UNESP - Universidade Estadual Paulista
“Júlio de Mesquita Filho”
Faculdade de Odontologia de Araraquara

Analú Barros de Oliveira

Bioprospecção de produtos naturais em terapia fotodinâmica contra micro-organismos de interesse médico-odontológico

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Dissertação apresentada à Universidade Estadual Paulista (Unesp), Faculdade de Odontologia de Araraquara para obtenção do título de Mestre em Ciências Odontológicas, na Área de Odontopediatria.

Orientadora: Prof[a] Dr[a] Fernanda Lourenção Brighenti
Coorientadora: Prof[a] Dr[a] Carla Raquel Fontana

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"Tudo posso naquele que me fortalece"
(Filipenses 4:13)

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“Ensinar não é transferir conhecimento,
mas criar possibilidades para a sua produção ou a sua construção.
Quem ensina aprende ao ensinar e quem aprende ensina ao aprender.”

(Paulo Freire)

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[*] Autor desconhecido

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[*] Autor não identificado. Traduzido por Seme Draibe. São Paulo; 1957.

Oliveira AB. Bioprospecção de produtos naturais em terapia fotodinâmica contra micro-organismos de interesse médico-odontológico [dissertação de mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2020.

RESUMO

Este trabalho está dividido em 2 publicações cujos objetivos foram: a) realizar uma revisão sistemática da literatura, seguida de uma metanálise sobre a eficácia da terapia fotodinâmica (TFD) nos micro-organismos responsáveis pela cárie dentária (Publicação 1); b) avaliar o potencial *in vitro* dos óleos essenciais de *Coffea arabica*, *Matricaria recutita* e *Eugenia uniflora* e dos extratos vegetais de *Senna splendida*, *Senna reticulata* e *Senna Macranthera* para serem utilizados como monoterapia na terapia fotodinâmica sobre suspensões de micro-organismos de interesse médico-odontológico. Publicação 1: A questão de pesquisa e as palavras-chave foram construídas de acordo com a estratégia do PICO. A pesquisa do artigo foi realizada nas bases de dados Embase, Lilacs, Scielo, Medline, Scopus, Cochrane Library, Web of Science, Science Direct e Pubmed. Ensaios clínicos randomizados e estudos in vitro foram selecionados na revisão. O estudo foi conduzido de acordo com as diretrizes do PRISMA para revisão sistemática. Publicação 2: Foram utilizadas as seguintes cepas de referência em suspensão: *Cutibacterium acnes* ATCC 6919, *Streptococcus mutans* ATCC 35688, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 e *Candida albicans* ATCC 90028. Os materiais vegetais foram testados nas concentrações de 50 μg/mL (extratos) ou 2% (óleos essenciais). Foram estudados cinco grupos: controle negativo, material vegetal sem exposição a luz (FS-luz), material vegetal com exposição a luz (FS+luz), controle de veículo sem exposição a luz (CV-luz) e controle de veículo com exposição a luz (VC+luz). A eficácia da terapia foi avaliada através da contagem de células viáveis após o tratamento. A produção de espécies reativas de oxigênio foi avaliada nos materiais vegetais que apresentaram melhor atividade antimicrobiana. Para este teste foram utilizadas as sondas fluorescentes 3′-p- (aminofenil) fluoresceína (APF; detecta principalmente o radical hidroxil [• OH]) e o Sensor de Oxigênio Singleto (SOSG; detecta oxigênio singleto [O_2]). Os dados foram analisados no programa IBM SPSS versão 20.0, com nível de significância de 5%. Para *C. acnes* e *S. mutans* foram observadas reduções microbianas totais em dois dos seis compostos vegetais testados. Já para *S. aureus* e *C. albicans* obtivemos redução microbiana total em 4 dos 6 compostos testados. Por último, foi observado redução total em apenas 1 dos 6 compostos naturais testados para *E. coli*. Os materiais vegetais de *S. macranthera* e *S. reticulata* foram os que mais produziram espécies reativas de oxigênio, seguido de *S. splendida*, *E. uniflora* e *M. recutita*. Os resultados da publicação 1 destacam que não há consenso sobre os protocolos de estudo para TFD contra micro-organismos cariogênicos, embora os resultados tenham mostrado que a TFD pode ser uma boa alternativa para o tratamento de cárie dentária. Já os resultados da publicação 2 mostraram que a terapia fotodinâmica antimicrobiana (TFDa) mediada por diferentes substâncias naturais foi eficiente na redução microbiana de suspensões de micro-organismos de interesse médico-odontológico.

Palavras chave: Fotoquimioterapia. Plantas medicinais. Infecções bacterianas e micoses. Produtos com ação antimicrobiana.

Oliveira AB. Bioprospecting of natural products in photodynamic therapy against microrganisms of medical and dental interest [dissertação de mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2020.

ABSTRACT

This study is divided into 2 publications whose objectives were: a) to carry out a meta-analysis about antimicrobial photodynamic therapy (PDT) in Denstistry (Publication 1) and b) To evaluate the in vitro potential of essential oils of *Coffea arabica*, *Matricaria Recutita* and *Eugenia uniflora* and plant extracts of *Senna Splendida*, *Senna Reticulata* and *Senna Macranthera* for use as monotherapy in photodynamic therapy on suspensions of microorganisms of medical and dental interest. Publication 1: A research question and how keywords were constructed according to the PICO strategy. A search of the article was carried out in the Embase, Lilacs, Scielo, Medline, Scopus, Cochrane Library, Web of Science, Science Direct and Pubmed databases. Randomized controlled trials and in vitro studies were selected in the review. The study was conducted according to the PRISMA guidelines for systematic review. Publication 2: The following references strains were used in suspension: *Cutibacterium acnes* ATCC 6919, *Streptococcus mutans* ATCC 35688, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 90028. The plant materials were tested at 50 µg / mL (extracts) or 2% (essential oils). Five groups were studied: negative control, plant material without exposure to light (FS-light), plant material with exposure to light (FS + light), vehicle control without exposure to light (CV-light) and vehicle control with exposure the light (VC + light). The effectiveness of the therapy was assessed by counting viable cells after treatment. The production of reactive oxygen species was evaluated in plant materials that showed the best antimicrobial activity. For this test, 3′-p- (aminophenyl) fluorescent probes (APF; mainly detects the radical hydroxyl [• OH]) and the Singlet Oxygen Sensor (SOSG; detects singlet oxygen [O_2]) were used. The data were analyzed using the IBM SPSS version 20.0 program, with a significance level of 5%. For *C. acnes* and *S. mutans*, total microbial reduction was observed in 2 of the 6 plant materials tested. For *S. aureus* and *C. albicans,* total microbial reduction was observed in 4 of the 6 plant materials tested. A total reduction of 1 out of 6 natural materials was observed for *E. coli*. *S. macranthera* and *S. reticulata* were produced the greatest amount of reactive oxygen species, followed by *S. splendida*, *E. uniflora* and *M. recutita*. In conclusion, Publication 1 highlights that there is no consensus on the study protocols for PDT against cariogenic microorganisms, although the results shown that a PDT may be a good alternative for the treatment of dental caries. Publication 2 shows that antimicrobial photodynamic therapy (PDT) mediated by different natural substances was efficient in reducing microbial suspensions of microorganisms of medical and dental interest.

Keywords: Photochemotherapy. Medicinal plants. Bacterial infections and mycoses. Products with antimicrobial action.

SUMÁRIO

1 INTRODUÇÃO

Os micro-organismos são componentes importantes da biosfera e desempenham um papel vital na manutenção dos ecossistemas[1]. Para sobreviver, os micro-organismos desenvolveram mecanismos que lhes permitiram responder à pressão seletiva exercida por diversos fatores[2].

Os seres humanos expuseram continuamente populações microbianas patogênicas a antibióticos, anti-sépticos e outros agentes antimicrobianos, na tentativa de controlar doenças infecciosas. No entanto, esses micro-organismos responderam desenvolvendo uma variedade de mecanismos de resistência tornando os atuais tratamentos antimicrobianos pouco eficazes[3]. A utilização de tratamentos não convencionais para o combate de micro-organismos de interesse médico-odontológico, que possibilitem menor chance de causar resistência é de grande relevância, e é neste ponto que as técnicas de terapia fotodinâmica (TFD) destacam-se, uma vez que tem se mostrado uma ferramenta eficaz no tratamento de diversas enfermidades[4].

O princípio da terapia fotodinâmica foi descrito pela primeira vez em 1990 por Oscar Raab e seu professor, Herman Von Tappeiner, que investigando a ação de um corante (acridina) em culturas de paramécios comprovaram que a associação deste corante com a luz era letal para estes microrganismos[5]. Posteriormente, outros estudos realizados pelo professor Herman Von Tappeiner permitiram criar o termo “ação fotodinâmica” para descrever uma reação dependente de oxigênio após fotossensibilização, pois observaram que a luz e o corante isoladamente não apresentavam qualquer efeito citotóxico sobre as culturas dos protozoários[6].

