

Evaluation of Antibacterial Activity of Combined Ethanolic Extracts from *Callistemon citrinus* Leaves and *Mangifera indica* Bark Against *Streptococcus pneumoniae*

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Background: *Streptococcus pneumoniae* is a leading cause of respiratory infections, including pneumonia, meningitis, and otitis media. The rise in antimicrobial resistance has complicated treatment strategies, highlighting the need for alternative therapies. Plant-based remedies are increasingly being explored for their antibacterial potential, particularly in traditional medicine.

Methods: This study evaluated the antibacterial activity of ethanolic extracts from *Mangifera indica* bark and *Callistemon citrinus* leaves, individually and in combination, against *S. pneumoniae*. The agar well diffusion method was employed to assess zones of inhibition. A combination effect was analyzed by calculating the increase in fold area of inhibition.

Results: Both individual extracts demonstrated antibacterial activity. However, the combination of the two extracts produced larger zones of inhibition than either extract alone. The calculated increase in fold area of inhibition was 0.659, suggesting a synergistic effect.

Conclusion: The combination of *Mangifera indica* and *Callistemon citrinus* extracts shows enhanced antibacterial efficacy against *S. pneumoniae*, supporting their traditional use in managing respiratory infections. Further research is needed to determine the mechanism of action, in vivo efficacy, and safety profile before clinical application.

Keywords: *Callistemon citrinus* leaves, *Mangifera indica* bark, *Streptococcus pneumoniae*, antibacterial effect

Introduction

Streptococcus pneumoniae is a Gram-positive, extracellular pathogen and a major cause of global morbidity and mortality. It particularly affects children under five, the elderly, and immunocompromised individuals. In 2013, pneumonia accounted for approximately 935,000 deaths among children under five, with Sub-Saharan Africa being heavily affected.¹ In Uganda, pneumonia and malaria are the leading causes of death in children under five, exacerbated by delays in diagnosis and treatment.²

Penicillin and related antibiotics have historically served as the first line of treatment for pneumococcal infections. However, the global rise in antibiotic resistance—especially to penicillin and erythromycin—has become increasingly problematic.¹ These trends underscore the urgent need for alternative, affordable antibacterial agents derived from traditionally used medicinal plants.³

Callistemon citrinus, commonly known as lemon bottlebrush, belongs to the Myrtaceae family and is native to Australia but widely cultivated in tropical regions, including Uganda.⁴ It is a 6-meter-tall evergreen shrub with bright red brush-like flowers and lemon-scented leaves. Traditionally, it has been used to manage bronchitis, tuberculosis, urinary incontinence, excessive menstruation, diarrhea, and mucosal discharges.⁵ A study in communities near Uganda's Mabira Forest found *C. citrinus* was cited with 100% fidelity for cough treatment.⁶ Phytochemical analysis reveals its richness in polyphenols, alkaloids, tannins, monoterpenoids, and triterpenoids, many of which exhibit antimicrobial and antioxidant activity.⁷ Its antibacterial effect is superior to some synthetic antibiotics such as miconazole and clotrimazole.⁵

Mangifera indica (mango), a member of the Anacardiaceae family, is widely cultivated across the tropics and valued for its fruit, wood, and medicinal bark.⁸ In Uganda, the bark—locally known as Omuyembe—treats diarrhoea, asthma, bronchitis, hypertension, toothaches, and piles.⁹ It contains bioactive constituents such as flavonoids, tannins, alkaloids, glycosides, and saponins.^{10,11} demonstrated that extracts from *M. indica* exhibited antimicrobial activity against various pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

While the individual antibacterial effects of *C. citrinus* and *M. indica* have been previously established, little is known about the outcome of combining these two extracts against *S. pneumoniae*. In pharmacology, such combinations can result in synergistic, additive, or antagonistic interactions.¹² Investigating such interactions is vital in validating traditional polyherbal remedies and developing new treatments for resistant infections.

This study aims to evaluate the in vitro antibacterial activity of ethanolic extracts from *Callistemon citrinus* leaves and *Mangifera indica* bark—individually and in combination—against *Streptococcus pneumoniae*, to determine whether their interaction is synergistic, additive, or antagonistic. The findings are expected to contribute to the scientific validation of traditional medicine and offer a potential alternative to conventional antibiotics.

