, there are three trials conducted.

Table 1 shows the descriptive measurements of the zone of inhibition produced by the guava leaves extract in each different percent concentrations and the control. These results clearly show that the concentration of the extracts have immense effect on the zone of inhibition produced. Guava leaves extract against Staphylococcus aureus showed inhibition in an average of 15.67, 18.33, and 21.33 mm in concentrations 50%, 75%, and 95%. On the other bacteria Escherichia coli, guava leaves extract showed inhibitions in an average of 16.33, 19.67 and 22.33 mm in concentrations 50%, 75%, and 95%. Based on the measurements, 95% of guava leaves extract presents a greater effect in both pathogenic bacteria than the other percentage of guava leaves extract. Penicillin as control, presents 16 mm in Staphylococcus aureus and 18.67 in Escherichia coli. The result implies that guava leaf extracts are very active against S. aureus, thus making up important potential sources of new antimicrobial compounds (Neto et al., 2008).

Antimicrobial Effect of the varying percentage or concentrations of Guava Leaves extract against Staphylococcus aureus

Table 2

Effect of % Guava Leaves Extracts Against Staphylococcus aureus: One-way ANOVA Test

Guava Leaves Extracts	Mean ± SD	F-value (df)	p-value	Remarks
Control	16.00 ± 1.00			
50%	15.67 ± 1.53			
75%	18.33 ± 1.16	11.746** (3,8)	0.003	Significant
95%	21.33 ± 1.53			

Note: ** significant (P ≤ 0.01)
2-same letter means statistically indifference (based on Duncan Test)

Table 2 shows the effect of different percentages of the Guava leaves extracts against Staphylococcus aureus. It shows that there are significant differences in the values of their treatment means. It clearly shows that the higher the concentration the extract is the more inhibitory effect it has. With a concentration of 50%, an average inhibition zone of 15.67±1.53 mm is produced while the 95% concentration, a much larger average zone of inhibition of 21.33±1.53 is created. 95% guava leaves extracts showed significant inhibitory effect against Staphylococcus aureus. As explained by Biswas (2013) study has shown that inhibition of Gram-

positive bacteria, Staphylococcus aureus, suggests that guava possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using methanol or ethanol as the solvent.

Antimicrobial Effect of the varying percentage or concentrations of Guava Leaves extract against Escherichia coli

Table 3

Effect of % Guava Leaves Extracts Against Escherichia coli: One-way ANOVA Test

Guava Leaves Extracts	Mean[2] ± SD	F-value (df)	p-value	Remarks
Control	18.67 ± 1.16			
50%	16.33 ± 1.53			
75%	19.67 ± 2.52	3.833 (3,8)	0.057	Not Significant
95%	22.33 ± 3.06			

Note: not significant (P ≥ 0.05)
2-same letter means statistically indifference (base on Duncan Test)

Table 3 shows the effect of different percentages of the guava leaves extracts against E. coli. It shows that there are minimal differences in the values of their treatment means. The results obtained shows that 95% guava leaves extract has the highest zone of inhibition with 22.33±3.06 mm, followed by 75% with 19.67±2.52 then the control (penicillin) with 18.67±1.16, and 50% guava leaves extract with 16.33±1.53 mm. The values obtained were close to each other. The guava leaves extract showed inhibitory effects to Escherichia coli, but its concentration is not significant to its antimicrobial effects. It shows that the guava leaves extract with its different concentrations show insignificant inhibitory effects against E. coli. As explained by Biswas et al. (2013), it indicates that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active components against the organisms.

Varying percentage or concentrations of Guava Leaves extracts have antimicrobial effect against the two tested microorganisms

Table 4

Two-way ANOVA test for Testing the Effects of Guava Leaves and Tested Bacteria on the Zone of Inhibition

Source	Sum of Square	df	Mean Square	F-value	p-value	Remarks
Bacteria	12.042	1	12.042	3.658	0.074	Not significant
Concentration	113.792	3	37.931	11.523	<0.001	Significant
Bacteria × Concentration	3.458	3	1.153	0.350	0.790	Not significant
Error	52.667	16	3.292			
Total	181.958	23				

Note: ***Significant (p ≤ 0.001). Same letter means statistically insignificant difference based on Duncan test.

