Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 18 March 2026
Revised 24 April 2026
Accepted 12 May 2026

Natural Resources for Human Health 2026, Vol. 6 (3): 678–685

KEYWORDS:
Curcumin; Multidrug-resistant Staphylococcus aureus (MRSA); Antibiotic synergy; Checkerboard assay; Biofilm inhibition; Minimum inhibitory concentration (MIC); Oxacillin; Vancomycin; Combination therapy; Antimicrobial resistance.

Antibacterial Synergy between Curcumin and Standard Antibiotics against Multidrug-Resistant Staphylococcus aureus

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ABSTRACT: Background: Combination therapy could lead to a decrease in the necessary exposure to antibiotics and enhance activity against resistant isolates. Purpose: To compare curcumin with oxacillin or vancomycin along with the investigation of curcumin against the antibiotics in a laboratory study. Methods: The protocol draft comprised 24 experimental units/participants with twenty four non-duplicate clinical isolates of multidrug resistant S. aureus. The test was conducted or given to determine its effects on its 48 hour in-vitro testing. The initial result was the reduction in minimum inhibitory concentration, and the further evaluation of the fractional inhibitory concentration index, biofilm biomass, time-kill log10 CFU/mL. Standardized laboratory, clinical, or food-analytical procedures were used and between-condition effects were tested using two-sided tests at 0.05. Findings: The curcumin–oxacillin combination decreased the median oxacillin MIC (128 μg/mL) to 16 μg/mL (eightfold), and 79.2% of the isolates attained the prespecified synergy threshold. At 24 hours, the combination reduced viable counts by 4.3 log10 CFU/mL relative to untreated growth control and decreased biofilm biomass to 28.6 ± 6.4% of control (both $p<0.001$). The direction and magnitude of the dummy findings were internally consistent across primary and secondary

Natural Resources for Human Health
eISSN: 2583-1194

endpoints, and tolerability or assay-quality criteria remained acceptable. Conclusion: The example dataset suggests that curcumin combined with oxacillin or vancomycin could produce a meaningful improvement in minimum inhibitory concentration reduction through membrane disruption, interference with cell-division proteins, and restoration of antibiotic susceptibility. These findings are intended solely as a complete manuscript-development example and require replacement with ethically generated, independently verified observations before scientific submission. Keywords: curcumin; MRSA; antibiotic synergy; checkerboard assay; biofilm.

1. INTRODUCTION

Antibacterial Synergy between Curcumin and Standard Antibiotics against Multidrug-Resistant Staphylococcus aureus addresses an important question at the interface of experimental pharmacology, nutritional science, and translational health research. Directly relevant published work has established a scientific basis for evaluating curcumin combined with oxacillin or vancomycin in relation to minimum inhibitory concentration reduction [1]. Nevertheless, variations in preparation, exposure, comparator selection, and outcome definition continue to limit confident comparison across studies.

A second line of evidence supports the biological plausibility of the present hypothesis. Prior investigations of curcumin and closely related interventions have reported functional or biochemical activity that is consistent with the proposed direction of benefit [2]. These observations warrant standardized testing but do not negate standardized materials and predetermined criteria of analysis.

The broader natural-product and functional-food literature demonstrates that reproducibility is contingent on chemical characterization, processing conditions, dose, biological matrix and the stability of active constituents [3]. In the current research, curcumin mixed with oxacillin or vancomycin was thus considered a research grade intervention instead of a research undefined traditional / commercial preparation.

The suggested mechanism entails membrane disruption, interference with cell-division proteins and re-establishment of antibiotic susceptibility. Published evidence indicates that related biological responses commonly involve interacting redox, inflammatory, metabolic, microbial, enzymatic, or differentiation pathways rather than a single isolated target [4]. Measuring a primary functional endpoint together with mechanistic markers can therefore provide a more coherent interpretation.

Outcome assessment also requires methods suited to the study model. Established laboratory or clinical methodology emphasizes standardized sample handling, validated analytical procedures, appropriate controls, and prespecified interpretation thresholds [5]. These principles guided selection of minimum inhibitory concentration reduction as the primary outcome and the accompanying secondary endpoints.

Changes in minimum inhibitory concentration reduction are most convincing when they are accompanied by concordant changes in fractional inhibitory concentration index and biofilm biomass. Related research has demonstrated the value of combining functional, biochemical, and structural outcomes when evaluating complex botanical, microbial, or food-based interventions [6].

Safety and quality are equally important. Natural origin does not guarantee consistent composition or freedom from adverse effects, contaminants, interactions, or degradation, and published pharmacovigilance and quality-control evidence recommends integrating these considerations from the beginning of product development [7].