Materials and Methods

Collection and Authentication of Plant Materials

Fresh leaves of *Callistemon citrinus* and bark of *Mangifera indica* were collected from Rukararwe Eco-Tourism and Herbal Medicine Centre (0°34'19.7"S, 30°11'31.0"E) in Bushenyi District, Southwestern Uganda. Botanical identification and authentication were performed by Mr. Tumushabe Emmanuel, a taxonomist at Mbarara University of Science and Technology Herbarium, where voucher specimens were deposited under accession numbers CCL-2024-01 and MIB-2024-02, respectively.

Drying, Pulverization, and Storage

Plant materials were shade-dried at room temperature ($25 \pm 2^\circ\text{C}$) until constant weight was achieved (two weeks for leaves; four weeks for bark), then ground into fine powder using an electric blender (Panasonic MX-AC400, Japan). The powders were stored in air-tight amber containers in a cool, dry place until extraction.

Extraction Procedure

For each plant, 100 g of powdered material was extracted using a Soxhlet apparatus (Borosil Glass Works Ltd., India) with 500 mL of 96% ethanol (Loba Chemie, analytical grade, purity $\geq 99.5\%$) for 12 hours. Extracts were concentrated under reduced pressure using a rotary evaporator (Heidolph Hei-VAP Core, Germany) at 45°C and stored at 4°C .

Test Organism and Authentication

A clinical isolate of *Streptococcus pneumoniae* was obtained from sputum samples at Mulago National Referral Hospital, Kampala, Uganda. The isolate was authenticated via Gram staining, optochin sensitivity, and bile solubility tests by the microbiology laboratory at Makerere University School of Health Sciences. Identity was confirmed using the VITEK[®] 2 Compact System (bioMérieux, France).

Preparation of Culture Media

Brain Heart Infusion (BHI) agar (Oxoid Ltd., UK) was prepared according to the manufacturer's instructions by dissolving 52.0 g of powder in 1 L of distilled water, sterilized at 121°C for 15 minutes, and poured to a uniform depth (4 mm) in sterile Petri dishes.

Preparation of Serial Dilutions and Combination Ratio

Stock solutions were made by dissolving 3 mg of each dry extract in 2 mL of distilled water. Serial twofold dilutions (1:1, 1:2, 1:4, 1:8) were prepared. The combination was formulated by mixing equal volumes of 1:1 stock solutions of each extract (ie, 1:1 ratio). This ratio was selected based on traditional co-use in respiratory ailments and prior pilot studies indicating optimal preliminary efficacy. No additional ratio optimization was attempted in this phase.

Antibacterial Testing (Agar Well Diffusion Method)

A bacterial suspension matching a 0.5 McFarland standard ($\sim 1.5 \times 10^8$ CFU/mL) was prepared in sterile BHI broth. The inoculum was evenly spread onto BHI agar plates. Wells (5 mm diameter) were punched and filled with 100 μ L of each dilution of the extracts and their combination. Plates were labeled: “B” (*Mangifera indica*), “L” (*Callistemon citrinus*), and “LB” (combination). Each test was performed in duplicate. Plates were incubated at 37°C for 14 hours.

Measurement of Zones of Inhibition

After incubation, diameters of inhibition zones were measured in millimeters using a digital Vernier caliper (Mitutoyo, Japan). Mean values and standard deviations were calculated from duplicate results.

Calculation of Increase in Fold Area (IFA)

The IFA was calculated to evaluate potential synergistic effects using the formula adapted from¹³

$$A1 = \frac{Y - X}{X}$$

$$A2 = \frac{Y - Z}{Z}$$

$$A = A1 + A2$$

Where:

- Y = zone of inhibition for the combination (mm),
- X = zone for *Mangifera indica* (mm),
- Z = zone for *Callistemon citrinus* (mm),
- A = total increase in fold area.

Interpretation¹³

- If A1 and A2 > 0 and A < 2 → additive effect,
- If A1 and A2 > 0 and A > 2 → synergistic effect,
- If A1 or A2 < 0 and A > 0 → partial antagonism,
- If A1 and A2 < 0 → complete antagonism.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics Version 26 (IBM Corp., USA). Descriptive statistics were computed, and ANOVA was used to compare mean zones of inhibition. A p-value < 0.05 was considered statistically significant.