No mesmo ano, o cientista dinamarquês Niels Finsen, publicou o livro “Phototerapy”, que demonstrava relatos de sucesso do uso da aplicação de fontes de luz no tratamento de pacientes portadores de lúpus cutâneo, sendo premiado em 1903 com o Prêmio Nobel na área da Medicina[7].

Em 1907, von Tappeiner e colaboradores, publicaram um livro-texto sobre TFD, definindo-a como um processo de fotossensibilização dependente de oxigênio, criando assim o termo “ação fotodinâmica”. Neste livro, os autores relataram suas experiências com eosina tópica a 5% e luz artificial para tratamento de câncer cutâneo não melanoma e de outras dermatites, como lúpus e condiloma. Com estudos adicionais, von Tappeiner presumiu que a eosina, assim como a acridina, após incorporação a célula alvo, poderia produzir uma reação citotóxica quando exposta a uma fonte de luz adequada na presença de oxigênio[8].

Porém, devido a sua toxicidade e potencial carcinogênico, os corantes acridina e eosina foram logo descartados. Então, em 1908, surgiram os primeiros casos envolvendo o uso de porfirinas como agente fotossensível, o que causou um grande alvoroço na comunidade científica. Alguns anos após esta descoberta, Albert Policard detectou a emissão de fluorescência de tumores expostos a porfirinas quando eram irradiados, presumindo-se que as porfirinas se concentravam preferencialmente em neoplasias. A partir de então, diversas pesquisas foram desenvolvidas para estudar este mecanismo[9].

Em paralelo às diversas pesquisas que estudavam a aplicabilidade da TFD, Albert Einstein, em 1916, criava a teoria “os princípios da luz pela emissão estimulada da radiação”, que, posteriormente, resultaria no desenvolvimento do primeiro dispositivo de laser por Theodore Maiman, proporcionando assim uma série de pesquisas envolvendo a interação de luz e tecido[10].

Em 1925, Policard avaliou a capacidade de porfirinas produzirem efeito fototóxico. Posteriormente Auler e Banzer confirmaram as observações de Policard, sugerindo a aplicação de porfirinas para o diagnóstico e detecção inicial de tumores malignos[11].

Em 1976, Weishaupt e colaboradores propuseram que o oxigênio singleto, originado da sensibilização a luz, a partir da transferência da energia do agente fotossensível no estado tripleto excitado para o oxigênio molecular em estado fundamental, era o agente responsável pela citotoxicidade e desativação das células alvo[11].

Em 1998, a FDA (*Food and Drug Administration/* EUA) aprovou o uso da TFD para tratamento de câncer, seguido de vários países como Holanda, Japão, Alemanha entre outros[12].

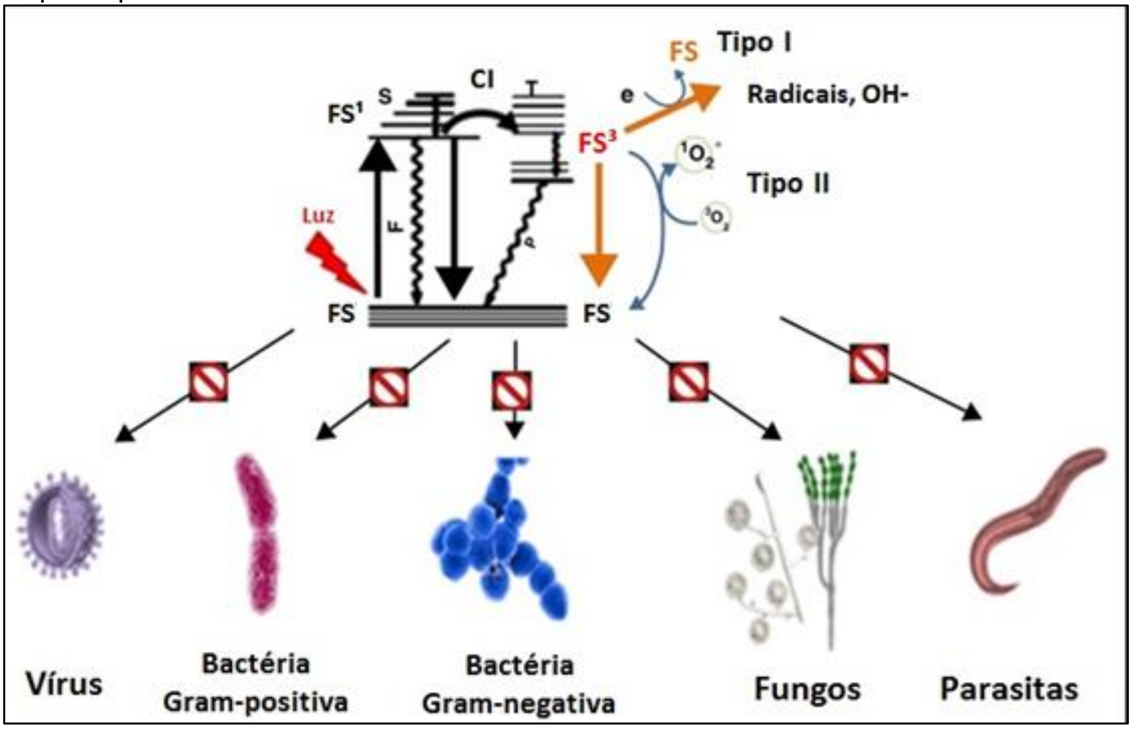
A implantação da TFD no Brasil envolveu uma equipe composta, principalmente, por físicos do Instituto de Física de São Carlos (USP) e médicos do Hospital Amaral Carvalho de Jaú - São Paulo, sob coordenação do Prof. Dr. Vanderlei Salvador Bagnato (Projeto FAPESP nº 98/14270-8). Porém, apenas em 2006 a TFD foi aprovada para tratamento de câncer de pele não melanoma no Brasil. Nos últimos 10 anos, seu uso ampliou para diversas outras indicações[13].

Resumidamente, a TFD é uma modalidade de tratamento que associa mecanismos foto-físicos e fotoquímicos gerando resposta biológica através do uso de fontes de luz específicas e de um agente fotossensibilizador associado ao oxigênio[8]. Para o sucesso dessa terapia, é necessário que o agente fotossensibilizador (FS) esteja disponível em sua forma pura e que sua composição química seja conhecida, que seja sintetizável a partir de precursores disponíveis e facilmente reproduzidos, que possua alto rendimento quântico de oxigênio singleto, que seja estável cinética e termodinamicamente, seja seletivo para induzir a morte apenas das células alvo, possuir atividade biológica apenas quando exposto à fonte de luz, estável e solúvel nos fluidos dos tecidos do corpo e de fácil entrega aos tecidos alvos através de injeção ou de outros métodos e que seja excretado facilmente após a conclusão do tratamento[14].

Os processos fotoquímicos da TFD iniciam após a absorção de fótons pelo agente fotossensibilizador, ocasionando a transição do estado fundamental para o excitado. Em seu estado fundamental (S0) a molécula do agente fotossensibilizador apresenta-se como singleto, que passa para seu estado singleto excitado de vida curta (S1) em decorrência da absorção de luz. Neste momento, esta molécula em S1 pode voltar ao estado fundamental, emitindo fluorescência, ou sofrer uma transição para o estado tripleto de vida mais longa através do cruzamento inter-sistemas[15].

No estado tripleto excitado, o agente fotossensibilizador é passível de reagir com moléculas biológicas através de dois principais mecanismos que são dependentes de oxigênio, denominados de reação tipo I e reação tipo II[11,16]. Na reação tipo I, o agente fotossensibilizador reage com biomoléculas como, por exemplo, lipídios, proteínas e ácidos nucléicos, transferindo elétrons para dar origem a radicais e íons radicais. A reação com o oxigênio molecular resulta na produção de espécies reativas de oxigênio (EROs) como por exemplo, o ânion superóxido, radicais hidroxila e peróxido de hidrogênio. Já na reação tipo II, o agente fotossensibilizador transfere energia diretamente ao oxigênio em seu estado tripleto fundamental, em um fenômeno chamado aniquilação tripleto-tripleto. Há a formação de oxigênio singleto, que é altamente reativo e citotóxico, e que pode reagir com um grande número de substratos biológicos e induzir dano oxidativo na membrana celular e na parede celular[11,16]. O oxigênio singleto tem uma vida curta em sistemas biológicos e causa uma resposta localizada sem afetar células ou órgãos distantes[17]. A Figura 1 ilustra os tipos de reações acima citados e suas ações em TFDa.