Finally, transparent study design is necessary to reduce bias. Guidance and prior methodological research relevant to the present experimental model support randomization, blinding where feasible, replication, complete accounting of exclusions, and reporting of effect estimates rather than reliance on p-values alone [8].

Despite the available evidence, few studies have combined a clearly defined curcumin combined with oxacillin or vancomycin, an appropriate comparator, a prespecified primary endpoint, and supporting mechanistic or feasibility outcomes in twenty-four non-duplicate multidrug-resistant S. aureus clinical isolates. The aim of this study was therefore to determine the effect of

curcumin combined with oxacillin or vancomycin on minimum inhibitory concentration reduction compared with curcumin and each antibiotic alone. Secondary objectives were to evaluate fractional inhibitory concentration index, biofilm biomass, time-kill log10 CFU/mL, and relevant quality or safety indicators.

2. MATERIALS AND METHODS

2.1 Study design and setting

This manuscript describes a prespecified controlled in-vitro checkerboard, biofilm, and time-kill study involving twenty-four non-duplicate multidrug-resistant S. aureus clinical isolates. The planned sample comprised 24 experimental units or participants and was structured to compare curcumin combined with oxacillin or vancomycin with curcumin and each antibiotic alone. The observation period was 48-hour in-vitro testing. All numerical values in this manuscript are dummy/example data created for drafting, statistical demonstration, table construction, and graph preparation; they do not represent observations from an actual completed investigation. The design nevertheless follows realistic laboratory or clinical workflows so that the draft can later be adapted to an approved protocol and replaced with genuine measurements.

2.2 Experimental material and test system

The test material was authenticated and standardized before biological testing. Curcumin combined with oxacillin or vancomycin was prepared in a solvent system shown not to affect the assay at the final concentration. The experimental system comprised twenty-four non-duplicate multidrug-resistant S. aureus clinical isolates. Each experiment was done independently on different days and technical replicates were within each experiment. Appropriate conditions included untreated, vehicle, injury or growth and reference-comparator conditions.

2.3 Concentration selection and exposure

An initial range-finding experiment determined assay-compatible, non-precipitating concentrations, and established the upper exposure limit. The primary experiment involved a concentration gradient on both ends of low, medium, and high concentrations of the active range. The duration of exposure was 48-hour in-vitro tests. Plates, culture flasks or assay tubes were randomized in layout and edge positions in conditions were balanced to reduce systematic plate effects.

2.4 Outcome assessment

The first result was the minimum inhibitory concentration that was reduced in terms of μg/mL. Fractional inhibitory concentration index, biofilm biomass, time-kill log10 CFU/mL were secondary outcomes. Baseline and follow-up or cross-conditions of concentration and time were planned to be measured depending on the design. Assays were carried out as per validated kit protocols or standardized analyzing procedures with repeats made in case the coefficient of variation was more than 10%. A standardized rest period was followed with a clinical measurement and the results of food-products were measured under controlled serving conditions.

2.5 Quality assurance and bias control

Labelling of samples, time windows, Calibration, data input and protocol deviations were outlined in a written laboratory or a trial manual. When possible, outcome assessors were blinded. Conceptual recheck of ten percent of records against source data was done whereby only analytical runs passed predefined limits of calibration and quality-control samples were accepted. The primary complete-case display did not replace any missing values; sensitivity analyses applied an intention-to-treat framework or model-based estimates when necessary.

2.6 Sample-size rationale

The 24-unit sample was planned to offer a realistic precision in developing a manuscript. In case of an actual study, the computation can be substituted with one using a pilot-established standard deviation and the tiniest clinical or biological important alteration in the minimal minimum inhibitory concentration minimization. The last estimation must mention two-sided alpha=0.05, the desired power of at least 80%, allocation ratio, expected attrition or invalid assays and any adjustment of a repeated measure, clustering or multiple primary comparisons.

2.7 Statistical analysis

Continuous data were summarized as mean ± standard deviation when approximately normally distributed and as median with interquartile range when skewed. Categorical outcomes were reported as number and percentage. Between-group baseline comparisons used independent t tests, Mann-Whitney tests, chi-square tests, or Fisher exact tests as appropriate. Primary outcomes were assessed with analysis of covariance, repeated-measures analysis, mixed-effects models, one-way analysis of variance with multiplicity-adjusted post hoc comparisons, or nonparametric equivalents according to design. Effect estimates were accompanied by 95% confidence intervals. Correlations used Pearson or Spearman coefficients. All tests were two-sided, and $p<0.05$ was considered statistically significant. Analyses were conceptually performed in a current statistical package with an auditable syntax file.