Results

Percentage Yield of Ethanol Extracts

The percentage yield of ethanol extracts was calculated based on the weight of the extract obtained relative to the weight of the plant powder used. Tables 1 and 2 summarize the percentage yield for *Callistemon citrinus* and *Mangifera indica*, respectively. The yield from *C. citrinus* was notably higher (31.8%) compared to *M. indica* (22.85%).

Antibacterial Susceptibility Testing

The antibacterial assay included both plant extracts, their combination, a standard positive control (ciprofloxacin 5 μ g), and a negative control (DMSO). The zones of inhibition were measured for *M. indica*, *C. citrinus*, the combination, ciprofloxacin, and DMSO against *Streptococcus pneumoniae*. Table 3 presents the mean inhibition zones at different concentrations for the plant extracts and combination, while Figure 1 visually represents the comparative activity.

Table 1 Percentage Yield of Ethanol Extract of *Callistemon Citrinus*

Extract	Weight of Powder Residue (g)	Weight of Extract (g)	Percentage Yield (%)
Ethanol 96%	200	63.6	31.80

Table 2 Percentage Yield of Ethanol Extract of *Mangifera Indica*

Extract	Weight of Powder Residue (g)	Weight of Extract (g)	Percentage Yield (%)
Ethanol 96%	200	45.7	22.85

Table 3 Mean Zones of Inhibition (Mm) of *M. Indica*, *C. Citrinus*, and Their Combination

Concentration (mg/mL)	<i>M. indica</i>	<i>C. citrinus</i>	Combination
1.00	16.85	12.00	19.95
0.50	14.40	9.50	16.50
0.25	13.25	8.30	14.75
0.125	10.00	6.45	11.00

Statistical Comparison of Antibacterial Activity

Table 4 provides a statistical summary, showing the mean zones of inhibition and standard deviations for each test substance, including ciprofloxacin and DMSO. The p-values were derived from one-way ANOVA using GraphPad Prism version 9.5.1 (GraphPad Software, LLC, USA).

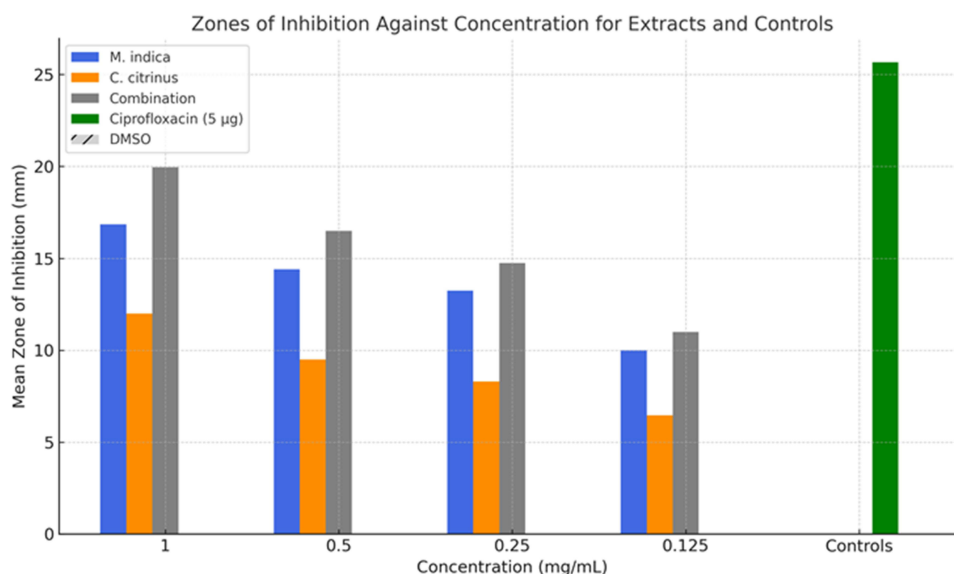


Figure 1 Comparative bar graph of mean zones of inhibition for *Mangifera indica*, *Callistemon citrinus*, their combination, ciprofloxacin (positive control), and DMSO (negative control) against *Streptococcus pneumoniae* at various concentrations.

