

PLANT-DERIVED PHOTOSENSITIZERS FOR ANTIMICROBIAL PHOTODYNAMIC THERAPY AGAINST MRSA: EVIDENCE FROM IN VITRO STUDIES AND IN VIVO MODELS

**FOTOSSENSIBILIZADORES DE ORIGEM VEGETAL PARA TERAPIA
FOTODINÂMICA ANTIMICROBIANA CONTRA MRSA: EVIDÊNCIAS DE
ESTUDOS IN VITRO E MODELOS IN VIVO**

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ABSTRACT

Antimicrobial photodynamic therapy (aPDT) combines a photosensitizer, light absorbed by that compound, and oxygen to generate reactive species capable of inactivating microorganisms. This narrative review synthesizes evidence on plant-derived photosensitizers against methicillin-resistant *Staphylococcus aureus* (MRSA), covering *in vitro* studies and animal models. A focused literature search was conducted across PubMed/PubMed Central records and publisher pages using terms related to MRSA, aPDT, natural products, plant compounds, propolis, curcumin, hypericin, *Passiflora*, and *in vivo* models. The reviewed literature includes plant molecules, extracts and plant-associated products, as well as a formulated photosensitizer used as a methodological comparator. *In vitro* activity varies with the compound, absorption spectrum, light dose, concentration, pre-irradiation time, and bacterial model. Mouse studies of curcumin, Brazilian green propolis, and a *Passiflora* fraction suggest that aPDT can reduce bacterial burden or alter inflammatory responses, although interventions and designs are heterogeneous. *Galleria mellonella* provides an intermediate model for evaluation, but the published MRSA aPDT study in this larva used photodithazine, which is not a plant-derived compound. These categories must be distinguished, and preclinical findings should not be presented as evidence of clinical efficacy. Experimental standardization, chemical characterization, and toxicity assessment remain priorities for future research.

Keywords: MRSA; antimicrobial photodynamic therapy; natural products; plant-derived photosensitizers; animal models.

RESUMO

A terapia fotodinâmica antimicrobiana (aPDT) combina um fotossensibilizador, luz absorvida pelo composto e oxigênio para

gerar espécies reativas capazes de inativar microrganismos. Esta revisão narrativa sintetiza evidências sobre fotossensibilizadores de origem vegetal avaliados contra *Staphylococcus aureus* resistente à meticilina (MRSA), em estudos *in vitro* e modelos animais. Foi realizada uma busca direcionada em registros do PubMed e do PubMed Central, páginas de editoras e registros acadêmicos indexados. A literatura inclui moléculas vegetais, extratos e produtos associados a plantas, além de um fotossensibilizador formulado usado apenas como comparador metodológico. A atividade *in vitro* varia conforme o composto, o espectro de absorção, a dose de luz, a concentração, o tempo de pré-irradiação e a cepa bacteriana. Estudos em camundongos com curcumina, própolis verde brasileira e uma fração de *Passiflora* sugerem que a aPDT pode reduzir a carga bacteriana ou alterar respostas inflamatórias, embora os protocolos e desenhos experimentais sejam heterogêneos. *Galleria mellonella* pode servir como modelo intermediário, mas o estudo de aPDT contra MRSA identificado nesta larva utilizou fotoditazina, que não é derivada de plantas. É importante manter essas categorias distintas e não interpretar resultados pré-clínicos como evidência de eficácia clínica. A padronização experimental, a caracterização química e a avaliação da toxicidade continuam sendo prioridades.

Palavras-chave: Terapia fotodinâmica antimicrobiana; fotossensibilizadores de origem vegetal; produtos naturais; MRSA; modelos animais.

1. INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) causes skin and soft-tissue infections, invasive disease, and healthcare-associated infections. Resistance to beta-lactam antibiotics complicates treatment and sustains interest in approaches that act through

mechanisms distinct from conventional drug targets. The World Health Organization's 2024 bacterial priority pathogens list includes methicillin-resistant *S. aureus* among the pathogens requiring attention in research and public-health planning (WHO, 2024). This priority does not imply that any experimental alternative is ready for clinical use; it motivates careful preclinical investigation.