Table 4, the two-way ANOVA analysis revealed that extract concentration significantly influenced the zone of inhibition (p < 0.001), indicating that higher concentrations of guava leaf extract produce

stronger antibacterial effects. In contrast, the type of bacteria alone did not significantly influence the inhibition zone ($p = 0.074$), suggesting that both microorganisms respond similarly to the extract under the experimental conditions. Furthermore, the interaction between bacterial species and extract concentration was not statistically significant ($p = 0.790$), indicating that the effect of concentration on antimicrobial activity was consistent across both tested organisms.

Table 5
Post-hoc test for varying percentage or concentrations of guava leaves extract based on homogeneous subsets

% Extract	N	Subset 1	Subset 2	Subset 3
50%	6	16.00		
Control	6	17.33	17.33	
75%	6		19.00	
95%	6			21.83

p-value:
Subset 1 = 0.221
Subset 2 = 0.131
Subset 3 = 1.000

Table 5 in Post-hoc analysis using homogeneous subset testing further clarified the differences among extract concentrations. The 95% concentration produced the largest mean zone of inhibition (21.83 mm), indicating the strongest antibacterial activity. The 75% concentration showed moderate inhibition (19.00 mm), while the 50% concentration exhibited the lowest inhibition (16.00 mm). The control treatment showed a mean inhibition zone of 17.33 mm, indicating baseline antimicrobial activity from the standard drug used for comparison.

These findings suggest a dose-dependent antimicrobial response, where increasing concentrations of guava leaf extract enhance bacterial growth inhibition. This observation supports previous studies indicating that guava leaves contain bioactive compounds such as flavonoids, tannins, and phenolic compounds, which contribute to their antimicrobial properties.

Previous research has documented that guava leaves are traditionally used to treat intestinal disorders, wounds, and skin infections. According to D'Cruz (2011), guava leaves have long been prepared as herbal remedies, either brewed as decoctions to treat gastrointestinal issues or applied topically as poultices for wounds and skin rashes. Recent studies have also identified lectins and other compounds in guava that may bind to bacterial cells and inhibit their adhesion to intestinal walls, thereby reducing infection and preventing diarrhea.

The present results are consistent with earlier reports demonstrating the antibacterial activity of guava leaf extracts against enteropathogenic bacteria, particularly Staphylococcus aureus and Escherichia coli. In this study, the highest inhibition was observed against Staphylococcus aureus at the 95% extract concentration, with a mean zone of inhibition of 21.33 mm, while Escherichia coli showed relatively lower sensitivity, particularly at lower concentrations (e.g., 7.33 mm inhibition at 50% concentration). This difference in sensitivity may be attributed to structural differences in bacterial cell walls between Gram-positive and Gram-negative bacteria.

Beyond antimicrobial effects, guava leaves have also been reported to exhibit a wide range of pharmacological properties, including antioxidant, hepatoprotective, anti-allergic, antidiabetic, anti-inflammatory, antispasmodic, cardioprotective, anticough, and analgesic activities (Gutierrez et al., 2008). These properties further support the potential therapeutic value of guava leaves in traditional and modern medicine.

The findings of this study indicate that guava leaf extracts possess promising antimicrobial potential, particularly at higher concentrations. However, additional studies are recommended to further evaluate the antimicrobial efficacy of guava leaf extracts against a broader range of bacterial strains

and to identify the specific bioactive compounds responsible for the observed effects.

X. CONCLUSION

The findings of study demonstrate that ethanolic guava (Psidium guajava) leaf extract possesses measurable antimicrobial activity against Staphylococcus aureus and Escherichia coli. The results revealed that increasing concentrations of the extract produced larger zones of inhibition, indicating a concentration-dependent antibacterial effect. Among the treatments tested, the 95% guava leaf extract exhibited the strongest inhibitory activity against both bacterial species. Statistical analysis further confirmed that extract concentration significantly influenced the zone of inhibition, particularly against Staphylococcus aureus, while the differences in antibacterial response against Escherichia coli were not statistically significant.

Overall, the study supports the potential of guava leaf extract as a natural antimicrobial agent and highlights its possible application as an alternative or complementary treatment for bacterial infections. Further research is recommended to isolate the specific bioactive compounds responsible for the antibacterial activity and to evaluate its effectiveness against a broader range of pathogenic microorganisms.

XI. ETHICAL CONSIDERATIONS

The study strictly adhered to ethical standards for handling pathogenic microorganisms. All experimental procedures complied with biosafety guidelines to ensure the safe handling, storage, and disposal of bacterial cultures. Additionally, the study was conducted with approval from the college's institutional review board to maintain ethical integrity.