Table 4 Antibacterial Activity of Test Substances Against *S. Pneumoniae*

Test Substance	Mean $\pm$ SD (mm)	p-value
<i>M. indica</i>	13.87 $\pm$ 3.19	0.0000
<i>C. citrinus</i>	9.56 $\pm$ 2.16	0.0000
Combination	15.03 $\pm$ 3.19	0.0000
Ciprofloxacin 5 μ g	25.67 $\pm$ 1.52	0.0000
DMSO	0.00 $\pm$ 0.00	—

Concentrations of Extracts in Combination

Each 1:1 combination was prepared to contain equal concentrations of both extracts. Table 5 presents the actual concentrations of each extract in the mixture at various dilution levels.

Comparative Activity: Visual Analysis

Figure 1. Comparative bar graph illustrating the mean zones of inhibition for *Mangifera indica*, *Callistemon citrinus*, their combination, ciprofloxacin (positive control), and DMSO (negative control) against *Streptococcus pneumoniae* at varying concentrations. The inhibition zones decreased with declining concentration, with ciprofloxacin exhibiting the highest antibacterial activity. (Graph generated using GraphPad Prism 9.5.1)

The inhibition zones for all test substances decreased with declining concentration. Across all concentrations, the combination demonstrated the largest inhibition zones among plant samples, indicating enhanced activity. Ciprofloxacin showed the highest zone of inhibition overall, while DMSO had no activity.

Regression Analysis

Best fit values were generated to assess the effect of concentration on the zones of inhibition for each extract and their combination. The statistical parameters for each regression model are summarized in Table 6 for *M. indica*, Table 7 for *C. citrinus*, and Table 8 for the combination treatment.

Fold Increase Analysis

The increase in fold area was calculated using inhibition zone values to evaluate the interaction effects of the combination.

Let X = 13.87 (*M. indica*), Y = 15.03 (Combination), Z = 9.56 (*C. citrinus*)

- $A1 = (Y - X)/X = (15.03 - 13.87)/13.87 = 0.084$
- $A2 = (Y - Z)/Z = (15.03 - 9.56)/9.56 = 0.576$
- $A = A1 + A2 = 0.084 + 0.576 = 0.660$

Interpretation: Since $A1$ and $A2 > 0$ and $A < 2$, the combination effect is additive.¹³

Table 5 Component Concentrations in Extract Combinations

Total Combination Concentration (mg/mL)	<i>M. indica</i> (mg/mL)	<i>C. citrinus</i> (mg/mL)
1.00	0.50	0.50
0.50	0.25	0.25
0.25	0.125	0.125
0.125	0.0625	0.0625

Table 6 Regression Values for *M. Indica*

Parameter	Value
p-value	0.0004
CI	[-14.942, -5.884]
F-stat	33.564
R ²	0.810
Adj R ²	0.761
RMSE	1.714

Table 7 Regression Values for *C. Citrinus*

Parameter	Value
p-value	0.0033
C.I	[-11.989, -2.354]
F-stat	13.576
R ²	0.612
Adj R ²	0.524
RMSE	1.553

Table 8 Regression Values for the Combination

Parameter	Value
p-value	0.0001
C.I	[-17.422, -7.744]
F-stat	49.125
R ²	0.864
Adj R ²	0.822
RMSE	1.263

Discussion

The ethanol extraction of *Callistemon citrinus* and *Mangifera indica* yielded 31.8% and 22.85%, respectively. This difference can be attributed to the physicochemical nature of the plant materials and the efficiency of ethanol as a solvent of intermediate polarity, which enhances the extraction of a wide range of phytochemicals.¹⁴ Despite its lower yield, *M. indica* demonstrated superior antibacterial activity compared to *C. citrinus*. When the two extracts were combined in equal proportions, the resulting mixture exhibited enhanced antibacterial activity against *Streptococcus pneumoniae*, as evidenced by consistently higher zones of inhibition across all tested concentrations.

The observed additive effect was further confirmed by fold increase calculations and supported by statistical analysis using one-way ANOVA ($p < 0.0001$), performed with GraphPad Prism version 9.5.1. The absence of inhibition zones in

DMSO confirmed that the observed effects were due to the plant extracts and not the solvent. Notably, ciprofloxacin (5 µg), the positive control, exhibited the highest inhibition zone (25.67 ± 1.52 mm), validating the responsiveness of the test organism and serving as a benchmark for comparison.