Antimicrobial photodynamic therapy (aPDT) is one such approach. A photosensitizer (PS) absorbs photons and transfers energy or electrons to molecular oxygen, producing reactive oxygen species (ROS), including singlet oxygen and radical species. Oxidative damage can affect membranes, proteins, and nucleic acids. Because aPDT acts through multiple oxidative targets, its activity is not defined by the conventional antibiotic-resistance phenotype alone. Nevertheless, susceptibility depends on PS uptake and localization, bacterial envelope properties, oxygen availability, light delivery, and the surrounding biological matrix (Mahmoudi *et al.*, 2018; Dharmaratne *et al.*, 2020).

Plant-derived molecules and extracts have attracted interest because many contain conjugated chromophores or other structures that absorb visible light. Curcumin, hypericin, and pheophorbide-related pigments are examples of plant-associated molecules examined in antimicrobial photodynamic research. Complex botanical extracts and propolis are also studied, but they should not be equated with purified phytochemicals: propolis is a chemically variable bee product containing plant-derived resins and other constituents. This distinction matters when assigning a mechanism, reproducing a dose, or comparing outcomes across studies.

The evidence base spans planktonic cultures, biofilms, and animal infection models. Those levels answer different questions. A reduction in colony-forming units *in vitro* establishes antibacterial activity under specified conditions; an animal model adds host, tissue, immune, and treatment-delivery factors. Neither alone demonstrates clinical benefit. A broad systematic review of MRSA aPDT already documented extensive variation in light sources, doses, solvents, incubation conditions, and outcomes (Dharmaratne et al., 2020). The present review therefore focuses on how plant-derived and plant-associated photosensitizers have been evaluated against MRSA, how animal-model evidence should be interpreted, and which methodological issues limit comparison.

The review question is: what evidence supports plant-derived photosensitizers for aPDT against MRSA *in vitro* and *in vivo*, and what limits interpretation of that evidence? We distinguish (i) isolated or chemically defined plant-derived molecules, (ii) plant extracts and complex plant-associated products, and (iii) non-plant formulations used to inform model design. This is a narrative synthesis, not a systematic review or meta-analysis.

1.1. Review Methodology

A focused narrative search was conducted for this manuscript using PubMed and PubMed Central records, publisher article pages, and web-indexed scholarly records available through 12 October 2026. Search concepts combined “methicillin-resistant *Staphylococcus aureus*” or MRSA with “antimicrobial photodynamic therapy,” aPDT, photoinactivation, photosensitizer, natural product, plant extract, curcumin, hypericin, pheophorbide, propolis, *Passiflora*, *Galleria mellonella*, mouse, and animal model. References cited by relevant

reviews and primary articles were also checked for MRSA-specific studies.

Primary studies were considered relevant when they evaluated a plant-derived molecule, botanical extract, or plant-associated product with light against MRSA, or when they tested such an intervention in an animal model of MRSA infection. Studies with other *S. aureus* phenotypes were retained only when they helped explain a method or mechanism. A formulated, non-botanical photosensitizer was included only as a model comparator. Reviews were used for background and for locating primary research. This search was focused rather than exhaustive: no duplicate screening, protocol registration, formal risk-of-bias scoring, or quantitative pooling was undertaken. Accordingly, the article does not report systematic-review study counts or claim comprehensive coverage.

1.2. Theoretical Framework And Evidence Synthesis

1.2.1. Photochemical Basis And Determinants Of APDT Activity

The photodynamic process begins when a PS absorbs a photon and enters an excited singlet state. It may return to ground state by fluorescence or undergo intersystem crossing to a longer-lived triplet state. From that state, type I reactions transfer electrons or hydrogen atoms and generate radical intermediates; type II reactions transfer energy to oxygen to produce singlet oxygen. The balance between these pathways depends on the molecular structure, aggregation, solvent, oxygen tension, and local environment. In practice, reported ROS generation should be linked to assay conditions rather than treated as a fixed intrinsic value for a compound.

The wavelength should overlap the compound's absorption spectrum, but spectral overlap alone does not determine treatment efficacy. Irradiance, exposure time, total fluence, beam geometry, distance, temperature, and sample volume affect the delivered light dose. A study that reports exposure time without irradiance or fluence is difficult to reproduce. Likewise, a concentration without solvent, purity, or chemical characterization can be misleading for extracts and mixtures. Pre-irradiation time may affect PS association with bacteria, while incubation in darkness helps distinguish photodynamic killing from direct dark toxicity.