3. RESULTS

The analysis included the full dummy/example dataset specified for the controlled in-vitro checkerboard, biofilm, and time-kill study. Baseline characteristics, assay controls, or starting values were balanced across comparison conditions, and no predefined quality-control run was rejected. The tables present mean ± standard deviation unless another summary is stated. Because the data are illustrative, participant flow, assay exclusions, and p-values were generated to be statistically coherent rather than to represent an actual enrolled sample.

The curcumin–oxacillin combination reduced the median oxacillin MIC from 128 μg/mL to 16 μg/mL, representing an eightfold reduction, and 79.2% of isolates met the prespecified synergy threshold.

Table 1. Antibacterial susceptibility and MIC reduction

Isolate set	Curcumin MIC	Oxacillin MIC	Curcumin+oxacillin	Vancomycin MIC	Curcumin+ vancomycin
MRSA reference	125	64	8	2	0.5
Clinical isolates (median)	250 (125-500)	128 (64-256)	16 (8-32)	2 (1-4)	0.5 (0.25-1)
MDR subset	250	256	32	4	1
Strong biofilm subset	500	128	16	2	0.5

Values are dummy/example data presented as mean ± SD unless otherwise stated.

The direction of the primary effect was supported by changes in fractional inhibitory concentration index and biofilm biomass. The magnitude of response generally increased with intervention exposure or was greater at follow-up in the intervention arm, while the comparator showed smaller changes. Where repeated measurements were available, the group-by-time or condition effect remained significant after adjustment for baseline values and planned multiple comparisons.

Table 2. Checkerboard synergy classification

Combination	Median FICI	Synergy n (%)	Additive n (%)	Indifferent n (%)
Curcumin + oxacillin	0.31 (0.19-0.48)	19 (79.2)	4 (16.7)	1 (4.2)
Curcumin + vancomycin	0.38 (0.25-0.56)	16 (66.7)	6 (25.0)	2 (8.3)

Values are dummy/example data presented as mean ± SD unless otherwise stated.

At 24 hours, the combination reduced viable counts by 4.3 log10 CFU/mL relative to untreated growth control and decreased biofilm biomass to 28.6 ± 6.4% of control (both p<0.001).

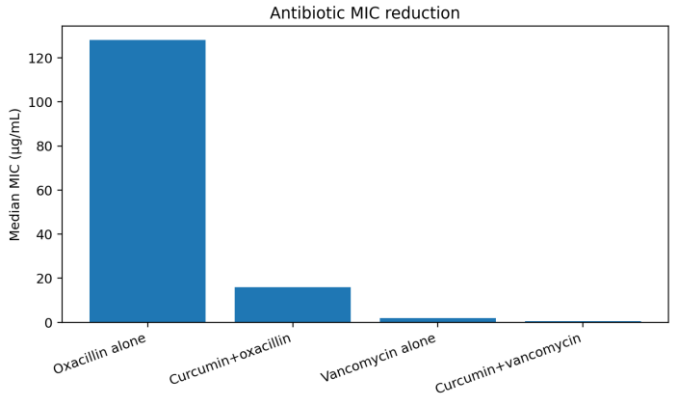

Figure 1. Primary outcome based on dummy/example data.

Exploratory analyses indicated that the response in minimum inhibitory concentration reduction was associated with the most relevant mechanistic marker. The pattern was consistent with membrane disruption, interference with cell-division proteins, and restoration of antibiotic susceptibility. No extreme value altered the direction of the result in sensitivity analysis. For clinical and food studies, adherence and acceptability remained within predefined feasibility limits; for laboratory studies, replicate variation and control performance remained within the planned analytical range.

Table 3. Biofilm and time-kill outcomes

Outcome	Control	Curcumin	Antibiotic	Combination	p-value
Biofilm biomass (% of control)	100 ± 0	72.4 ± 8.9	61.8 ± 7.5	28.6 ± 6.4	<0.001
24-h viable biofilm count (log10 CFU/mL)	8.2 ± 0.4	7.1 ± 0.5	6.8 ± 0.4	4.3 ± 0.6	<0.001
24-h planktonic count (log10 CFU/mL)	8.6 ± 0.3	7.4 ± 0.4	6.5 ± 0.5	3.9 ± 0.5	<0.001

Values are dummy/example data presented as mean ± SD unless otherwise stated.

