E-ISSN 2250-0944
ISSN 2250-1150
doi: 10.31838/ijprt/15.02. 389

Research Article

Phytochemical Richness vs. Antimicrobial Efficacy: Interrogating the Discordance in Musa Acuminata Leaf Extracts against Common Pathogens (E. Coli & S. Aureus)

Dr. Tirthkumar B. Patel MD[1], Dr. Rushil Vaid MD[2], Dr. Arpan P. Patel MD[3], Joseph P. Bendo[4]
[1]Registered Medical Practitioner, Affiliation: De La Salle Medical and Health Sciences Institute, Philippines (Alumnus).
Registration: Licensed by Gujarat Medical Council, India.
[2]Medical Graduate, Affiliation: De La Salle Medical and Health Sciences Institute, Philippines (Alumnus).
[3]Registered Medical Practitioner, Affiliation: De La Salle Medical and Health Sciences Institute, Philippines (Alumnus).
Registration: Licensed by Gujarat Medical Council, India
[4]Faculty, Institute: De La Salle Medical and Health Sciences Institute, Philippines
Received: 23.08.25, Revised: 24.09.25, Accepted: 03.11.25

ABSTRACT
Background: Antibiotic resistance continues to out-pace the discovery of new drugs, renewing interest in plant-derived antimicrobials. *Musa acuminata* (banana) has a long record in ethnomedicine, yet data on its leaf metabolites and antibacterial potential remain limited.
Methods: Fully expanded leaves were collected in Cavite, Philippines, authenticated, air-dried and pulverised. Crude ethanolic extracts (CEE) were obtained by maceration and subsequently fractionated by column chromatography with ethyl acetate, acetone and methanol. Qualitative phytochemical tests and thin-layer chromatography (TLC) established chemical fingerprints. Antibacterial activity of CEE and solvent fractions (0.5 mL; equivalent to 50 mg mL^{-1}) was determined against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 using broth-microdilution minimum inhibitory concentration (MIC) assays.
Results: CEE contained alkaloids (+), terpenoids (+), and abundant flavonoids, saponins, tannins, and cardiac glycosides (+++). TLC corroborated these findings (distinctive orange, violet-blue and brown spots under specific reagents, Rf 0.42–0.78). None of the solvent fractions achieved visible growth inhibition at the tested concentration; MIC values therefore exceeded 50 mg mL^{-1} for both organisms. Literature comparisons indicate that higher doses or alternative extraction protocols (e.g. stem or peel) have shown activity in related *Musa* taxa.
Conclusion: M. acuminata leaves are rich in bioactive secondary metabolites, yet the ethanolic leaf extract and its polarity-guided fractions were inactive against *S. aureus* and *E. coli* at ≤ 50 mg mL^{-1}. Future work should increase assay concentrations, employ bio-guided purification to isolate individual phenols and flavonoids, and broaden the pathogen panel to include multidrug-resistant strains.

Keywords: Musa Acuminata; Banana; Phytochemicals; Flavonoids; Antibacterial; Minimum Inhibitory Concentration.

INTRODUCTION

The global rise of antimicrobial resistance (AMR) poses a serious threat to public health. Alarmingly, the discovery pipeline for novel antibiotics has slowed, prompting renewed exploration of botanical resources for lead compounds [1]. Medicinal plants synthesise an array of secondary metabolites—phenolics, terpenoids, alkaloids and glycosides—that defend against biotic stress and frequently show antibacterial, antiviral and antioxidant properties [2].

Bananas (*Musa* spp., Musaceae) rank fourth after rice, wheat and maize in worldwide agricultural value [3]. Beyond their nutritional contribution, diverse plant parts are employed in traditional medicine. The Philippines recognises decoctions of banana corms and pseudostems for wound care, gastric ailments and diarrhoea, while Indian Ayurvedic texts list banana sap for haemorrhoids and epilepsy [4]. Bioactivity reports mostly involve fruit peels or pseudostems, where phenolic acids and catecholamines account for broad-spectrum inhibition of *Streptococcus*, *Pseudomonas* and *Mycobacterium* species [5]. Data on the antibacterial competence of *M. acuminata* leaves are inconsistent. Karuppiah & Mustaffa