Figura 1 - Diagrama de Jablonski ilustrando a produção fotoquímica de diferentes espécies reativas de oxigênio durante a TFD e suas propriedades antimicrobianas de amplo espectro

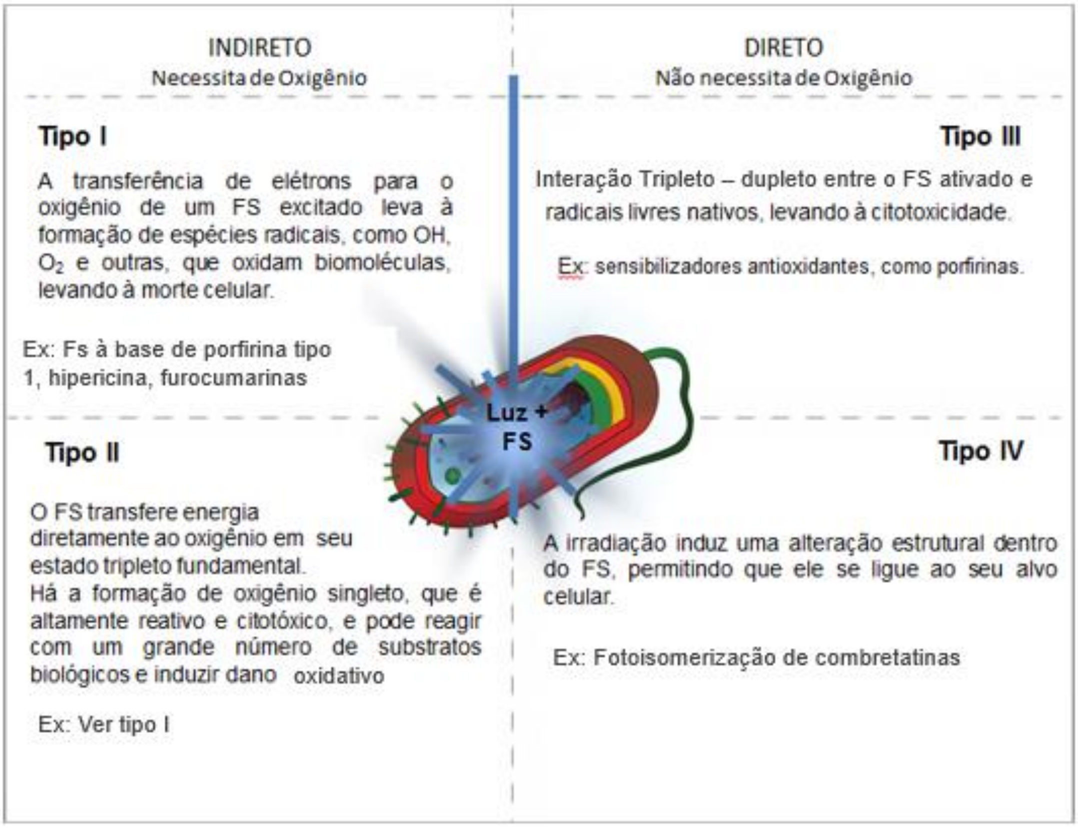


O estado fundamental FS absorve um fóton para formar o estado excitado singleto FS^1 que pode passar pelo cruzamento inter-sistema (CI) para formar o estado tripleto FS^3. Esta espécie de vida longa pode sofrer transferência de energia (Tipo II) para formar oxigênio singleto O_2* ou transferência eletrônica (Tipo I) para formar radicais hidroxilas (OH-). Ambas são capazes de matar um amplo espectro de patógenos.

Fonte: Adaptado de Hamblin et al.[29].

Além dos mecanismos indiretos (que requerem oxigênio), existem os mecanismos diretos (independentes de oxigênio) de fotossensibilização na TFD. Esses mecanismos vêm sendo discutidos na literatura cientifica, de acordo com do FS utilizado (Figura 2). Tratam-se das reações de tipo III e reações de tipo IV, que confiam na ativação de fotossensibilizadores para induzir a morte celular sem a dependência do oxigênio para transferência de energia ou carga[15].

Figura 2. Mecanismos diretos (independentes de oxigênio) e indiretos (que requerem oxigênio) de fotossensibilização na TFD, dependendo do FS utilizado.



Fonte: Adaptado de Scherer et al.[15] 2017.

A maioria dos fotossensibilizadores utilizados na clínica demonstra a presença do mecanismo Tipo II, mas fotossensibilizadores que utilizam o mecanismo Tipo III e IV também são eficazes, especialmente nas situações em que a fotossensibilização dos Tipos I e II é limitada. Geralmente, os fotossensibilizadores do tipo III transportadores antioxidantes que diminuem a concentração do radical nas células-alvo e geram oxigênio singleto. Na reação do tipo IV, os fotossensibilizadores não podem se ligar ao alvo molecular e, após irradiação, pode ocorrer um processo chamado fotoisomeração. Esse processo causa remodelação intramolecular, que facilita a ligação do fotossensibilizador ao alvo celular. Contudo, mais estudos devem ser realizados para melhor entendimento dessas reações[15].

A terapia fotodinâmica antimicrobiana (TFDa), é indicada em várias modalidades da Medicina e da Odontologia, uma vez que a maioria das enfermidades infecciosas são localizadas e possibilitam o acesso e a ação direta dos agentes fotossensibilizadores[18]. A formação de oxigênio singleto é a principal via de dano celular microbiano[17].

Doenças de origem microbiana são consideradas um problema de saúde pública em todo o mundo, tais doenças ocupam um lugar de destaque no panorama mundial de saúde, uma vez que em consequência dessas doenças, podem ocorrer não só injúrias na pele, mucosa bucal e vaginal, pulmões, mas também, septicemias e endocardites[19]. Dentre os principais agentes etiológicos de doenças de interesse, destacam-se *Streptococcus mutans*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Candida albicans* e *Cutibacterium acnes*, cuja importância médico-odontológica está descrita no (Quadro 1).

Quadro 1 - Características morfotintoriais e importância médico-odontológica das espécies de micro-organismo em estudo

Espécie	**Características morfotintoriais**	**Importância médico-odontológica**
C. acnes	Gram-positivo	Principal agente etiológico da acne vulgar presentes em lesões endodônticas secundárias, nos casos de periodontite agressiva e está associada ao aparecimento de pneumonia em pacientes sob ventilação mecânica[19,20,21].
C. albicans	Células esféricas, Gram-positivo	Infecções Orais e Vaginais[22].
E. coli	Bastonete Gram-negativo	*Relacionada a diversas doenças intestinais e infecções em feridas.* Também encontrado em infecções endodônticas[23,24].
S. aureus	Coco Gram-positivo	*Principal agente causador de infecções hospitalares em todo o mundo, na cavidade oral está relacionada com a ocorrência de doença periodontal,* mucosite peri-implantar e peri-implantite[25,26].
S. mutans	Coco Gram-positivo	Frequentemente isolado de lesões de cárie dentária[27].

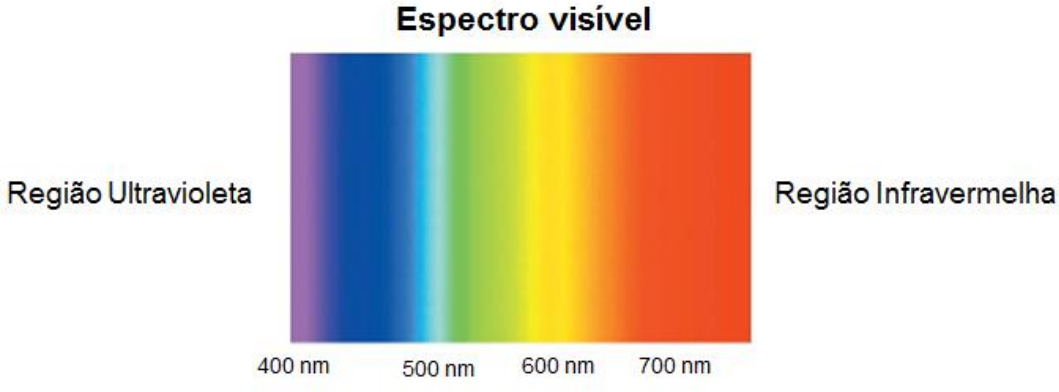
Fonte: Elaboração própria.

A terapia fotodinâmica antimicrobiana já vem sendo utilizada com sucesso em casos de micro-organismos resistentes a antibióticos ou em associação com os medicamentos existentes para aumentar sua eficácia[28,29]. Neste contexto, uma das vantagens da TFDa em relação à antibioticoterapia, é que até o momento não há relatos de desenvolvimento de resistência microbiana a essa modalidade de tratamento, uma vez que o seu mecanismo de ação é baseado através da produção de oxigênio singleto, causando a eliminação destes micro-organismos em um curto espaço de tempo[4,18].

A natureza dos agentes fotossensibilizadores utilizados em TFDa é muito vasta, podendo existir compostos de origem sintética ou natural[30-35]. Apesar da maior estabilidade presente pelos compostos sintéticos, compostos naturais têm sido amplamente estudados e aceitos, principalmente porque eles são menos propensos a efeitos colaterais e interações medicamentosas[31,33,36-39].

Para que um composto seja considerado um fotossensibilizador, além da absorção dentro do espectro de luz visível incidente, é necessário que ele apresente outras características (Figura 3). Idealmente, o fotossensibilizador deve apresentar baixa toxicidade no escuro, causando dano celular apenas quando irradiado, permanecer no estado excitado tempo suficiente para sua interação e seletividade com as células alvo, alto rendimento quântico, baixo tempo de meia vida para rápida eliminação dos tecidos, composição química bem definida, não causar mutagenicidade ou carcinogenicidade e ser cineticamente e termodinamicamente estável para conferir um tempo de prateleira adequado[40].