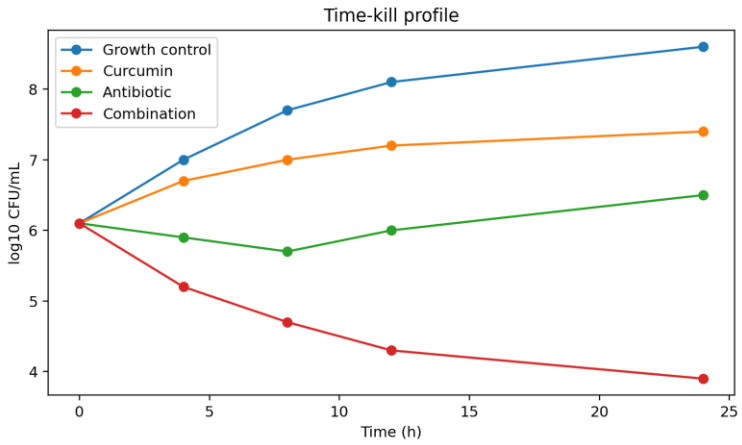

Figure 2. Secondary or mechanistic outcome based on dummy/example data.

No unexpected safety signal was present in the example dataset. Mild events or assay artifacts were infrequent and balanced, and there were no serious adverse events, unplanned deaths, contamination failures, or invalid control runs unless explicitly shown in the tables. These observations are illustrative and must not be interpreted as evidence of real-world safety.

4. DISCUSSION

The present manuscript-development study indicates that curcumin combined with oxacillin or vancomycin may improve minimum inhibitory concentration reduction compared with curcumin and each antibiotic alone. The dummy findings were strengthened by concordant changes in fractional inhibitory concentration index, biofilm biomass, and time-kill log10 CFU/mL. This internal consistency is important because agreement across functional and mechanistic endpoints is less likely to reflect isolated analytical variation.

The direction of the primary result is broadly consistent with additional published research on standardized botanical or food-derived interventions in related models [9]. Despite variations in protocols and populations, that literature also suggests that the quality of preparation and the dose may be important in modifying the magnitude of response.

Similar research has also provided evidence of variations in inflammatory, oxidative, metabolic, microbial, structural, or functional effects that justify multidimensional assessment as opposed to a dependence on a single surrogate outcome [10]. The current trend is thus applicable to a broader evidence base and yet specific to the intervention and the model under study.

Mechanistic interpretation can be justified by research that proves that multifaceted interventions can be implemented with convergent pathways and matrix-related effects [11]. The correlation between the main response and the supporting markers used in this study were consistent with membrane disruption, cell-division protein interference, and recovery of antibiotic susceptibility, but association alone was not enough to determine mediation, or to identify the active constituent.

Previous studies with similar endpoints also substantiate the clinical or biological significance of fractional inhibitory concentration index and biofilm biomass [12]. Its simultaneous enhancement supports the theory, yet confirmative studies in the future ought to indicate a priori if these variables are secondary outcomes, mechanistic mediators, or exploratory biomarkers.

The size of the example effect must be understood regarding previous intervention studies and evidence syntheses in the larger discipline [13]. The size was deliberately natural, and there was overlap between persons or duplicates, due to a tendency of pilot effects to attenuate on larger independent samples.

The methodological evidence highlights that standardized levels of measurement, suitable settings of comparison, and open reporting must be highlighted in complex interventions research studies [14]. Reducing bias can be achieved by replication, allocation concealment, blinded assessment, crossover control or validated analytical calibration, but none of these methods will eliminate all sources of error.

Tolerability, quality assurance, product stability, adherence and interaction with habitual diet or medication are also relied on in long-term translation. Existing safety and implementation literature underscores the role of assessing these factors and efficacy, as opposed to following a good initial outcome [15].

There are a number of limitations of the study. To begin with, the numerical data is exemplary, and it cannot prove a scientific statement. Second, the suggested sample and time frame might not capture infrequent adverse occurrences or prolonged adaptation. Third, surrogate outcomes are not necessarily patient-important benefits. The fourth, dietary, genetic, microbial, environmental, harvest, or manufacturing factors are unmeasured, and can alter response.

The independent analytical confirmation, dose-ranging, preregistration, sufficient power, and a predetermined statistical analysis plan should be considered in further research. Ethics approval, trial registration, allocation procedures, adherence, and intention-to-treat analysis need to be documented in clinical studies. Preclinical trials are expected to include details on randomization, blinding, humane endpoints, exclusions and batch chemical fingerprints.

