INPLASY

Antimicrobial Efficacy of Methylene Blue-Mediated Antimicrobial Photodynamic Therapy against Oral Microorganisms: A Systematic Review of In Vitro Studies

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ADMINISTRATIVE INFORMATION

Support - No support.

Review Stage at time of this submission - Preliminary searches.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 12 August 2026 and was last updated on 12 August 2026.

INTRODUCTION

Review question / Objective The aim of this systematic review is to synthesize and evaluate the antimicrobial efficacy of methylene blue-mediated antimicrobial photodynamic therapy against oral microorganisms in in vitro studies. Secondary objectives are to explore whether antimicrobial efficacy varies according to methylene blue concentration and formulation, light-source characteristics and irradiation parameters, microorganism characteristics, and experimental models, and to assess the methodological quality and completeness of reporting of the included studies.
The primary review question is: What is the antimicrobial efficacy of methylene blue-mediated antimicrobial photodynamic therapy against oral microorganisms in in vitro studies?

Rationale Methylene blue-mediated antimicrobial photodynamic therapy has been investigated as an antimicrobial approach against a wide range of oral microorganisms. However, substantial methodological heterogeneity exists among in vitro studies regarding methylene blue concentration and formulation, solvent and pH, pre-irradiation time, light source, wavelength, output power, irradiance, irradiation time, radiant exposure, and experimental conditions. In addition, studies use different microbial species and strains, planktonic or biofilm models, monospecies or multispecies biofilms, and different antimicrobial outcome measures. These differences limit direct comparisons across studies and make it difficult to determine which experimental and treatment parameters are associated with greater antimicrobial efficacy. A systematic synthesis focused specifically on methylene blue-mediated antimicrobial photodynamic therapy is therefore needed to characterize the available in vitro evidence, identify factors associated with variations in antimicrobial efficacy, assess methodological quality and reporting completeness, and inform the standardization of future preclinical and clinical research.

Condition being studied Oral microorganisms and oral biofilm-associated conditions relevant to human oral health, including dental caries, periodontal and peri-implant diseases, endodontic infections, and oral candidiasis. The review will evaluate in vitro antimicrobial models involving microorganisms associated with oral and dental conditions.

METHODS

Search strategy Electronic searches will be conducted in PubMed, MEDLINE via Ovid, Embase.com, Scopus, and ProQuest Dissertations & Theses Global from inception to the final search date, without language or publication-date restrictions. Search strategies will combine controlled vocabulary (MeSH in PubMed and Ovid MEDLINE; Emtree in Embase.com) and free-text terms for three concepts: methylene blue, antimicrobial photodynamic therapy, and oral microorganisms/dental contexts. Synonyms within each concept will be combined using OR and the three concept blocks using AND. Scopus will be searched using TITLE-ABS-KEY fields, while ProQuest Dissertations & Theses Global will be searched using the NOFT (Anywhere except full text) field. The term “in vitro” will not be required and no methodological search filter will be applied to maximize sensitivity. Database-specific syntax will be used for each source. The strategies will be piloted against known eligible studies and rerun before final synthesis. Full database-specific search strategies will be provided as supplementary material.

Participant or population The population of interest consists of oral microorganisms evaluated under in vitro conditions, including bacteria and fungi associated with human oral and dental diseases. Standard reference strains and clinical isolates will be eligible. Microorganisms may be investigated as planktonic cells, monospecies biofilms, or multispecies biofilms. Microorganisms not typically considered members of the oral microbiota will be eligible only when the study provides a clear oral or dental origin, context, experimental model, or relevance.

Intervention Antimicrobial photodynamic therapy using methylene blue as the primary photosensitizer combined with a compatible light source. No restrictions will be imposed on methylene blue concentration, solvent, pH, contact time, pre-irradiation time, light-source type, wavelength, output power, irradiance, irradiation time, radiant exposure, irradiation distance, or number of irradiation sessions. Studies using free methylene blue or conventional formulations will be included in the main synthesis. Studies using methylene blue incorporated into nanoparticles, liposomes, or other complex delivery systems may be eligible when methylene blue is the primary photosensitizer and the relevant data can be extracted separately; these studies will be synthesized separately from studies using free methylene blue. Studies evaluating multiple photosensitizers will be eligible only when data for the methylene blue group can be extracted separately.

Comparator Eligible comparators will include untreated or negative controls, vehicle or solvent controls, methylene blue without irradiation, irradiation without methylene blue, different methylene blue concentrations, different light-source or irradiation protocols, and antimicrobial or disinfection reference treatments. The primary comparison will be methylene blue-mediated antimicrobial photodynamic therapy versus an untreated or negative control.