Figura 3 - Visualização do espectro de luz visível



Fonte: Adaptado de Soukos e Godson[41] (2011).

Entretanto, ainda não há disponível um FS que reúna todas as características ideais. Portanto, nos últimos anos, houve um aumento no interesse de compostos naturais para aplicação como fotossensibilizadores em TFD[42]. Marrelli[43] e colaboradores realizaram uma revisão da literatura observando diferentes compostos à base de plantas utilizados em TFD para tratamento de câncer de pele com resultados promissores.

Um fator que contribui para utilização de compostos naturais em TFD é a facilidade com que essas substâncias conseguem aderir ou atravessar a membrana citoplasmática e o seu potencial em produzir espécies reativas de oxigênio[44]. Outra vantagem de se utilizar compostos naturais, é que, embora o isolamento desses compostos não seja fácil, a quantidade de matéria prima disponível para ser estudada é enorme, em especial no Brasil, onde há uma rica flora avaliada como fonte incalculável de riquezas naturais e que atrai pesquisadores de todo o mundo[45].

Desse modo, o estudo de materiais vegetais pode ser uma fonte interessante para a descoberta de agentes fotossensibilizantes para serem utilizados em TFD. Portanto, esse trabalho propõe estudar o potencial *in vitro* de óleos essenciais e extratos vegetais para serem utilizados em terapia fotodinâmica antimicrobiana sobre suspensões e biofilmes de micro-organismos de interesse médico-odontológico.

2 PROPOSIÇÃO

O objetivo deste trabalho foi:

1) Realizar uma revisão sistemática da literatura em diversas bases de dados, avaliando em estudos *in vitro* e *in vivo* a eficácia da terapia fotodinâmica contra microrganismos associados à etiologia da cárie;

2) Avaliar o potencial *in vitro* dos óleos essenciais de *Coffea arabica, Matricaria recutita* e *Eugenia uniflora* e dos extratos vegetais de *Senna splendida*, *Senna reticulata* e *Senna macranthera* para serem utilizados como monoterapia na terapia fotodinâmica sobre suspensões de *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* e *Cutibacterium acnes*.

3 PUBLICAÇÕES

3.1 Publicação 1

de Oliveira AB, Ferrisse TM, Marques RS, de Annunzio SR, Brighenti FL, Fontana CR. Effect of Photodynamic therapy on microorganisms responsible for dental caries: a systematic review and meta-analysis. Int J Mol Sci. 2019; 20(14).

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Review

Effect of Photodynamic Therapy on Microorganisms Responsible for Dental Caries: A Systematic Review and Meta-Analysis

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Abstract: The aim of this study was to perform a systematic review of the literature followed by a meta-analysis about the efficacy of photodynamic therapy (PDT) on the microorganisms responsible for dental caries. The research question and the keywords were constructed according to the PICO strategy. The article search was done in Embase, Lilacs, Scielo, Medline, Scopus, Cochrane Library, Web of Science, Science Direct, and Pubmed databases. Randomized clinical trials and in vitro studies were selected in the review. The study was conducted according the PRISMA guideline for systematic review. A total of 34 articles were included in the qualitative analysis and four articles were divided into two subgroups to perform the meta-analysis. Few studies have achieved an effective microbial reduction in microorganisms associated with the pathogenesis of dental caries. The results highlight that there is no consensus about the study protocols for PDT against cariogenic microorganisms, although the results showed the PDT could be a good alternative for the treatment of dental caries.

Keywords: dental caries; photodynamic therapy; antimicrobial; microorganism; systematic and meta-analysis review

1. Introduction

Dental caries is a hard dental tissue disease resulting from a chronic process that arises with the presence and interaction of factors such as microorganisms, diet, and host [1]. The most important factors for dental caries development is the interaction between a high sugar diet and specific oral bacteria within the oral biofilm. These bacteria produce acid through the fermentation of carbohydrates consumed by the host, which causes a sustained decrease in the oral cavity pH. Consequently, the enamel pH also reduces, causing its mineral dissolution [2]. If not properly treated, it may result in consequences for dental elements as well as chewing, talking, smiling, and on a patient's life quality [3].

There are several available treatments for dental biofilm removal. These treatments include mechanical biofilm removal, antiseptics, and the use of chemoprophylactic agents [4]. However, the search for therapies that inhibit biofilm formation has led to significant research efforts to discover new treatments [5]. Photodynamic therapy (PDT) is as an effective tool in the treatment of various diseases [6] and a promising adjunctive treatment for dentin infection [7].

The PDT consists of a photosensitive molecule that absorbs an adequate wavelength light. This light-excited molecule, the photosensitizer (PS), can induce two reactions that may happen simultaneously (Type I and II reactions). In Type I reactions, the excited triplet PS reacts with biomolecules such as nucleic acids, lipids, and proteins by transferring an electric charge that produces

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Table 1. Summary of the characteristics of the included studies.

	Study	Year	Study Design	Level of Evidence *	Sample Size	Irradiation Time **	Photosensitizer	Biofilm Inhibition	Wave-Length	Microorganism	Control Group	Biofilm Reduction (Log CFU/mL)
#1	Zanin et al. [12]	2006	In vitro	III	3	5 min	Toluidine blue	N/A	660 nm	*Streptococcus mutans*	Negative	<3
#2	Muller et al. [13]	2007	In vitro	III	9	1 min	Methylene blue	N/A	665 nm	Multispecies biofilm	Negative and chlorexidine digluconate 2%	<1
#3	Lutti Martin et al. [14]	2009	In vitro	III	N/A	1 min, 5 min, 15 min and 30 min	Fosfolipos and Hypericina	N/A	400 nm–505 nm	*Streptococcus mutans* and *Streptococcus subrinus*	Negative	3 (*S. subrinus*) and <3 (*S. mutans*)
#4	Mang et al. [15]	2012	In vitro	III	N/A	5 min	Porfimer sodium	N/A	630 nm	*Streptococcus mutans*	Negative	N/A
#5	Rolim et al. [16]	2012	In vitro	III	10	5 min	Methylene blue, Toluidine blue, Ortho and Malachite green	N/A	N/A	*Streptococcus mutans*	Negative	3
#6	Fekrazad et al. [17]	2013	In vitro	III	N/A	5 min	Toluidine blue, Radachlorine and Indocyanine green	N/A	660 mm and 810 nm	*Streptococcus mutans*	Negative	<3
#7	Spinei et al. [18]	2013	In vitro	III	N/A	N/A	Antocianine extract and methylene blue	N/A	625 nm–635 nm	*Streptococcus mutans, mitis, gordoni* and *sobrinus*	Negative	4.1
#8	Araujo et al. [19]	2014	In vitro	III	N/A	5 min	Curcumin	N/A	420 nm	*Streptococcus mutans* and *Lactobacillus acidophillus*	Negative	<1
#9	Manoil et al. [20]	2014	In vitro	III	12	5 min and 10 min	Curcumin	N/A	360 nm–550 nm	*Streptococcus mutans*	Negative	2
#10	Diniz et al. [21]	2015	In vitro	III	12	5 min	Methylene blue	N/A	660 nm	*Streptococcus mutans*	Negative	1.01
#11	Melo et al. [22]	2015	RCT	I	45	5 min	Toluidine blue	N/A	660 nm	Multispecies biofilm	Negative	<3

Table 1. *Cont.*

	Study	Year	Study Design	Level of Evidence *	Sample Size	Irradiation Time **	Photosensitizer	Biofilm Inhibition	Wave-Length	Microorganism	Control Group	Biofilm Reduction (Log CFU/mL)
#12	Soria-Lozano et al. [23]	2015	In vitro	III	N/A	1 min/ 1 h/3 h	Methylene blue, Rose Bengal, and Curcumin	N/A	N/A	*Streptococcus mutans*, *Streptococcus sanguinis* and *Candida albicans*	Negative	6.0 (*Streptococcus* spp), 5.0 (*C.albicans*)
#13	Cintia Lima et al. [24]	2017	In vitro	III	N/A	10 min	Methylene blue	N/A	660 nm	*Streptococcus mutans*	Negative	>3
#14	Fekrazad et al. [25]	2017	RCT	I	22	1 min	Toluidine blue	N/A	630 nm	*Streptococcus mutans*	Negative	0.68
#15	Hyung-Jung et al. [26]	2017	In vitro	III	N/A	N/A	Curcumin and Curcuma xanthorrhiza extract	N/A	405 nm	*Streptococcus mutans*	Negative	>3
#16	Leili Beytollahi [27]	2017	In vitro	III	N/A	5 min	Methylene blue and Green Indocyanine	Yes	635 nm	*Streptococcus mutans*	Negative	<3
#17	Nemezio et al. [28]	2017	In vitro	III	4	5 min	Methylene blue	N/A	660 nm	*Streptococcus mutans*	NaCL solution 0.9% and chlorhexidine digluconate 0.12%	1
#18	Péres-Laguna et al. [29]	2017	In vitro	III	N/A	N/A	Methylene blue and Rose Bengal	N/A	N/A	*Streptococcus mutans*and *sanguinis*	Negative	6
#19	Azizi et al. [30]	2018	In vitro	III	6	5 min	Indocyanine green and Methylene blue	N/A	660 nm and 808 nm	*Lactobacillus acidophillus*	Chlorexidine digluconate 0.2%, NaOCL2.5% and Penicilin 6.3.3	N/A