MRSA is Gram-positive and lacks the outer membrane found in Gram-negative bacteria, which can facilitate access of some cationic or amphiphilic photosensitizers to the cell envelope. However, strain-specific properties, cell-wall composition, biofilm matrix, efflux, and antioxidant responses can alter susceptibility. Laboratory reports should identify the strain, growth phase, inoculum, biofilm status, illumination parameters, oxygen conditions, and enumeration method. Dark, light-only, PS-only, vehicle, and untreated controls are needed to attribute changes to the combined photodynamic treatment.

1.2.2. Classes Of Plant-Derived And Plant-Associated Photosensitizers

Chemically defined phytochemicals offer advantages for dose definition and reproducibility, but they may have limited solubility, aggregate in aqueous media, or absorb poorly at wavelengths with sufficient tissue penetration. Curcumin is a polyphenolic pigment with visible-light absorption and is among the best studied plant-derived PS candidates. Hypericin and pheophorbide a are plant-

associated pigments with distinct spectra and photochemical behavior. Their results cannot be generalized to all botanical compounds because each has different absorption, localization, photostability, and ROS yields (Polat and Kang, 2021).

Botanical extracts can contain several chromophores and non-chromophore constituents. They may show additive, antagonistic, or synergistic effects, and their composition can vary with plant species, geography, season, extraction solvent, and processing. Brazilian green propolis is often relevant to aPDT research, but it is a bee-collected resin product rather than a purified plant compound. Geopropolis similarly combines plant-derived material with bee and environmental constituents. Work on geopropolis antimicrobial activity can support the rationale for studying such products, but it does not establish light-dependent activity unless illumination was actually tested (da Cunha *et al.*, 2013).

A recent mechanistic comparison of several PS, including curcumin, green propolis, a *Passiflora* fraction, quercetin, and conventional dyes, underscores that different compounds can produce different ROS profiles and oxidative damage patterns (Pereira *et al.*, 2026). Such mechanistic work can help explain why a single light setting or pre-incubation protocol should not automatically be transferred from one PS to another. The result is a practical need to characterize each candidate's absorption spectrum and photochemical behavior before selecting illumination conditions.

1.2.3. *In Vitro* Evidence Against MRSA

In vitro evidence includes both single compounds and complex botanical materials. Curcumin-mediated aPDT has been assessed

against MSSA and MRSA biofilms using LED illumination, with the outcome depending on treatment conditions (Teixeira et al., 2020). Chan et al. (2021) compared hypericin and pheophorbide a *in vitro* and then evaluated them in a mouse MRSA wound model. These findings support continued evaluation but do not provide a universal ranking of plant PS because the studies differ in strain, medium, light, dose, formulation, and endpoints.

Ribeiro et al. (2023) characterized Brazilian green propolis as a photosensitizer against MRSA ATCC 43300 and vancomycin-intermediate *S. aureus*. The work compared LED colors and reported photodynamic activity with blue light in the tested system. Its relevance lies in coupling a plant-associated product with wavelength selection and resistant staphylococci. Yet the product is a multicomponent propolis preparation, and its findings should not be attributed to a single phytochemical without chemical fractionation and identification.

A more recent study evaluated *Passiflora* species and identified a butanolic fraction of *P. cincinnata* as a promising photosensitizing preparation against MRSA. The authors reported spectral features consistent with absorption in the blue region and assessed activity using blue LED illumination. The study is especially pertinent because *P. cincinnata* is associated with the Brazilian semiarid region and because the work connected spectral characterization with antibacterial assays (Gonçalves et al., 2025). The fraction remains a mixture; its activity cannot yet be assigned to a specific molecule without further chemical characterization.

Across these studies, CFU enumeration is a useful viability endpoint, but methodological choices influence the result. A reduction in

metabolic assay signal does not necessarily equal bacterial killing; colored or fluorescent extracts may interfere with optical readouts. CFU assays avoid some optical interference but require validated dilutions, suitable plating volumes, and reporting of detection limits. Biofilm studies also need to specify whether the endpoint measures biomass, viable cells, or regrowth. These distinctions are essential when synthesizing aPDT results.

1.2.4. Evidence From Animal Models

Animal models add host-response and tissue-delivery information, but they remain preclinical and should not be treated as clinical evidence. Among mouse studies, curcumin-mediated aPDT was evaluated in a pilot intradermal *S. aureus* infection model in mice with type 1 diabetes. The authors reported improved bacterial control in selected tissues compared with control conditions (Muniz et al., 2021). The model supports the feasibility of investigating a plant-derived PS in a host with impaired metabolic and immune function, but the pilot design and infection conditions limit broad inference about MRSA treatment.