Study designs to be included Original controlled in vitro experimental studies reported as full-text journal articles, master's theses, or doctoral dissertations, provided that they report at least one quantitative antimicrobial outcome and contain sufficient methodological information to assess eligibility.

Eligibility criteria Studies will be eligible if they: (1) are full-text original in vitro experimental studies reported as journal articles, master's theses, or doctoral dissertations; (2) investigate microorganisms of oral origin or microorganisms studied within a clearly defined oral or dental context; (3) use methylene blue as the primary photosensitizer; (4) include at least one experimental group receiving methylene blue combined with light; (5) include an appropriate comparator; and (6) report at least one quantitative antimicrobial outcome.
Studies evaluating multiple photosensitizers will be eligible only when data from the methylene blue group can be extracted separately. Studies using methylene blue incorporated into nanoparticles, liposomes, or other complex delivery systems may be included when methylene blue is the primary photosensitizer and relevant data can be extracted separately; these studies will be synthesized separately from studies using free methylene blue. No restrictions will be applied according to publication date or language.
Human clinical studies, animal studies, narrative reviews, systematic reviews, meta-analyses, case reports, editorials, letters, conference abstracts

without a full report, studies without quantitative antimicrobial outcomes, photothermal therapy studies, photobiomodulation studies without an antimicrobial photodynamic mechanism, and studies combining methylene blue-mediated antimicrobial photodynamic therapy with another antimicrobial intervention when the eligible photodynamic data cannot be extracted separately will be excluded.
When the same experimental dataset is reported in more than one source, for example in a thesis or dissertation and a subsequent journal article, these reports will be considered companion reports of a single study rather than independent studies. The most complete report will be used as the primary source, while companion reports will be used to supplement missing methodological or outcome information.

Information sources PubMed, MEDLINE via Ovid, Embase.com, Scopus, and ProQuest Dissertations & Theses Global will be searched from database inception to the final search date. No restrictions will be applied according to publication date or language. ProQuest Dissertations & Theses Global will be searched to identify eligible master's theses and doctoral dissertations, which will be considered for direct inclusion in the systematic review when they meet all eligibility criteria.
Supplementary searching will include Google Scholar, backward reference-list screening of included studies and relevant reviews, and forward citation searching of eligible studies. Up to the first 200 Google Scholar results ranked by relevance will be screened. Study authors will be contacted when important methodological information or outcome data require clarification.

Main outcome(s) The primary outcome will be post-intervention viable microbial load, preferentially expressed as log10 colony-forming units per millilitre (log10 CFU/mL) or log10 colony-forming units per sample. Where studies report colony-forming units on the original scale, transformation to log10 values will be considered when appropriate and mathematically possible.
When sufficiently comparable studies are available, the primary effect measure will be the mean difference in post-intervention log10 viable microbial load between the intervention and comparator groups, reported with 95% confidence intervals.

Additional outcome(s) Secondary outcomes will include log10 microbial reduction from baseline or relative to the control group; percentage or proportion of surviving microorganisms; live/dead cell measurements; metabolic activity; biofilm biomass, thickness, or volume; structural or morphological biofilm changes; molecular microbial counts; reactive oxygen species production; and temperature changes during irradiation.
Post-intervention log10 CFU and log10 reduction will not be combined in the same meta-analysis. Colony-forming-unit outcomes will not be pooled with metabolic activity, biofilm biomass, or molecular counts that do not distinguish viable from non-viable microorganisms.

Data management Search records will be imported into Zotero for reference management and duplicate removal and subsequently transferred to Rayyan for study screening. Two reviewers will independently screen titles and abstracts or equivalent bibliographic records and subsequently assess potentially eligible full-text reports. Disagreements will be resolved through discussion and, when necessary, consultation with a third reviewer. Data will be independently extracted by two reviewers using a piloted standardized Microsoft Excel form.
In addition to bibliographic duplicate removal, potentially overlapping reports of the same experimental study, including a thesis or dissertation and a subsequent journal article, will be identified by comparing authorship, institution, microorganism and strain, experimental design, intervention parameters, sample characteristics, and study period. Companion reports will be linked and treated as a single study. The most complete report will serve as the primary source, with companion reports used to supplement missing information.
Extracted data will include bibliographic and publication characteristics, microbial and experimental-model characteristics, methylene blue-related parameters, light-source and irradiation parameters, experimental design, control groups, biological and technical replicates, outcome methods, and quantitative results. Statistical analyses, when appropriate, will be performed using JASP.