Table 1. *Cont.*

	Study	Year	Study Design	Level of Evidence *	Sample Size	Irradiation Time **	Photosensitizer	Biofilm Inhibition	Wave-Length	Microorganism	Control Group	Biofilm Reduction (Log CFU/mL)
#20	Darmani et al. [31]	2018	In vitro	III	N/A	5 min	Toluidine Blue	N/A	670 nm	*Streptococcus mutan*, *Streptococcus salivar*, *Lactobacillus casei* and *Actinomyces viscosus*	Negative	<1
#21	Esteban Florez et al. [32]	2018	In vitro	III	15	5 min	Methylene blue	N/A	660 nm	*Streptococcus mutans*	Negative and chlorexidine digluconate 2%	1,3
#22	Fumes et al. [33]	2018	In vitro	III	3	1 min, 2 min, and 5 min	Methylene blue	N/A	N/A	*Streptococcus mutans* and *Candida albicans*	Negative and chlorexidine digluconate 0.12%	<3
#23	Garcia et al. [34]	2018	In vitro	III	10	N/A	Fotoencitine and Photoditazine	N/A	660 nm	*Streptococcus mutans*	Negative and Methylene Blue	Complete eradication (Fotoencitine) and 6 (Photoditazine)
#24	Gholibegloo et al. [35]	2018	In vitro	III	3	5 min	Indocyanine green	Yes	N/A	*Streptococcus mutans*	Negative	<1
#25	Gomez et al. [36]	2018	RCT	I	10	3 min	Methylene blue	N/A	670 nm	*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia* and *Tannerella forsythia*	US technique	N/A
#26	Míndez et al. [37]	2018	In vitro	III	9	2 min	Curcumin	N/A	455 nm	*Streptococcus mutans*	Negative	<3
#27	Oliveira et al. [38]	2018	In vitro	III	6	2 min	Methylene Blue	N/A	630 nm	Multispecies biofilm from saliva	Negative	<3

Table 1. *Cont.*

	Study	Year	Study Design	Level of Evidence *	Sample Size	Irradiation Time **	Photosensitizer	Biofilm Inhibition	Wave-Length	Microorganism	Control Group	Biofilm Reduction (Log CFU/mL)
#28	Tokubo et al. [39]	2018	In vitro	III	3	5 min	Erythrosine and Methylene blue	N/A	N/A	*Streptococcus mutans*	Negative and chlorexidine digluconate 0.12%	4.3
#29	Trigo-Gutierrez et al. [40]	2018	In vitro	III	N/A	30 min	Cloroaluminium phthalocyanine nanoemulsion	N/A	N/A	*Candida albicans*, *Candida glabrata* and *Streptococcus mutans*	Negative	<3
#30	Alexandrino et al. [41]	2019	In vitro	III	N/A	N/A	Rose Bengal and Rose Bengal encapsulated with cyclodextrin	Yes	520 nm	*Streptococcus mutans*	NaCL solution 0.9% and chlorhexidine digluconate 0.12%	Complete eradication
#31	Alves et al. [42]	2019	RCT	I	20	5 min	Methylene blue	N/A	660 nm	*Streptococcus mutans*	Negative	2.8
#32	Esper et al. [43]	2019	In vitro	III	10	5 min	Hematoporfirine	N/A	420 nm and 480 nm	*Streptococcus mutans*	Negative	<1 (biofilm) and 3.8 and 6.78 (planktonic)
#33	Lamarke et al. [44]	2019	In vitro	III	4	2 min	Curcumin	N/A	420 nm	Multispecies biofilm	Negative and chlorexidine digluconate 0.12%	1.32
#34	Pourbajibagher et al. [45]	2019	In vitro	III	10	5 min	Cationic doped zinc oxide nanoparticle adhesive	Yes	435 nm	*Streptococcus mutans*	Negative	1.96

N/A: not available; min: minutes; h: hours; Negative: no treatment applied; * Level of evidence according to the Oxford Centre for Evidence-Based Medicine; ** Pre-irradiation time; RCT: randomized clinical trial.

2.2. Synthesis of Results

The systematic review showed that among the cariogenic microorganisms listed in the selected studies, the most studied microorganism was *Streptococcus mutans* (82%) [12,14–21,23–29,31–35,37,39–43,45].

The success of PDT depends on factors such as the administrated dose of light in the target cells and the time of exposure to light [46]. Considering these factors, we found that the most widely used light source was the red LED (32%) with wavelengths ranging from 625 to 670 nm [12,15–18,21,24,29,30,32,36,40], the most commonly used PS was methylene blue [13,16,18,21,23,24,27–30,32,33,36,38,39,42], and the most widely used pre-irradiation time was 5 min [12,14–17,19–22,27,28,30–33,39,42,43,45].

Regarding the ability to reduce the number of viable bacteria, most articles showed less than three logs of reduction [13,17,20–22,25,27–29,31,32,35,37,38,40,42,44,45]. The most commonly used control group was a negative control with no intervention. In addition, few studies have reported whether the biofilm inhibitory capacity of this treatment modality was tested [27,35,41,45].

2.3. Level of Evidence

According to the level of evidence (LoE) based on guidelines of the Oxford University Center for Evidence-Based Medicine [47], we noticed only four articles with level of evidence 1 and 28 articles with level of evidence 3. This difference between levels can be explained by different types of study and show the knowledge curve regarding photodynamic therapy on microorganisms associated with the pathogenesis of dental caries. Thus, there is a need to perform more randomized clinical studies in animals models and humans to increase the quality of scientific information.

2.4. Meta-Analysis

Only four studies were included in this analysis. Two of them among the in vitro studies [34,48] and more two studies related to randomized clinical studies [25,42]. One in vitro study was excluded due to a high standard deviation that was close to the mean of the data, which would possibly provide a non-parametric distribution of the data directly affecting the heterogeneity of the meta-analysis [45]. Furthermore, one other randomized clinical study was excluded due to the absence of the mean of the negative control [36]. Figure 2 illustrates the details about the statistical performance. Figure 2A shows the observed meta-analysis to the in vitro studies. The data showed a significant statistical difference to the experimental group that was formed by cariogenic microorganisms that received photodynamic therapy. The study of Lamarke et al. (2019) [44] presented more weight for analysis due to the larger sample size and lower standard deviation between the groups. Figure 2B shows the meta-analysis to the randomized clinical studies. Although there was a significant statistical difference to the experimental group, the heterogeneity among studies was $I^2 = 70\%$, which was considered too high to rely on the result of the statistical analysis. It is more likely that the heterogeneity found was due to the nature of the phenomenon evaluated for the type of study.

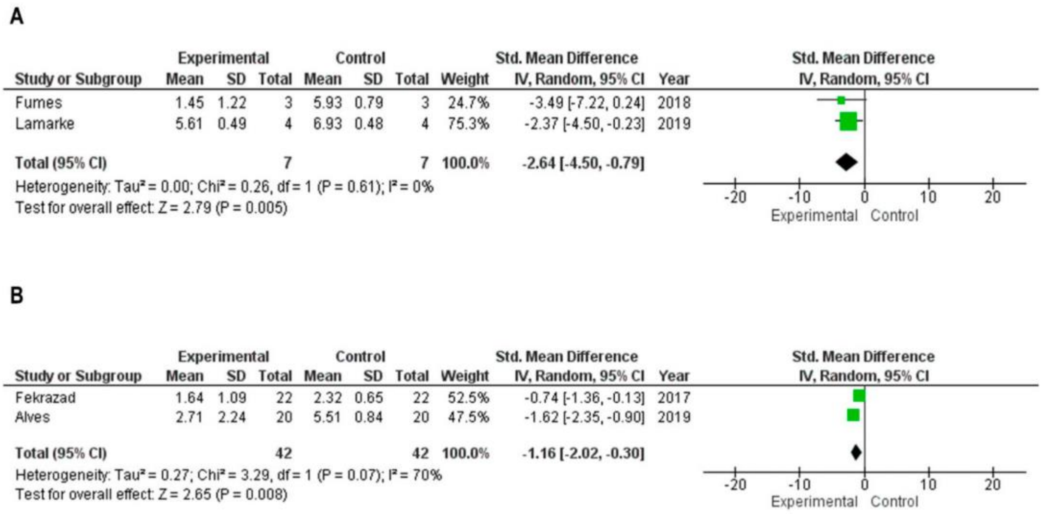

Figure 2. Results of the meta-analyses. The experimental group was formed based on colony forming units (CFU/mL) in the microorganisms that received photodynamic therapy (PDT). The control group was formed based on colony forming units (CFU/mL) in microorganisms that did not receive photodynamic therapy. (**A**) Meta-analysis in the in vitro study design. (**B**) Meta-analysis in the randomized clinical trials.