Acknowledgement

The researcher sincerely expresses gratitude to the institution for providing the necessary facilities, resources, and academic support that made this study possible. Appreciation is also extended to colleagues and friends who offered valuable assistance, encouragement, and constructive feedback throughout the research process. Above all, the researcher deeply acknowledges the unwavering support, understanding, and motivation from family members, whose encouragement played an essential role in the successful completion of this study.

REFERENCES

1. Biswas, B., Rogers, K., McLaughlin, F., Daniels, D. & Yadav, A. (2013). Antimicrobial activities of leaf extracts of guava (Psidium guajava L.) on two gram-negative and gram-positive bacteria. International Journal of Microbiology, 2013.
2. Capa. (2010). Analysis of Different Concentrations of "Tawa-Tawa" Crude Extract Against Selected Gram-positive Bacteria.
3. Cole, A. M., Tahk, S., Oren, A., Yoshioka, D., Kim, Y. H., Park, A. & Ganz, T. (2001). Determinants of Staphylococcus aureus nasal carriage. Clinical and Diagnostic Laboratory Immunology, 8(6), 1064-1069.
4. Cosgrove, S. E., Vigliani, G. A., Campion, M., Fowler, V. G., Abrutyn, E., Corey, G. R., et al. (2009). Initial low-dose gentamicin for Staphylococcus aureus bacteremia and endocarditis is nephrotoxic. Clinical Infectious Diseases, 48(6), 713-721.
5. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Review.
6. DCruz, M. Ma-Ke Bonsai Care Guide for Psidiumguajava. Ma-Ke Bonsai.net. Retrieved 2011 http://www.fruitipedia.com/guava.htm
7. Frieden (2014, May 20). Centers for Disease Control and Prevention. http://www.cdc.gov/ecoli/general/
8. Goncalves, F. A., Andrade Neto, M., Bezerra, J. N., Macrae, A., Sousa, O. V. D., Fonteles-Filho, A. A., & Vieira, R. H. (2008). Antibacterial activity of GUAVA, Psidium guajava leaf extracts on diarrhea-causing enteric bacteria isolated from seabob shrimp, Xiphopenaeus kroyeri (Heller). Revista do Instituto de Medicina Tropical de São Paulo, 50(1), 11-15.

9. Gutierrez, R.M. (17 April 2008). Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology.
10. James C. G. (2013). Antibiotic Susceptibility Testing. vlab.amrita.edu/?sub=3&brch=73&sim=1628&cnt=1
11. Last J. M. (2007). "Germ theory of disease", A Dictionary of Public Health, Westminster College, Pennsylvania: Oxford University Press.
12. Neto A.M. (2008). Antibacterial activity of GUAVA, Psidium guajava Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob shrimp, Xiphopenaeus kroyeri (Heller).
13. Okigbo, R. N., & Mmeka, E. C. (2008). Antimicrobial effects of three tropical plant extracts on Staphylococcus aureus, Escherichia coli and Candida albicans. African Journal of Traditional, Complementary and Alternative Medicines, 5(3), 226-229.
14. Pamplona-Roger, G.D. M.D. (2004). New Lifestyle Healthy Foods. Madrid, Spain. Editorial Safeliz.
15. Ribnicky et al. (2008). Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition.
16. Rogers K. (2013). Antimicrobial Activities of Leaf Extracts of Guava (Psidium guajava L.) on Two Gram-Negative and Gram-Positive Bacteria.
17. Ryan K.J. (2004). Sherris Medical Microbiology (4th ed.). ISBN 0-8385-8529-9.
18. Sanda, K.A. (2011). Pharmacological Aspects of Psidium guajava: An Update. https://scialert.net/?doi=ijp.2011.316.324
19. Sapkota, R., Dasgupta, R., & Rawat, D. S. (2012). Antibacterial effects of plants extract on human microbial pathogens & microbial limit tests. International Journal of Research in Pharmacy and Chemistry, 2(4), 2231-2781.
20. Singleton P. (1999). Bacteria in Biology, Biotechnology and Medicine (5th ed.). Wiley. pp. 444-454. ISBN 0-471-98880-4.
21. Tabanag (2010). Effectiveness of Carica papaya as antimicrobial agent.

About the Author

Professor, Department of General Education, School of Allied Health and Medical Sciences, Adventist Medical Center College, Iligan City, Philippines