
COMPARATIVE ANTIBACTERIAL EFFICACY OF SALA AND JAMAICAN CHERRY LEAVES: A BASELINE STUDY FOR NATURAL ORAL CARE APPLICATIONS

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ABSTRACT

Introduction: Because Gram-negative bacteria are often linked to serious tooth illnesses like periodontitis, the development of natural-based oral therapies necessitates the use of potent antibacterial medicines. Objective: Using Escherichia coli as a baseline model for Gram-negative bacterial pathogens, this study conducted to assess the antibacterial effectiveness of leaf extracts from Cynometra ramiflora L. (Sala) and Muntingia calabura L. (Jamaican Cherry), both separately and in combination. Methods: 96% ethanol was used to carry out the extraction process using maceration. The Kirby-Bauer disc diffusion method was used to assess the antibacterial activity. Sala and cherry leaf single extracts as well as their mixtures at ratios of 25:75, 50:50, and 75:25 made up the experimental groups. The positive and negative controls were 10% DMSO and tetracycline, respectively.

Results: Compared to the single Jamaican Cherry leaves extract (12.33 mm), the single ethanol extract of Sala leaves showed "very strong" antibacterial activity with a mean inhibition zone of 21.5 mm. On the other hand, the antagonistic impact of the two extract combinations produced lower inhibition zones between 12.5 and 13.0 mm, which were unable to outperform the effectiveness of the single Sala extract.

KEYWORDS

Cynometra ramiflora, Muntingia calabura, dentistry, herbal formulation, Gram-negative bacteria



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INTRODUCTION

Oral health is a fundamental component of general health and well-being. Among the most prevalent oral conditions globally is periodontal disease, a chronic inflammatory condition that destroys the supporting structures of the teeth, including the gingiva, periodontal ligament, and alveolar bone (Tattar et al., 2025). The primary etiology of periodontal disease is the accumulation of dental plaque biofilm, which is predominantly colonized by highly pathogenic Gram-negative bacteria, such as *Escherichia coli*, *Porphyromonas gingivalis*, *Treponema denticola*, and *Aggregatibacter actinomycetemcomitans* (Abdulkareem et al., 2023; Cruz et al., 2022; Łasica et al., 2024). These Gram-negative pathogens possess complex outer membranes containing lipopolysaccharides (LPS) that trigger severe localized inflammatory responses and tissue destruction in the oral cavity (Abdulkareem et al., 2023).

The management of periodontal infections typically involves mechanical debridement combined with the use of antimicrobial oral formulations, such as mouthwashes, local irrigants, or periodontal gels. Currently, Chlorhexidine (CHX) is considered the gold standard for chemical plaque control. However, the prolonged use of synthetic oral formulations like CHX is associated with several adverse effects, including tooth discoloration (brown staining), taste alteration, mucosal desquamation, and an increased risk of calculus formation (Poppo Deus & Ouanounou, 2022).

One major threat to world health is the emergence of antibiotic resistance. According to the Antimicrobial Resistant in Indonesia (AMRIN-Study), *Escherichia coli* accounted for 81% of the 781 hospital cases of antibiotic resistance in Indonesia (Yunita et al., 2022). Eliminating Gram-negative bacteria is essential in dentistry since they are frequently linked to serious periodontal disorders (Yusoff et al., 2024). Finding new plant-based antibacterial substitutes is crucial to creating safe oral treatments. The polyphenols and flavonoids found in *Muntingia calabura* L. (Jamaican Cherry) leaves have demonstrated antibacterial efficacy against oral infections such as *Porphyromonas gingivalis* and *Streptococcus viridans* (Dutta et al., 2023; Rizkita et al., 2025; Simamora et al., 2020). Similar to this, *Cynometra ramiflora* L. (Sala) leaves have potent antibacterial qualities against a variety of pathogens, such as *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* (Prabowo & Rahmatillah, 2025; Sabiha et al., 2022; Samadd et al., 2024; Siddique & Mukhleshur Rahman, 2024).