3. Discussion

Dental caries is a multifactorial disease that slowly progresses in most individuals. In the absence of treatment, it can progress to oral pain and tooth loss [1].

Dental biofilm is one of the main local etiological factors of dental caries, and its mechanical removal through brushing with dentifrice associated with diet sugar reduction, is a method of control and prevention of the disease [49]. However, according to Valkenburg et al. (2016) [48], the efficacy of this method depends on the individual's ability. Therefore, in some cases, as in special needs patients, this method needs complementary approaches. For this reason, the dental biofilm chemical control has been highly indicated. Chlorhexidine is known for its clinical and microbiological efficacy against various microorganisms present in the oral cavity [50]. However, its use has been questioned due to the adverse effects presented during its prolonged use [51].

Several studies have already demonstrated the susceptibility of cariogenic bacteria to photodynamic therapy [39,52,53], suggesting that this therapy may be useful as a minimally invasive adjuvant therapy for the control of dental caries [54] through cariogenic bacteria inactivation [55].

However, this therapy presents different challenges on the susceptibility of different microorganisms [56]. Most of the photosensitizers used in PDT are significantly more effective in inactivating Gram-positive bacteria than Gram-negative bacteria [57], which favors their use against dental caries microorganisms, since these caries lesions typically present the prevalence of Gram-positive strains [58].

For PDT to be successful, many variables should be considered such as the PSs used and the light dosimetry [59,60].

Among the evaluated articles, the most widely used photosensitizer (PS) was methylene blue (MB). This molecule belongs to the class of phenothiazine and presents solubility in water and ethanol. This PS efficiency in PDT is related to its intense absorption in the UV-visible region, whose maximum absorption wavelength is 664 nm, within the spectral region of 600 to 1000 nm (phototherapeutic window). It allows for the deep penetration of light in the biological tissues and expressive quantum yield for singlet oxygen formation [61,62]. The literature has already established the action of PDT mediated by MB, presenting its action against several bacteria associated with oral diseases [63,64].

MB has characteristics that promote good interaction with bacteria such as the positive charge on the molecule and low molecular mass. MB has action in both Gram-positive and Gram-negative bacteria, however, Gram-positive bacteria are more efficiently inactivated, due to the fact that the transport of positively charged molecules into the cell is facilitated. These bacteria have teichoic acids that give a negative charge to the outer surface [65], thus making this PS suitable for the inactivation of cariogenic microorganisms.

Aside from MB, in the reviewed articles, the phenothiazine dye toluidine blue was the most widely used PS, followed by curcumin (a natural compound), rose Bengal, and green indocyanine, respectively. The data suggest that phenothiazine dyes have been the most investigated to date. Thus, these photosensitive agents might be promising for the adjuvant treatment of dental caries. However, more clinical studies with these PS should be developed to confirm this result.

Considering the pre-irradiation time [66], which is the period where the PS will remain in contact with the samples and may bind to the plasma membrane and/or internalize the target cells prior to light treatment, different times were evaluated. Andrade et al. (2013) [67] verified that in planktonic cultures of *Candida spp.* the photodynamic action was not dependent on the pre-irradiation time. However, for biofilms, a longer pre-irradiation time was required for the internalization of curcumin in the samples. In this review, the pre-irradiation time of the studies ranged from 1 to 30 min for different photosensitizers. Among the 34 articles, two did not report the time used, although this parameter is considered an essential information to determine clinical protocols in PDT. Fumes et al. (2018) [33] verified that 1-min pre-irradiation of the MB PS was able to reduce *S. mutans* in biofilm, and presented no statistical difference in the microbial load reduction when compared with superior times (2 and 5 min). In this same study, the authors reported the challenge of keeping a child with their mouth open for 5 min in a pilot clinical study, demonstrating the need to evaluate shorter times. Thus, studies evaluating shorter pre-irradiation times are desirable because they may develop clinical protocols that minimize patient discomfort.

Regarding the antimicrobial effect of PDT, there are several microbiological techniques that determine whether a substance can be considered bactericidal or potentially bactericidal. This determination can be influenced by factors such as microorganism growth conditions, bacterial density, test duration, and number of bacteria reduction. For a substance to be considered as a bactericide, it is necessary for a total inhibition of microorganism growth or ≤99.9% decrease in the initial inoculum (3-log 10 reduction in colony forming units [cfu]/mL) in the subculture [68]. From the 34 articles analyzed, only 11 presented a reduction greater than or equal to 99.9% [14,16,18,23,24,29,34,39,41,43,45]. This fact proves that eliminating these microorganisms is a great challenge, especially when they are in the biofilm.

The microorganisms present a great impact on public health, especially when in biofilm form, because they present a greater resistance to antibacterial agents and disinfection methods when compared to microorganisms in planktonic form [69]. Inhibition of biofilm formation may be relevant in cariogenicity reduction and in preventing the onset of new lesions [27,70].

Extracellular polysaccharides are the main constituents of cariogenic biofilms matrix, and are directly related with the virulence in biofilms [71]. Moreover, Zhao et al. (2013) [72] showed that these glue-like substances promoted the development of biofilm by conditioning the surface of the substrate. This indicates that the inhibition of the growth of the microorganisms is not the only strategy in reducing the development of dental caries. The influence on the expression of genes responsible for the polysaccharide synthesis and the reduction of this synthesis seem to be reasonable paths for further investigation [73].

Despite the notorious influence of polysaccharides on biofilm virulence, only three studies have evaluated it. Zanin et al. (2006) [12] analyzed the insoluble polysaccharide concentration in biofilms treated with the association of toluidine blue as a photosensitizer and a light-emission diode laser of 638.8 nm as the light source. The biofilms were evaluated at different times and it was concluded that in older biofilms, the concentration of insoluble polysaccharides was higher, indicating that despite the treatment, the age of the biofilm had an influence on the biofilm cariogenicity.

Gholibegloo et al. (2018) [35] evaluated the PDT influence on *gtfB* gene expression and concluded that there was a significant difference in the reduction of gene expression between the irradiated and non-irradiated groups, pointing to PDT as a potential treatment to prevent the formation of cariogenic biofilms. Nemezio et al. (2017) [28] concluded that PDT reduced the insoluble extracellular polysaccharide and intracellular polysaccharide concentration by nearly three- and four-fold, respectively, when compared to the control. Moreover, this effect resembled that of chlorhexidine. However, due to the lack of studies evaluating polysaccharides produced by biofilms, more studies are needed to prove the efficacy of photodynamic therapy in controlling the virulence of cariogenic biofilms.

Despite the time and number of studies involving PDT, few articles in this review had used in vivo models. In vitro studies have great importance for the initial analyses of treatments, however, when dealing with dental caries, it is important to emphasize that the oral cavity is composed of more than 700 microorganism species [74] and some of these species can be lost when in vitro biofilm models are used to mimic the oral environment. This limitation may be important to encourage new studies using in vivo models.

Among the vitro studies, the biofilm models were more frequent [12,13,15,18–21,24,27,28,31–35,37–40,43–45] than the suspensions, which confirm the fidelity of the data, since in biofilms, the microorganisms interact with each other and are more resistant to the antimicrobial agents when compared to the microorganisms in suspension [75]. Many studies have used monotypic biofilms of *S. mutans*, but this model is less representative of the oral environment and underestimates the complexity of the dental biofilm [76], so we emphasize the importance of studies with multispecies biofilms.

Limited clinical information remains on the use of PDT against cariogenic microorganisms. The appropriate parameters of energy dose, photosensitizer concentration, pre-irradiation time, and exposure should be developed through additional studies.

4. Materials and Methods

4.1. Eligibility Criteria

The systematic review was undertaken following the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) guidelines [77]. The "PICO" strategy for systematic exploratory review guided the research question development [78]. This study aimed to answer the following question: Is photodynamic therapy effective against cariogenic microorganisms? The PICO strategy was: P (cariogenic microorganisms), I (photodynamic therapy), C (non-photodynamic therapy applied), and O (microbial reduction).

The inclusion criteria for our systematic review were: (i) All types of study design (in vitro, in situ, in vivo, randomized clinical trial, case cohort, and case control); (ii) Studies involving cariogenic biofilm models; (iii) Articles that evaluated the influence of photodynamic therapy on cariogenic microorganisms; and (iv) Articles published in English.

In this systematic review, the following study designs were not included: (i) Review articles, letters to the editor, personal opinions, book chapters, or conference abstracts; (ii) Studies that did not present a control group; (iii) Non-English language articles; and (iv) Articles where the full text was not freely available.

4.2. Search Strategy

Three independent examiners (ABO, RSM, and SRA) conducted an electronic search in the PubMed, Embase, SCOPUS, Lilacs, Science Direct, Web of Science, Medline, SCIELO, and Chochrane Library databases for articles published between December 1989 and March 2019.

The following search terms and combinations were used: (((Photochemotherapy OR Photodynamic Therapy)) AND (Streptococcus mutans OR Caries OR Carious Dentin OR Caries disease)) AND Cariogenic Biofilm.