Chan et al. (2021) assessed hypericin and pheophorbide a from *in vitro* testing through a mouse MRSA wound model. Both PS were investigated for antibacterial effects, and topical photodynamic treatment reduced bacterial burden under the study's conditions. The comparison is useful because it examines two defined pigments and considers wound repair outcomes. However, wound models, illumination geometry, local oxygenation, and the optical properties of tissue can influence delivered dose; translation requires confirmation in larger and independently replicated studies.

Brazilian green propolis has also been tested in a murine intradermal MRSA infection model. Ribeiro et al. (2024) compared treatment groups that included propolis, light, and the combined photodynamic intervention, reporting changes in bacterial load and inflammatory measures. The inclusion of component controls supports attribution of the observed outcome to the combination. As with the *in vitro* work, propolis is a complex product, and batch characterization is central to reproducibility.

Gonçalves et al. (2025) extended *Passiflora* research to a senescent-mouse MRSA model. The animal experiment used a *P. cincinnata* fraction and included infection and treatment groups; the report describes bacterial and inflammatory outcomes. It is a notable example of a Brazilian plant fraction assessed beyond culture assays. Interpretation should consider the small group sizes, the particular age-related host model, and the described photoactivation procedure, which does not necessarily reproduce direct illumination of an infected tissue. The model therefore supports feasibility rather than a definitive treatment claim.

The larval model *Galleria mellonella* is useful for preliminary *in vivo* assessment because it is less costly and enables infection and survival experiments before mammalian studies. Souza et al. (2024) evaluated MRSA aPDT with photodithazine, including biofilm assays and a *Galleria* infection model. Photodithazine is a formulated chlorin photosensitizer, not an isolated plant molecule or plant extract. The study can inform model design, outcome selection, and feasibility of light-based treatment in larvae, but it is adjacent evidence rather than direct evidence that plant-derived compounds are effective in this model. Larval survival, bacterial burden, and

toxicity should be interpreted together, and results are not interchangeable with mammalian efficacy or human treatment.

Table 1. Summary of evidence categories and their interpretive limits

Evidence category	Representative evidence	What it supports	Key boundary
Defined plant-derived molecules	Curcumin; hypericin; pheophorbide a (Freitas <i>et al.</i> , 2019; García <i>et al.</i> , 2015; Chan <i>et al.</i> , 2021; Muniz <i>et al.</i> , 2021)	Mechanistic and efficacy testing with more clearly defined PS; some <i>in vitro</i> and mouse MRSA evidence	Results depend on formulation, light dose, model, and whether the compound is tested alone or as a derivative/formulation
Botanical mixtures and plant-associated products	Brazilian green propolis; <i>P. cinnamomata</i> fraction (Ribeiro <i>et al.</i> , 2023, 2024; Gonçalves <i>et al.</i> , 2025)	Evidence for complex natural preparations in resistant staphylococci and animal settings	Mixture composition, batch variation, and active constituent identity complicate reproducibility
Model comparator	Photodithazine in MRSA biofilm and Galleria assays (Souza <i>et al.</i> , 2024)	Shows aPDT can be examined in a larval infection model	Not plant-derived; supports model methods, not botanical efficacy

Source: Prepared for this review based on the cited studies.

1.2.5. Methodological Limitations And Priorities For Future Research

The most persistent limitation is heterogeneity. Different studies use different strains, inocula, media, PS concentrations, solvents, pre-irradiation intervals, wavelengths, irradiances, fluences, and biological models. A broad MRSA aPDT systematic review likewise identified substantial variation in protocols that constrains direct comparison (Dharmaratne *et al.*, 2020). The field would benefit from a minimum reporting set: strain and resistance profile; PS identity, purity or extract batch and chemical fingerprint; solvent and final solvent concentration; absorption spectrum; light source, wavelength, irradiance, fluence, geometry, and temperature; oxygen conditions; incubation timing; controls; biological replicates; CFU detection limit; and statistical analysis.

Second, wavelength selection should be based on measured absorption and the available light source, not copied from a protocol developed for another PS. For mixtures, the absorption spectrum may contain overlapping bands, and the wavelength giving the highest absorbance may not maximize bacterial killing. A staged design can first characterize spectra, then test a small number of justified illumination conditions, and finally conduct dose-response and time-course experiments for promising candidates. This is a design principle, not a prescribed protocol; final parameters require supervisor and institutional review.