Escherichia coli (*E. coli*) is a good, standardized laboratory model for Gram-negative bacteria because of its strong structural similarities, the LPS-rich outer membrane to Gram-negative periodontal pathogens. Therefore, this study aims to evaluate the antibacterial activity of the ethanol extracts of *Muntingia calabura* L. and *Cynometra ramiflora* L. leaves, both individually and in combination, against *E. coli* as a baseline model. The results will serve as a basis for the future development of safe and effective herbal oral care formulations that target Gram-negative bacterial in dentistry.

RESEARCH METHOD

Tools and Materials

The tools used include glasswares, vacuum rotary evaporators, microscopes, and autoclaves and ovens, petri dishes, micropipettes, incubator shakers, incubators, blue tips, yellow tips, spreader glass, and Bunsen burners. *Muntingia calabura* L. and *Cynometra ramiflora* L. leaves were gathered from Karangrejo, Karanganyar, Central Java, Indonesia.

Extraction Process

For three days, the extraction process was carried out by maceration from dried simplisia (each 300 gram) with 2.5 L of 96% ethanol. A rotary evaporator was then used to concentrate the filtrate in order to get a thick extract.

Antibacterial Assay

Antibacterial Assay was conducted using the Kirby-Bauer diffusion method. Mueller-Hinton (MH) media were used to test the antibacterial activity. 10^8 CFU/mL *E. coli* bacterial suspension was inoculated in the media. Single extracts (10 μ L) and mixtures of Jamaican cherry and sala extracts in ratios of 25:75; 50:50; and 75:25 were assessed in the study. The positive control was tetracycline, and the negative control was 10% DMSO.

RESULT AND DISCUSSION

Efficacy and Antibacterial Activity of Single Extracts

The primary evaluation of the single extracts demonstrated a stark contrast in their antibacterial capabilities against the Gram-negative bacterial model, *Escherichia coli*. At a standardized volume of 10 μ L, the single ethanol extract of *Muntingia calabura* L. (Jamaican cherry leaves) produced a moderate inhibition zone of 12.33 mm. While effective, it was vastly outperformed by the single ethanol extract of *Cynometra ramiflora* L. (sala leaves), which yielded an impressive mean inhibition zone of 21.5 mm. According to standard antimicrobial susceptibility classifications, an inhibition zone greater than 20 mm is categorized as "very strong."

Table 1. Antibacterial Assay of Single Extract

Extracts	Concentration (%)	Inhibition zone (mm)
<i>Muntingia calabura</i> L.	40	12.33
	30	11.5
	20	12.0
	10	10.0
<i>Cynometra ramniflora</i> L.	40	21.5
	30	16.5
	20	15.0
	10	12.5

The profound efficacy of the sala leaves extract can be attributed to its high concentration of polyphenols and alkaloids. Gram-negative bacteria, such as *E. coli* and many periodontal pathogens (e.g., *P. gingivalis*), possess a highly complex outer membrane rich in lipopolysaccharides (LPS) that usually acts as a robust barrier against antibiotics. However, alkaloids are highly basic compounds that can intercalate with the bacterial DNA and interfere with cell division. Furthermore, the polyphenols in sala leaves likely induce bacterial cell death by binding to the lipid bilayer, causing severe membrane disruption, leakage of intracellular fluid, and ultimately, cellular lysis.

Phytochemical Incompatibility and Antagonistic Effects

A central objective of this study was to determine if combining these two herbal extracts would yield a synergistic effect, which is highly desirable in pharmacological formulations. The combinations were tested in cherry:sala ratios of 25:75, 50:50, and 75:25. Surprisingly, the combination groups yielded significantly lower inhibition zones ranging between 12.5 mm and 13.0 mm across all ratios.