Based on the titles and abstracts of the studies, the three independent researchers selected the articles. The Mendeley Reference Manager Software® was used to delete duplicate articles.

4.3. Data Extraction and Analysis

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement was followed during the data assessment and extraction [77]. The following data were extracted from the studies: (a) type of study; (b) sample size; (c) time of pre-incubation of the photosensitizer; (d) photosensitizer; (e) ability to inhibit biofilm; (f) wavelength; (g) microorganism; (h) group control; and (i) reduction capacity. The Level of Evidence (LoE) for each study was determined according to the guidelines of the Oxford University Center for Evidence-Based Medicine [47].

4.4. Statistical Analysis

A meta-analysis was conducted using Review Manager 5.2 (Cochrane Collaboration). The effect size utilized was the standardized mean difference and the statistical analysis was performed using the random effect model. Two meta-analyses were realized due to the different types of study (randomized clinical studies and in vitro study). The I^2 test evaluated the heterogeneity among the studies. A level of significance of 95% and level of reliability of 95% were chosen to perform the statistical analysis.

5. Conclusions

To date, photodynamic therapy has been suggested as a potential adjuvant to maximize the oral disinfection of microorganisms responsible for dental caries. However, additional studies are needed to determine the appropriate parameters for using this therapy as well as randomized and controlled clinical trials to verify the in vitro results in the in vivo models.

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Abbreviations

PDT	Photodynamic Therapy
PS	Photosensitizer
ROS	Reactive Oxygen Species
ACS	Antioxidant Carrier Sensitizers
LoE	Level of Evidence
CFU	Colony Forming Units
MB	Methylene Blue

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3.2 Publicação 2*

Bioprospecting of natural products in photodynamic therapy against microorganisms of medical and dental interest.

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ABSTRACT

Nowadays, the bacterial resistance caused by the indiscriminate use of antibiotics is one of the greatest problems worldwide. Thus, researches aiming to study new treatments for infections should be motived. In this context, antimicrobial photodynamic therapy (aPDT) was considered an effective and promising alternative therapy. The objective of this study was to evaluate the *in vitro* potential of essential oils and plant extracts to be used in photodynamic therapy as monotherapy against microorganisms of medical and dental interest. The following reference strains were used: *Cutibacterium acnes* ATCC 6919, *Streptococcus mutans* ATCC 35688, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 90028. Three essential oils (*Coffea arabica*, *Eugenia uniflora* and *Matricaria recutita*) and three plant extracts (*Senna splendida*, *Senna reticulata* and *Senna macranthera*) were included in the study. The plant materials were tested at 50µg/mL (extracts) or 2% (essential oils). Five groups were studied: negative control, plant material, photodynamic therapy (light + plant material), vehicle control without light exposure and vehicle control with light exposure. The effectiveness of therapy was assessed by counting viable cells after treatment. The production of reactive oxygen species was evaluated in plant materials that showed the best antimicrobial activity. For this test, 3′-p- (aminophenyl) fluorescent probes (APF; mainly detects the radical hydroxyl [• OH]) and the Singlet Oxygen Sensor (SOSG; detects singlet oxygen [O_2]) were used. The data were analyzed using the IBM SPSS version 20.0 program, with a significance level of 5%. For *C. acnes* and *S. mutans*, total microbial reductions were observed in 2 of the 6 plant compounds tested, whereas, in addition, for *S. mutans*, a microbial reduction of 3.19 CFU / mL logs was observed for another natural compound. For *S. aureus* and *C. albicans* obtain total microbial reduction in 4 of the 6 compounds tested. Finally, a total reduction of only 1 out of 6 natural compounds tested for *E. coli* was observed. The plant materials of *S. macranthera* and *S. reticulata* were the ones that produced the most reactive oxygen species, followed by *S. splendida*, *E. uniflora* and *M. recutita*. The results of the present study showed that antimicrobial photodynamic therapy (aPDT) mediated by different natural compounds was efficient in microbial suspension of microorganisms of medical and dental interest.

1. INTRODUCTION

The control and treatment of diseases caused by pathogenic microorganisms became a great challenge in medical sciences since 1990, as a consequence of the development of multi-drug resistance bacteria. Additionally, bacterial resistance increases in a rate higher than the industry is able to produce new drugs. These findings results in an increased morbidity from infections that were easily treated in the past (Liu et al., 2015).

Recent studies indicate that by the year 2050, increased bacterial resistance could cause over 300 million deaths worldwide (O'Neill, 2016). Therefore, it is of great relevance the development of unconventional treatments that fight against these microorganisms without selecting resistant strains.

The genera *Streptococcus, Staphylococcus, Enterococcus, Escherichia, Corynebacterium* and *Candida* are among the main etiological agents of medical and dental diseases. These microorganisms can cause injuries in skin, oral cavity and vaginal mucosa. Additionally, these microorganisms might be related to at least one of the following conditions: invasive infections in hospital patients, dental caries, aggressive periodontitis, endodontic lesions, gastro-urinary infections, and eventually sepsis and endocarditis (Araújo Navas et al., 2012; Ceci et al., 2015; Gangcuangco et al., 2015; Machado et al., 2011; Nobile and Johnson, 2015; Tong et al., 2015; Sanz et al., 2017).

Photodynamic therapy (PDT) combines photophysical and photochemical mechanisms that generates a biological response through the use of specific light sources and an oxygen-associated photosensitizing agent. Interestingly, when PDT is applied in treatment of microorganism, there is no resistance induction (Chilakamarthi et al., 2017).

The use of light as a therapeutic conduct has been performed since ancient times in ancient Greece, Egypt and India. However, only in the last century the term "photodynamic action" was first described by the German pharmacist Hermann von Tappeiner (Ackroyd et al., 2001; Rkein and Ozog 2014).

The photochemical processes of PDT start after photon absorption by the photosensitizing agent, causing the transition from ground to excited state. In its ground state (S0) the photosensitizing molecule appears as singlet state. As a result of light absorption, the molecule changes to its excited short-lived singlet state (S1). At this time, this molecule in S1 may return to ground state, emitting fluorescence, or transition to the longer life triplet state through intersystem crossing (Scherer et al., 2017).

In the excited triplet state, the photosensitizing agent reacts with biological molecules through two main oxygen-dependent mechanisms, called type I reaction and type II reaction

(Machado, 2000; Ribeiro et al., 2011). In type I reaction, the photosensitizing agent reacts with biomolecules such as lipids, proteins and nucleic acids, transferring hydrogen atoms to give rise to radical ions. Reaction with molecular oxygen results in the production of reactive oxygen species such as superoxide anion, hydroxyl radicals and hydrogen peroxide. In the type II reaction, the photosensitizing agent transfers energy directly to oxygen in its triplet funnel state, a phenomenon called triplet-triplet annihilation. There is the formation of highly reactive and cytotoxic singlet oxygen that can react with a large number of biological substrates and induce oxidative damage to the cell membrane and cell wall (Machado, 2000; Konopka and Goslinski, 2007; Ribeiro et al., 2011).

Singlet oxygen is short-lived in biological systems and causes a localized response without affecting distant cells or organs (Wilson, 2004). Thus, the PDT therapy can be indicated in many medical and dental modalities (Hamblin and Hasan, 2004; Konopka and Goslinski, 2007).

The nature of photosensitizing agents used in PDT is very wide and there are compounds of synthetic or natural origin. In recent years there has been a growth in the number of publications using natural products as a source of photosensitizing agents (Gasparetto et al., 2010; Andreazza et al., 2015; Agustí et al., 2015; Andreazza et al., 2016; Barroso et al., 2016; Santezi et al., 2016; Araújo et al., 2017; Marqués-Calvo et al., 2017). This fact can be explained due to the ability of these substances in adhering to or crossing the cytoplasmic membrane and their potential to produce reactive oxygen species (Nakamura et al., 2015).

Several studies shown that some photosensitizing agents have limitations, such as slow excretion of photosensitizer after use, prolonged sensitization of the patient, formation of aggregates that hinders light absorption by photosensitizer, low water solubility and different therapeutic window of photosensitizers. Thus, plant materials can be an interesting source for the discovery of photosensitizing agents that are safer and more efficient (Sun et al., 2016; Toratani et al., 2016; Chen et al., 2017). Thus, the aim of the present study was evaluated in vitro potential of different essential oils and plant extracts, as photosensitizer, for use in antimicrobial photodynamic therapy on suspensions of microorganisms of dental medical interest.

2. MATERIAL AND METHODS

2.1 Bacterial strains and culture conditions

The strains used in this study were *Cutibacterium acnes* (ATCC® 6919™), *Candida albicans* (ATCC® 90.028™), *Escherichia coli* (ATCC® 25.922™), *Staphylococcus aureus* (ATCC® 25.923™) and *Streptococcus mutans* (ATCC® 35.688™), obtained from the National Institute for Quality Control in Health (INCQS) of the Oswaldo Cruz Foundation (FIOCRUZ - Manguinhos, RJ, Brazil). Strain reactivation and microbial inoculum adjustment were performed according to the parameters described in table 1. The inoculum was adjusted by spectrophotometer (Biotek® ELx800 - Winooski, VT, USA) reading at 630 nm.