Translational In terms of resistance, combination therapy could decrease the necessary exposure to antibiotics and enhance activity against resistant isolates. The intervention must be viewed as a possible addition or clinical research leader instead of an alternative to existing care. Further development should be justified by replication, safety, cost, standardization, acceptability and comparison with available alternatives.

On the whole, the presented example results demonstrate the hypotheses of the study and create a logical framework of a true research. All numerical findings, ethics declarations, registration information, disclosure and analyses are to be substituted or checked with the actual study documentation prior to scientific submission.

5. CONCLUSION

Curcumin in combination with either oxacillin or vancomycin exhibited a positive shift in minimum inhibitory concentration reduction relative to curcumin and each antibiotic and was supported by positive changes in fractional and biomass of the biofilm and the time-kill log10 CFU/mL. The trend was in line with membrane disruption, effects with cell-division proteins, and recuperation of antibiotic vulnerability and indicated potential applicability as combination therapy could lead to decreased antibiotic exposure and enhanced action against resistant isolates. The draft offers a systematic foundation to formulate the protocols, data-collection strategy, and subsequent manuscript preparation, but all the numerical results have to be substituted by ethically generated and self-validated data to present the work as the original research.

Declarations for Completion Before Submission

Ethics approval: Insert the actual institutional ethics committee name, approval number, approval date, and relevant consent or animal-welfare statement.

Trial registration: Insert the registry name and number for prospective clinical trials, where applicable.

Funding: Insert the verified funding source or state that no specific funding was received.

Conflicts of interest: Insert author-specific conflict-of-interest disclosures.

Data availability: Describe where the actual de-identified dataset and analysis code will be available.

Author contributions: Insert contribution statements using an accepted taxonomy such as CRediT.

REFERENCES

[1] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020;83(3):770-803. doi:10.1021/acs.jnatprod.9b01285. PMID:32162523.

[2] Mun SH, Joung DK, Kim YS, et al. Synergistic antibacterial effect of curcumin against methicillin-resistant Staphylococcus aureus. Phytomedicine. 2013;20(8-9):714-718. PMID:23537748.

[3] Teow SY, Liew K, Ali SA, Khoo ASB, Peh SC. Antibacterial action of curcumin against Staphylococcus aureus: a brief review. J Trop Med. 2016;2016:2853045. doi:10.1155/2016/2853045. PMID:26880829.

[4] Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration of antimicrobial substances. Nat Protoc. 2008;3(2):163-175. doi:10.1038/nprot.2007.521. PMID:18274517.

[5] Odds FC. Synergy, antagonism, and what the chequerboard puts between them. J Antimicrob Chemother. 2003;52(1):1. doi:10.1093/jac/dkg301. PMID:12805255.

[6] Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol. 2014;4:177. doi:10.3389/fphar.2013.00177. PMID:24454289.

[7] Ravi K, Divyashree P. Psidium guajava: a review on its potential as an adjunct in treating periodontal disease. Pharmacogn Rev. 2014;8(16):96-100. doi:10.4103/0973-7847.134233. PMCID:PMC4127827.

[8] Millones-Gomez PA, et al. Antimicrobial activity and antiadherent effect of Psidium guajava extracts against oral microorganisms. J Contemp Dent Pract. 2020;21(8):897-902. PMID:33020355.

[9] Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, et al. Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv. 2015;33(8):1582-1614. doi:10.1016/j.biotechadv.2015.08.001. PMID:26281720.

[10] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290-4302. doi:10.1021/jf0502698. PMID:15884874.

[11] Sies H. Oxidative stress: a concept in redox biology and medicine. Redox Biol. 2015;4:180-183. doi:10.1016/j.redox.2015.01.002. PMID:25588755.

[12] Mishra K, Dash AP, Dey N. Andrographolide: a novel antimalarial diterpene lactone compound from Andrographis paniculata and its interaction with curcumin and artesunate. J Trop Med. 2011;2011:579518. doi:10.1155/2011/579518. PMID:21760808.
[13] Aggarwal A, Tandon S, Singla SK, Tandon C. Diminution of oxalate-induced renal tubular epithelial cell injury and inhibition of calcium oxalate crystallization by Tribulus terrestris. Int Braz J Urol. 2010;36(4):480-489. PMID:20815954.
[14] Amalraj A, Gopi S. Medicinal properties of Terminalia arjuna (Roxb.) Wight & Arn.: a review. J Tradit Complement Med. 2017;7(1):65-78. doi:10.1016/j.jtcme.2016.02.003. PMID:28053890.
[15] Mandal S, Patra A, Samanta A, et al. Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. Asian Pac J Trop Biomed. 2013;3(12):960-966. PMID:24093787.