Table 2. Antibacterial Assay of Combined Extract

Extracts (%)	Mean of Inhibition zone (mm)
25: 75 (Jamaican Cherry: Sala)	12.5
50: 50 (Jamaican Cherry: Sala)	13.0
75: 25 (Jamaican Cherry: Sala)	13.0
Tetracycline (Positive Control)	23.67
DMSO (Negative Control)	6

Because the combined efficacy was drastically lower than the most active single agent (sala extract at 21.5 mm), this interaction is strictly classified as antagonistic. This biological antagonism is most likely rooted in phytochemical incompatibility. Cherry leaves are rich in tannins, flavonoids, and saponins. In phytochemistry, it is well-documented that tannins have a strong affinity to bind with alkaloids (present in the sala extract) to form macromolecular, insoluble complexes or precipitates. It is highly probable that when these two extracts were mixed, the tannins from the cherry leaves bound to the active alkaloids from the sala leaves. Consequently, the active compounds neutralized each other *before* they could penetrate the bacterial cell wall, drastically reducing the overall bioavailability of the antibacterial agents at the target site.

Implications for Novel Herbal Dental Formulations

The findings of this in vitro study provide critical translational insights for the development of alternative dental pharmacology. The current trend in dentistry leans heavily toward polyherbal formulations under the assumption that "more herbs equal stronger effects." However, this study proves that empirical testing is vital, as arbitrary mixing can lead to chemical antagonism.

For the development of oral care therapeutics targeting Gram-negative pathogens—such as antibacterial mouthwashes, periodontal pocket gels, or root canal irrigants—formulators must be cautious of phytochemical interactions. Based on these results, *Cynometra ramiflora* L. (sala leaf) extract should be prioritized as a single active ingredient. Utilizing sala extract alone not only prevents the risk of antagonistic interactions but also maximizes the antimicrobial potency (achieving >20 mm clearance). Furthermore, a single-extract formulation simplifies the manufacturing process, reduces production costs, and minimizes the risk of adverse tissue reactions in the oral mucosa, making it a highly viable candidate to replace or supplement synthetic agents like Chlorhexidine in the future.

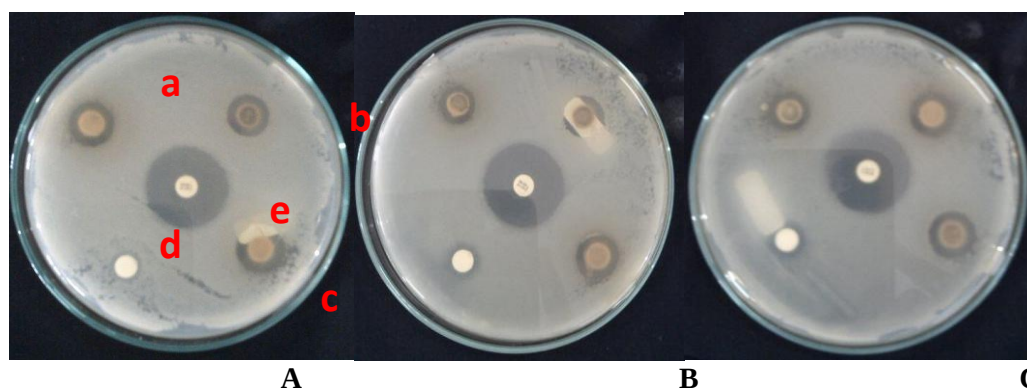


Figure 1. Antibacterial assay of *Muntingia calabura* L and *Cynometra ramiflora* L.
Note: (a) Sala leaves extract 40%; (b) Jamaican cherry leaves extract 40%; (c). Combination extract Jamaican cherry: Sala (25:75) in A, Combination extract Jamaican cherry: Sala (50:50) in B; Combination extract Jamaican cherry: Sala (75:25) in C; (d) Negative control (DMSO 100%), (e) Postive Control (Tetracycline)

CONCLUSION

Ethanol extracts of Jamaican cherry and Sala leaves have inhibitory activity against the growth of *E. coli* bacteria. Testing the ethanol extract on each extract showed strong inhibitory power for *E. coli* growth (10-20 mm). The ethanol extract of sala leaves had better activity than the other test groups, namely 21.2 mm, which is categorized as very strong (>20 mm).

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