Regression analysis demonstrated a strong concentration-response relationship, particularly for the combination extract ($R^2 = 0.864$), indicating increased efficacy at higher concentrations. These findings are in agreement with previous studies reporting antibacterial activity for both *M. indica* and *C. citrinus*, attributed to bioactive constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and glycosides.¹⁵ The additive interaction observed may be due to complementary mechanisms of action or synergy among secondary metabolites, offering a potential for reduced toxicity and improved efficacy, as previously described in herbal combination therapies.¹³

Conclusion

In conclusion, the combination of *M. indica* and *C. citrinus* offers a promising natural alternative against *S. pneumoniae*, and its enhanced efficacy over individual extracts underscores the potential value of phytotherapeutic synergy in antimicrobial development.

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Disclosure

The authors declare no conflicts of interest. No financial or commercial benefits have been received or will be received in connection with this study.

References

1. Qazi S, Aboubaker S, MacLean R, et al. Ending preventable child deaths from pneumonia and diarrhoea by 2025: development of the integrated global action plan for the prevention and control of pneumonia and diarrhoea. *Arch Dis Child*. 2015;100(Suppl 1):S23–S28. doi:10.1136/archdis-child-2013-305429
2. Ministry of Health. Annual health sector report 2012/2013. 2014.
3. Ashok VG, Priya SB, Pranita AG. Original research article evaluation of antibacterial and phytochemical analysis of *Mangifera indica* bark extracts. *Int J Curr Microbiol App Sci*. 2014;3(5):567–580.
4. Bottlebrush R, Gilman EF, Watson DG. *Callistemon citrinus*. 1993;2–4.
5. Bhandari NL, Khadka S, Dhungana BR, et al. Study of phyto- and physicochemical analysis, antimicrobial and antioxidant activities of essential oil extract of *Callistemon citrinus* (Curtis) Skeels leaves. *Adv J Chem B Nat Prod Med Chem*. 2021;3(2):109–119.
6. Asiimwe S, Namukobe J, Byamukama R, Imalingat B. Ethnobotanical survey of medicinal plant species used by communities around mabira and mpanga central forest reserves, Uganda. *Trop Med Health*. 2021;49(1). doi:10.1186/s41182-021-00341-z
7. Sowndhararajan K, Deepa P, Kim S. A review of the chemical composition and biological activities of *Callistemon lanceolatus* (Sm.) Sweet. *J Appl Pharm Sci*. 2021;11(12):65–73. doi:10.7324/JAPS.2021.1101204
8. Mahalik G, Jali P, Sahoo S, Satapathy KB. Ethnomedicinal, phytochemical and pharmacological properties of *Mangifera indica* L: a review. *Structure*. 2020;29:31.
9. Kalita P. An overview on *Mangifera Indica*: importance and its various pharmacological action. *PharmaTutor*. 2014;2(12):72–76.
10. Abubakar EM, Shaari K, Paetz C, Stanslas J, Abas F, Lajis NH. Antibacterial efficacy of stem bark extracts of *Mangifera indica* against some bacteria associated with respiratory tract infections. *Nat Product Commun*. 2009;4(10):1031–1037.
11. Ouf SA, Galal AMF, Ibrahim HS, et al. Phytochemical and antimicrobial investigation of the leaves of five Egyptian Mango cultivars and evaluation of their essential oils as preservatives materials. *J Food Sci Technol*. 2020;58(8):3130–3142. doi:10.1007/s13197-020-04816-5
12. Clisi M, Campeau S, Abmm D. m pl e.
13. Shaughnessy EMO, Meletiadiis J, Stergiopoulou T, Demchok JP, Walsh TJ. Antifungal interactions within the triple combination of amphotericin B, caspofungin and voriconazole against *Aspergillus* species. *J Antimicrobial Chemother*. 2006;58(6):1168–1176. doi:10.1093/jac/dkl392
14. Azwanida NN. Medicinal & aromatic plants a review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med aromat plants*. 2015;4(3):3–8. doi:10.4172/2167-0412.1000196
15. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. *Internationale Pharmaceutica Scientia*. 2011. 1(1):98–106.

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