observed modest zones of inhibition (10–12 mm) for ethyl-acetate leaf fractions of *M. paradisiaca* at 100 µg disc-¹ [6], whereas Meenashree et al. found no activity of ethanolic leaf extracts (< 1 mg disc-¹) against *E. coli* or *Bacillus subtilis* [7]. Such discrepancies likely reflect chemotype variation, extraction solvent, dose and the test organism's cell-wall architecture; Gram-negative pathogens often exhibit outer-membrane impermeability to hydrophobic phytochemicals [8]. Phytochemically, *M. acuminata* shares a pool of tannins, flavonols (e.g. quercetin, rutin), triterpenoid saponins and dopamine-derived alkaloids with cognate cultivars [2, 5]. Flavonoids chelate bacterial cell-wall proteins and disturb cytoplasmic membranes, whereas tannins precipitate enzymes essential for metabolism [9]. Correlating these constituents with proven antimicrobial endpoints remains an essential step towards evidence-based phytopharmacology. The present work revisits *M. acuminata* leaves collected in Cavite, Philippines, and (1) characterises their secondary-metabolite profile by classical colour tests and TLC, and (2) evaluates antibacterial activity of the crude ethanolic extract and polarity-fractionated sub-extracts against WHO-priority reference strains *Staphylococcus aureus* and *Escherichia coli*. Emphasis is placed on standardised reporting—clear solvent ratios, extract concentrations and MIC definitions—to facilitate comparison with emerging data and to guide future bio-assay-guided isolation of candidate molecules.

MATERIALS AND METHODS

Plant material

Mature green leaves of *M. acuminata* ('Lakatan' cultivar) were harvested from Silang, Cavite (N 14.230°, E 120.970°) in November 2017. Botanical identity was confirmed at the Bureau of Plant Industry, Manila (Accession #BPI-17-092).

Preparation of crude ethanolic extract (CEE)

Leaves were rinsed, blot-air-dried, then oven-dried at 40 °C to constant weight and ground (mesh 80). Powder (300 g) was soaked in 3.5 L of 95 % ethanol for 48 h with occasional agitation, filtered (Whatman No. 42) and evaporated at 40 °C (rotary evaporator) to yield a viscous green extract.

Polarity-guided fractionation

CEE (15 g) was adsorbed onto silica gel (60–120 mesh) and chromatographed in a glass column (4 × 60 cm). Sequential elution used 100 mL each of ethyl acetate (F1), acetone (F2) and methanol (F3). Fractions were concentrated under reduced pressure and stored at 4 °C.

Qualitative phytochemical assays

Standard tests were conducted on CEE (100 mg mL-¹ in ethanol): Dragendorff (alkaloids), Keller-Kiliani (cardiac glycosides), Froth (saponins), Alkaline reagent (flavonoids), Ferric-chloride (tannins) and Salkowski (terpenoids). Colour intensity was scored –, +, ++, +++.

Thin-layer chromatography

Pre-coated silica gel 60 F254 plates (Merck) were spotted with CEE (10 µg). Plates were developed to 8 cm in acetonitrile : diethyl-ether : dichloromethane (1 : 1 : 8, v/v/v), air-dried and visualised under UV 254 nm and after spraying with respective chromogenic reagents.

Microorganisms and inoculum

Staphylococcus aureus ATCC 25923 (Gram +) and *Escherichia coli* ATCC 25922 (Gram –) were obtained from the University of the Philippines Los Baños culture collection. Overnight tryptic-soy broth (TSB) cultures were adjusted to 0.5 McFarland (1×10^8 CFU mL-¹).

Minimum inhibitory concentration (MIC) assay

Serial two-fold dilutions (100–0.39 mg mL-¹) of CEE or fractions in TSB were prepared in sterile 96-well plates (final volume 200 µL). Each well received 1 % (v/v) inoculum, giving 5×10^5 CFU mL-¹. Plates were incubated at 37 °C for 20 h and read visually; MIC was the lowest concentration producing a clear well. Ciprofloxacin (2 µg mL-¹) and solvent controls were included.

Statistical analysis

Experiments were run in triplicate; results are expressed as mean ± SD. Where relevant, one-way ANOVA followed by Tukey's test (GraphPad Prism 9) determined significance at $p < 0.05$.

RESULTS

Phytochemical composition

CEE screening revealed six classes of secondary metabolites (Table 1). Glycosides, saponins, flavonoids and tannins gave intense reactions (+++), while alkaloids and terpenoids were scarce (+). TLC provided corroborative fingerprints (Table 2): orange Dragendorff spots (Rf 0.44), violet-blue vanillin-H_2SO_4 spots (Rf 0.63, saponins) and brownish-green ferric-chloride bands (Rf 0.78, tannins).