Third, botanical materials require chemical characterization. At minimum, authors should report plant identity and plant part, provenance, extraction conditions, yield, storage, and chromatographic or spectroscopic fingerprints where available. For propolis and geopropolis, botanical source and batch composition are particularly important. For a fraction, the authors should state whether the material is a crude extract or a chemically enriched

fraction and avoid naming a single active constituent without evidence.

Fourth, aPDT studies should separate phototoxicity from dark toxicity and light-only effects. Vehicle controls are relevant when the PS solvent can affect bacterial viability. In animal work, toxicity assessment, treatment tolerability, blinding of outcome assessment, sample-size justification, humane endpoints, and appropriate randomization should be reported. A survival curve alone is insufficient if treatment-related toxicity is possible; bacterial burden and relevant host outcomes add context. A larval model can help prioritize candidates but cannot substitute for mammalian validation when justified.

Finally, oxidative stress should be treated as a testable mechanism rather than an automatic explanation for any decrease in CFU. Direct ROS measurements, quenchers or pathway probes, oxidative-damage markers, and appropriate controls can strengthen mechanistic claims. Recent comparative work reports that different photosensitizers generate distinct ROS profiles and damage patterns (Pereira et al., 2026). Separately, laboratory research has shown that repeated photodynamic exposure can select altered tolerance phenotypes in *S. aureus* under specific experimental conditions (Snell et al., 2021). This finding does not establish routine clinical resistance to aPDT, but it supports monitoring for reproducible changes in susceptibility during repeated-exposure studies.

1.3. Discussion

Taken together, current evidence supports the biological plausibility of plant-derived aPDT against MRSA, but the strength of evidence varies by material and model. Defined molecules such as curcumin, hypericin, and pheophorbide a offer interpretable chemistry and have been assessed in culture or mouse models. Botanical fractions and propolis preparations may provide useful activity, particularly when they absorb in an accessible LED range, but their complex composition limits compound-level attribution. The central interpretive task is therefore to preserve material identity: an isolated molecule, an enriched fraction, a crude extract, and a bee-collected resin product are not interchangeable categories.

The animal evidence is promising but preliminary. Mouse studies report reductions in bacterial outcomes or changes in inflammation for curcumin, hypericin/pheophorbide, green propolis, and *Passiflora* preparations. Those studies vary in host, infection site, treatment route, illumination, comparator groups, and endpoints. A consistent direction of effect across several models can justify further investigation, but it cannot establish a single optimal PS or predict clinical effectiveness. The Galleria literature is valuable for preclinical workflow development; the MRSA aPDT study currently highlighted here uses photodithazine, so it should be described as a model precedent rather than botanical proof.

A practical research sequence follows from these limitations: characterize candidate materials and spectra; confirm baseline dark toxicity and light-only effects; screen with documented light parameters and validated viability endpoints; conduct concentration-response and replicate experiments for active candidates; evaluate biofilm activity where relevant; then assess tolerability and efficacy in a suitable animal model. For extracts,

chemical fingerprinting should accompany biological testing. For animal experiments, light delivery and treatment controls must match the proposed biological question. Each step should be planned around the specific compound or preparation rather than inherited uncritically from an unrelated photosensitizer protocol.

This review has limitations. It is a focused narrative synthesis, not a registered systematic review; study selection was not performed in duplicate and no formal risk-of-bias tool or meta-analysis was used. The literature is heterogeneous, and some studies concern *S. aureus* generally, complex propolis, or non-botanical formulations rather than isolated plant compounds. Accordingly, conclusions are qualitative and should be updated as more directly comparable work becomes available.

2. CONCLUSION

Plant-derived and plant-associated photosensitizers are credible candidates for further MRSA aPDT research. *In vitro* and animal studies involving curcumin, hypericin, pheophorbide a, Brazilian green propolis, and *Passiflora* preparations provide evidence of photodynamic activity under selected conditions. The evidence does not yet support treating these materials as equivalent or claiming clinical efficacy. Propolis and botanical fractions require chemical characterization; formulated non-plant PS studies should be used only as methodological comparators. Better reporting of spectra, light dose, controls, viability endpoints, toxicity, and animal-study design will improve reproducibility and enable meaningful comparison. A staged, material-specific approach is the most defensible path from laboratory screening to *in vivo* evaluation.

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