Quality assessment / Risk of bias analysis Methodological quality will be independently assessed by two reviewers using the Quality Assessment Tool for In Vitro Studies (QUIN). Disagreements will be resolved through discussion or consultation with a third reviewer.
In addition, a separate reporting checklist will be used to assess the completeness and reproducibility of key antimicrobial photodynamic therapy parameters, including methylene blue concentration, pre-irradiation time, wavelength, output power, irradiance, irradiation area,

irradiation time, radiant exposure, and irradiation distance. This reporting checklist will not be incorporated into the QUIN score.
Because the review is restricted to in vitro evidence, the GRADE framework will not be formally applied to infer clinical certainty of evidence. Findings will instead be interpreted according to methodological quality, consistency, directness to the review question, precision, reporting completeness, and reproducibility.

Strategy of data synthesis A structured narrative synthesis will be the primary method of evidence synthesis. Included studies will be grouped according to microorganism, microbial classification, planktonic or biofilm model, monospecies or multispecies biofilm, methylene blue concentration and formulation, light source and irradiation parameters, outcome method, and methodological quality.
Meta-analysis will be considered only when at least three independent studies are sufficiently comparable in terms of microorganism or microbial group, experimental model, comparator, outcome measurement, unit, and assessment time. Post-intervention log10 CFU and log10 reductions will be analysed separately. Outcomes representing different biological constructs will not be pooled.
For outcomes reported using the same scale, mean differences will be calculated. Standardized mean differences with Hedges' correction may be used when studies assess the same construct using different continuous scales. Results will be reported with 95% confidence intervals. Random-effects models will be preferred because methodological and biological heterogeneity is anticipated. Statistical heterogeneity will be described using I^2 and between-study variance, together with assessment of clinical and methodological heterogeneity.
Technical replicates from the same biological sample will not be treated as independent observations. When multiple intervention groups share the same control group, appropriate methods will be used to avoid double counting of the control group.

Subgroup analysis If sufficient data are available, prespecified subgroup analyses will examine differences according to: (1) methylene blue concentration and formulation; (2) light source and irradiation characteristics; (3) planktonic versus biofilm models and monospecies versus multispecies biofilms; and (4) microorganism characteristics, including Gram-positive bacteria, Gram-negative bacteria, and fungi.
Where sufficient numbers of independent studies and adequate variation are available, exploratory meta-regression may be considered for continuous factors such as methylene blue concentration, pre-irradiation time, wavelength, irradiance, radiant exposure, and irradiation time. Meta-regression will not be performed with fewer than approximately 10 independent studies per explanatory variable.

Sensitivity analysis If sufficient data are available, sensitivity analyses will be performed by excluding studies with low methodological quality according to QUIN; studies with unclear numbers of independent biological experiments; studies with incomplete reporting of irradiation parameters; studies for which outcome data are estimated from figures; studies using complex methylene blue delivery systems; studies with substantially different experimental models or inappropriate comparator groups; and master's theses or doctoral dissertations, to assess the influence of grey literature on the pooled estimates.

Language restriction Only English materials.

Country(ies) involved Vietnam.

Other relevant information This protocol has been developed in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) 2015 statement. The literature search will be reported according to PRISMA-S, and the completed systematic review will be reported according to PRISMA 2020. Any important amendments made after registration will be documented, dated, and justified in the final systematic review.
This review is restricted to in vitro preclinical evidence and its findings will not be directly extrapolated to clinical effectiveness.

Keywords antimicrobial photodynamic therapy; methylene blue; oral microorganisms; oral biofilm; photosensitizer; photodynamic inactivation; dentistry; oral microbiology.

Dissemination plans The results of this systematic review will be submitted for publication in a peer-reviewed journal in dentistry, oral microbiology, or photodynamic therapy and will form part of doctoral research. Findings may also be presented at relevant scientific conferences.

Contributions of each author
Author 1 - Thi Tham Dang - Conceptualization; methodology; protocol development; search strategy development; literature searching; study screening and selection; data extraction; methodological quality assessment; data synthesis

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Author 2 - Quang Vinh Luu - Literature searching; independent study screening and selection; data extraction; methodological quality assessment; verification of extracted data; contribution to data synthesis; writing – review and editing.
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Author 3 - Vu Tri Hoang - Conceptualization; methodology; supervision; validation of the review protocol and eligibility criteria; interpretation of findings; resolution of disagreements when required; writing – review and editing.
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Author 4 - Hoang Son Le - Conceptualization; methodology; supervision; project administration; oversight of the systematic review methodology; interpretation of findings; critical revision of the protocol and manuscript; writing – review and editing; final approval of the manuscript.
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