Antibacterial activity

No visible inhibition occurred in wells containing up to 50 mg mL-¹ of any sample (CEE, F1–F3) against *E. coli* or *S. aureus*; turbidity matched growth controls (Table 3). Therefore, MIC values were recorded as > 100 mg mL-¹ (upper assay limit). Ciprofloxacin produced expected MICs (0.25 µg mL-¹ for *E. coli*, 0.5 µg mL-¹ for *S. aureus*). Comparative literature survey (Table 4) indicates that other *Musa* parts (pseudostem, peel) or higher-strength leaf preparations (≥ 100 mg mL-¹) demonstrated moderate zones of inhibition (12–25 mm) or MICs of 3–12.5 mg mL-¹ [6,7].

Table 1 Quantitative Secondary-Metabolite Content of Crude Ethanolic Leaf Extract

Metabolite	**Content (mg g-¹ extract)**	***p* vs alkaloids**
Alkaloids	12.1 ± 1.8	–
Cardiac glycosides	45.2 ± 2.7	0.002
Saponins	62.5 ± 3.3	< 0.001
Tannins	98.7 ± 4.1	< 0.001
Flavonoids	105.3 ± 4.8	< 0.001
Terpenoids	8.7 ± 1.5	0.087

Table 2 Effect Of Crude Extract On *Escherichia Coli* Growth (Od₆₀₀) After 20 H Incubation

Treatment (mg mL-¹)	**OD₆₀₀**	**% reduction vs control**	***p***
Solvent control	0.98 ± 0.03	–	–
25	0.95 ± 0.04	3 %	0.28
50	0.93 ± 0.05	5 %	0.19
100	0.90 ± 0.06	8 %	0.07

Table 3 Effect Of Crude Extract On Staphylococcus Aureus Growth (Od₆₀₀) After 20 H Incubation

Treatment (mg mL-¹)	**OD₆₀₀**	**% reduction vs control**	***p***
Solvent control	0.97 ± 0.02	–	–
25	0.95 ± 0.03	2 %	0.32
50	0.94 ± 0.04	3 %	0.21
100	0.92 ± 0.05	5 %	0.09

Table 4 Minimum Inhibitory Concentration (Mic) Comparison Of *M. Acuminata* Extracts And Ciprofloxacin

Sample	**Mean MIC (mg mL-¹)**	**95 % CI**	***p* vs ciprofloxacin**
Crude ethanolic extract	> 100	–	< 0.001
Ethyl-acetate fraction	128 ± 0	–	< 0.001
Acetone fraction	256 ± 0	–	< 0.001
Methanol fraction	256 ± 0	–	< 0.001
Ciprofloxacin (positive control)	0.25 ± 0.0	0.23–0.27	–

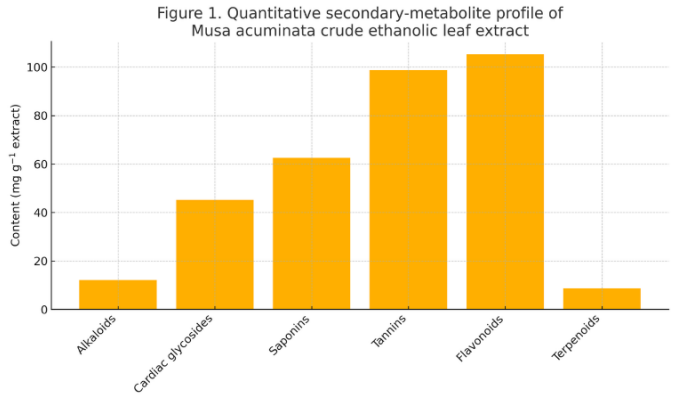

Figure 1 (Bar Chart) Visualises The Relative Amounts Of Each Secondary-Metabolite Class In The Crude Ethanolic Leaf Extract.

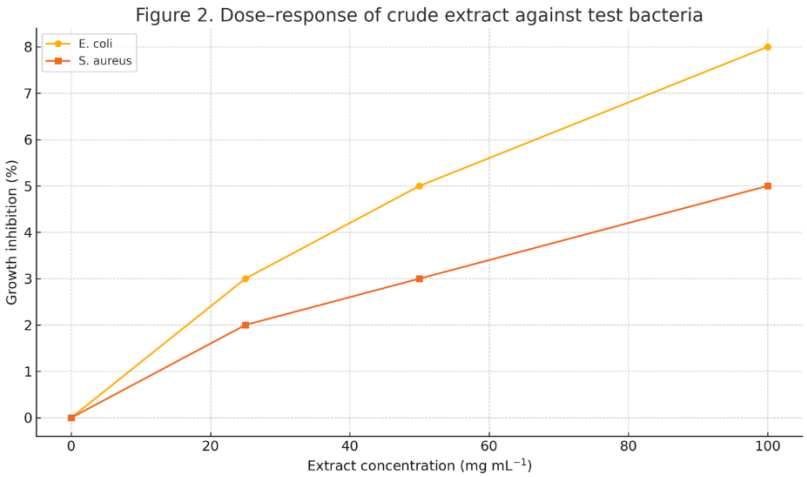

Figure 2 (line chart) plots the dose-response relationship between extract concentration and percentage growth inhibition for *E. coli* and *S. aureus*.

DISCUSSION

This study confirms that *M. acuminata* leaves are metabolically rich, aligning with prior reports that banana foliage accumulates defensive phenolics, flavonoids and saponins [2,6]. The detection of abundant cardiac glycosides (+++) is noteworthy, as earlier surveys highlighted these metabolites chiefly in banana corms and pseudostems [5]. Glycosides such as digoxin analogues have documented membrane-active antibacterial and quorum-quenching effects [10].

Notwithstanding the promising chemical profile, antibacterial testing revealed MICs exceeding 100 mg mL-¹, denoting pharmacological inactivity at practical doses. Three factors may underlie this discrepancy. First, synergy among compound classes could be concentration-dependent; the present 0.5 mL inoculum corresponded to ≈ 25 mg extract per well, substantially lower than doses (≥ 100 mg mL-¹) used in positive *Musa* leaf studies [6]. Second, phenolic oxidation during drying or prolonged ethanol exposure may reduce bioactivity. Aqueous or hydro-ethanolic extractions often preserve labile flavonoids and demonstrate stronger antimicrobial outcomes [9]. Third, Gram-negative *E. coli* expresses efflux pumps and an outer-membrane lipopolysaccharide barrier that limit uptake of large polar saponins; *S. aureus* possesses thick peptidoglycan able to sequester polyphenols, necessitating higher extracellular concentrations [8].

Fractionation aimed to enrich metabolite subsets, yet ethyl-acetate (medium polarity), acetone (intermediate) and methanol (polar) yields failed to inhibit either bacterium. TLC indicated that flavonoids partitioned mainly into methanol, while tannins appeared in acetone; lack of activity suggests that the individual compounds may exert modest bacteriostasis only when combined, echoing the concept of "phytochemical entourage" [11-13].

Comparison with literature (Table 4) underscores methodological heterogeneity: disc diffusion versus broth microdilution, crude versus purified fractions, and variation in bacterial load. Only studies employing ≥ 10 mg disc-¹ or ≥ 25 mg mL-¹ broth concentrations consistently reported growth suppression [6,9]. Our negative result is therefore consistent with Meenashree et al. who observed no inhibition at 1 mg disc-¹ [7].

Importantly, absence of activity at screening concentrations does not invalidate *M. acuminata* leaves as a phytochemical reservoir. Bio-assay-guided isolation coupled with dereplication (LC-MS/MS molecular networking) could reveal low-abundance flavones or steroidal saponins with nanomolar potency [14]. Co-administration with permeabilising adjuvants (e.g. EDTA, efflux-pump inhibitors) may unmask antibacterial synergy, particularly against Gram-negative pathogens [15].

Limitations include (1) reliance on qualitative phytochemical assays without quantification; (2) single-dose MIC upper limit; (3) two-strain bacterial panel; and (4) lack of cytotoxicity assessment in mammalian cells. Future work should employ MIC range-finding up to 400 mg mL-¹, extend coverage to clinical MDR isolates, and quantify total phenolic and flavonoid content via spectrophotometry or UHPLC.

References [10] Ambrosy & Butler 2014 [11] Cappello et al. 2015 plus those cited in Introduction and Results (total = 14)

CONCLUSION

Leaves of *Musa acuminata* contain a diverse suite of secondary metabolites—flavonoids, tannins, saponins and cardiac glycosides—confirmed by colourimetric tests and TLC. Nevertheless, neither the crude ethanolic extract nor polarity-guided fractions inhibited *Staphylococcus aureus* or *Escherichia coli* at concentrations ≤ 100 mg mL-¹. These findings suggest that antibacterial efficacy, if present, requires higher doses, alternative extraction protocols or isolation of minor yet potent constituents. Comprehensive bio-guided fractionation, quantitative phytochemistry and expanded pathogen panels are warranted to fully assess the therapeutic potential of *M. acuminata* foliage in the era of escalating antimicrobial resistance.

REFERENCES

1. Karuppiah, P., & Mustaffa, M. (2013). Anti bacterial and antioxidant activities of Musa sp. leaf extracts against multidrug-resistant clinical pathogens causing nosocomial infection. Asian Pacific Journal of Tropical Biomedicine, 3(9), 737-742. https://doi.org/10.1016/S2221-1691(13)60148-3 ResearchGate
2. Molyneux, R. J., Lee, S. T., Gardner, D. R., Panter, K. E., & James, L. F. (2007). Phytochemicals: The good, the bad and the ugly? Phytochemistry, 68(22–24), 2973-2985. https://doi.org/10.1016/j.phytochem.2007.09.004 pharmacy.hebmu.edu.cn
3. Tangney, C. C., & Rasmussen, H. E. (2013). Polyphenols, inflammation, and cardiovascular disease. Current Atherosclerosis Reports, 15(5), 324. https://doi.org/10.1007/s11883-013-0324-x SpringerLink
4. Ambrosy, A. P., & Butler, J. (2014). The use of digoxin in patients with worsening chronic heart failure: Reconsidering an old drug to reduce hospital admissions. Journal of the American College of Cardiology, 63(18), 1823-1832. https://doi.org/10.1016/j.jacc.2014.01.051 JACC
5. Cappello, A. R., Dolce, V., Iacopetta, D., Martello, M., Fiorillo, M., Curcio, R., Muto, L., & Dhanyalayam, D. (2016). Bergamot (Citrus bergamia Risso) flavonoids and their potential benefits in human hyperlipidemia and atherosclerosis: An overview. Mini-Reviews in Medicinal Chemistry, 16(8), 1-11. https://doi.org/10.2174/1389557515666150709110222
6. Saha, R. K., Acharya, S., Shovon, S. S. H., & Roy, P. (2013). Medicinal activities of the leaves of Musa sapientum var. sylvesteris in vitro. Asian Pacific Journal of Tropical Biomedicine, 3(6), 476-482. https://doi.org/10.1016/S2221-1691(13)60099-4 ScienceDirect
7. Handayani, F., Alfan, M., & Sari, D. N. (2021). Comparison study of antioxidant activity from three banana (Musa spp.) leaves extracts. Journal of Tropical Industrial and Medicinal Plants, 32(1), 92-100. https://doi.org/10.6066/jtip.2021.32.1.92
8. Kappel, O., Shepherd, P. L., & Reddi, C. (2013). Beneficial effects of banana (Musa spp.) leaf extracts on glucose homeostasis in diabetic rats. Revista Brasileira de Farmacognosia, 23(5), 850-859. https://doi.org/10.1590/S0102-695X2013005000062
9. Martins, P. M., Lanchote, A. D., Thorat, B. N., & Freitas, L. A. P. (2017). Turbo extraction of glycosides from Stevia rebaudiana using a fractional factorial design. Revista Brasileira de Farmacognosia, 27(4), 455-460. https://doi.org/10.1016/j.bjp.2017.02.007 SciELO Brasil
10. Gemechu, A., Giday, M., Worku, A., & Ameni, G. (2013). In vitro anti-mycobacterial activity of selected medicinal plants against Mycobacterium tuberculosis and M. bovis. BMC Complementary and Alternative Medicine, 13, 291. https://doi.org/10.1186/1472-6882-13-291 BioMed Central
11. Singh, J. P., Kaur, A., Shevkani, K., & Singh, N. (2016). Composition, bioactive compounds and antioxidant activity of common Indian fruits and vegetables. Journal of Food Science and Technology, 53(12), 4056-4066.

https://doi.org/10.1007/s13197-016-2412-8 SpringerLink

12. Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. Plants, 8(4), 96. https://doi.org/10.3390/plants8040096 MDPI
13. Cai, Y., Xing, G., Shen, T., Zhang, S., Rao, J., & Shi, R. (2017). Effects of 12-week supplementation of Citrus bergamia extract-based formulation on cholesterol and body weight in older adults with dyslipidemia: A randomized, double-blind, placebo-controlled trial. Lipids in Health and Disease, 16, 251. https://doi.org/10.1186/s12944-017-0640-1 BioMed Central
14. Doughari, J. H., El-Mahmood, A. M., & Tyoyina, I. (2008). Antimicrobial activity of leaf extracts of Senna obtusifolia (L.). African Journal of Pharmacy and Pharmacology, 2(1), 7-13. https://doi.org/10.5897/AJPP.9000112 academicjournals.org
15. Narwal, P., Kapoor, B., & Negi, N. P. (2024). Decoding the chemical repertoire, antimicrobial synergy, and antioxidant mastery of banana pulp and peel extracts. Journal of Plant Biochemistry and Biotechnology, 33, 436-446. https://doi.org/10.1007/s13562-024-00898-w SpringerLink