Table 1: Microbial Strains and Growth Conditions.

Strain	Culture médium (liquid / solid)	Incubation conditions	Inoculum
C. acnes	TSB enriched with haemine (5 μg/mL)/ RCM	Anaerobiosis at 37 ºC/ 24 h	10^5 CFU/mL
C. albicans	TSB / BHI	Aerobiosis at 37 ºC/ 48 h	10^6 CFU/mL
E. coli	TSB / TSA	Aerobiosis at 37 ºC/ 24 h	10^7 CFU/mL
S. mutans	BHI /BHI	Anaerobiosis at 37 ºC/ 48 h	10^7 CFU/mL
S. aureus	TSB / BHI	Aerobiosis at 37 ºC/ 24 h	10^8 CFU/mL

RCM: *Reinforced Clostridium Medium.* TSB: soy tryptone broth. TSA: soy tryptone agar. BHI: brain and heart infusion.

2.2 Plant materials

In the present study three commercial essential oils (*Coffea arabica*, *Eugenia uniflora* e *Matricaria recutita*) were purchased from Laszlo Aromaterapia Ltda. (Belo Horizonte-MG, Brazil) and three plant extracts (*Senna macranthera*, *Senna splendida* and *Senna reticulata*) were obtained from traditional plants of northeastern Brazil. Detailed information on the plant materials included in the present study is described in table 2.

For the production of *Senna macranthera*, *Senna splendida* and *Senna reticulata* extracts, the raw materials used were leaves, leaves and branches respectively. Plant material was collected in May 2016 in the state of Ceará, and the botanical certification was issued by Herbarium Prisco Bezerra-UFC, where exsiccates are deposited. To obtain the plant extracts, initially the plant materials were dehydrated at 40 °C for 36 h then crushed and finally extracted in an ultrasonic bath in a ratio of 100 mg of powder to 3 mL of methanol. Next, the extracts were filtered and concentrated in vacuum on a rotary evaporator (Bueno et al., 2015). The mass yield of the extracts was determined by the equation (yield = [final mass / initial mass] x100).

Table 2: General information about plant materials included in the present study

Family	Scientific name	Source	Voucher n.	Mass yield	Coordinates
Asteraceae	*Matricaria recutita*	Flowers; steam drag / Emporium Laszlo	N/A	N/A	N/A
Myrtaceae	*Eugenia uniflora* L.	Leaves; steam distillation / Emporium Laszlo	N/A	N/A	N/A
Rubiaceae	*Coffea arabica* L.	Seeds; pressing / Emporium Laszlo	N/A	N/A	N/A
Fabaceae	*Senna splendida* (Vogel) H.S.Irwin & Barneby	Leaves / NUBBE	60416	6.4%	S(0513974) W(4053621)
	Senna reticulata (Willd.) H.S.Irwin & Barneby	Branches / NUBBE	60420	16.3%	S(0403454) W(3901531)
	Senna macranthera (Collad.) H.S.Irwin & Barneby	Leaves / NUBBE	60422	9.75%	S(0258347) W(3953439)

NUBBE: Núcleo de Bioensaios, Biosíntese e Ecofisiologia de Produtos naturais, , UNESP - Institute of Chemistry, Araraquara. N/A: Not applicable. S: South; W: West.

Visible light absorption spectrum measurement was performed with a Synergy H1M microplate fluorescence reader (Synergy H1a Multi-Mode Reader, Biotek, Winooski, VT, USA).

The essential oils were diluted in Tween 80 at 1:1:8 ratio (essential oil: Tween 80: culture medium) and tested at 2% concentration. The plant extracts were diluted in dimethyl sulfoxide (DMSO; Labsynth, Brazil) (5 mg / mL) and then diluted in culture medium to give a final concentration of 50 µg/mL. All solutions of the plant materials were freshly prepared in a light protected environment.

The light sources consisted of 48 variable intensity LEDs mounted as a compact lighting system with a homogeneous lighting area and a cooling system to prevent overheating (IrradLED® - biopdi, São Carlos, SP, Brazil). All plant materials were light activated at 450 nm (151 mW / cm^2). The distance between the LEDs and the plate allowed for even distribution of light in each well, and light was provided below the plates.

2.3. Antimicrobial Photodynamic Therapy

The Antimicrobial Photodynamic Therapy (aPDT) was performed by broth microdilution using 96 well plates. Microbial suspensions were prepared as described in item 2.1 by optical densities assessment (λ = 630 nm). A volume of 50 µL of the prepared inoculum were transferred to each of the wells plates containing 50 µL of the plant materials, resulting in a final volume of 100 µL per well.

Five groups were studied: negative control, plant material, photodynamic therapy (light + plant material), vehicle control without light exposure and vehicle control with light exposure. Plant material and PDT groups were pre-incubated with plant materials for 5 min. Light and negative control group were pre-incubated in culture medium for 5 min. Irradiation was performed in groups light and PDT at 450 nm and 80 J/cm^2. The irradiation time was calculated from the light dose formula (J/cm^2) = I (W/cm^2) xt (sec), totaling 18 minutes of fractional mode irradiation. To avoid any interference during irradiation, each plate received only one type of treatment. Plant material and negative control groups were not irradiated and kept at room temperature for 18 min.

After the treatment, the suspensions were diluted and plated by the agar drop method (de Freitas et al., 2018) as described in Table 1. The number of cultured colonies was counted after 24 or 48 hours of incubation under the conditions described in Table 1.

2.4 Reactive oxygen species (ROS) detection

Cell-free system (solution)

For the detection test of reactive oxygen species we used the fluorescent probes 3′-p-(aminophenyl) fluorescein (APF; detects mainly hydroxyl radical [•OH]) and Singlet Oxygen Sensor Green (SOSG; detects singlet oxygen [O_2]) (Invitrogen, Thermo Fischer Scientific, Waltham, MA USA) and handled according to the manufacturer's manual.

The solutions containing the plant materials were prepared in phosphate buffer (0.1 M; pH 7.2) and added to the solutions of the 3 μM of APF or SOSG in wells of a black flat-bottom 96-well plate (Corning™) and irradiated from above to achieve the desired energy dose. Fluorescence readings were taken immediately after irradiation using the Synergy H1M (Synergy H1 Multi-Mode Reader, BioTek, Winooski, VT, USA). Excitation/emission wavelengths for APF were 490/515 nm, and for SOSG 505/525 nm.

2.5 Statistical Analysis

The sample size was obtained with Bioestat 5.0, after pilot study, with the following parameters: minimum difference between means (0.0186), standard deviation (0.0243), number of repetitions (5), power of analysis = 0.80 and α= 0.05, for each of the studied microorganisms (n = 10).

Data were analyzed using the IBM SPSS version 20.0 program. To analyze the results, a descriptive analysis of the data was performed followed by the verification of the normal distribution and homoscedasticity of the same ones, by the Shapiro-Wilk and Levene's tests, respectively. After verifying the above parameters, two-way analysis of variance (TWO-WAY ANOVA) was applied to each microorganism. For microbial viability, presence and absence of light and natural products were independent factors. Comparative analysis was performed by estimating means with a 95% confidence interval. For ROS detection, type of fluorescent probes and natural products were independent factors. In addition, a correlation study was also performed between the probe that showed the best results in ROS detection and the natural products that showed the highest CFU reduction. For that, the Pearson's correlation coefficient was calculated.

3 RESULTS

3.1. Visible light absorption spectrum

Supplementary data 1 shows the absorption spectrum graphs of plant materials from this study.

The three essential oils showed light absorption spectrum in the 450 nm range. In addition, *M. recutita* also showed light absorption spectrum in 640 nm region. The three plant extracts showed the absorption spectrum in the region of 400 nm, indicating a potential for application of this six plant materials in a TFD.

3.2 Evaluation of natural products in PDT against suspensions of microorganisms of medical and dental interest

Statistically significant differences were observed for the treatment with natural substances and exposure to light (p<0.0001) and for the interaction between natural substances and exposure to light (p<0.0001) for all microbial species studied (Supplementary data 2; Table 3).

Table 3. Summary of two-way ANOVA results for bacterial viability in microbial suspensions. The variables analyzed were: natural substances ("Natural") and exposure to light ("Light").

Source	df	SS	MS	F	p	Partial Eta-squared
C. albicans						
Natural	5	41.962	8.392	40454.530	<0.0001	0.999
Light	4	807.071	201.768	972589.111	<0.0001	1.000
Natural*Light	20	236.269	11.813	56944.944	<0.0001	1.000
C. acnes						
Natural	4	363.708	90.927	96920.627	<0.0001	0.999
Light	6	206.363	34.394	36660.921	<0.0001	0.999
Natural*Light	24	617.759	25.740	27436.665	<0.0001	1.000
E. coli						
Natural	5	83.123	16.625	67991.599	<0.0001	0.999
Light	4	128.007	32.002	130881.197	<0.0001	1.000
Natural*Light	20	443.141	22.157	90618.118	<0.0001	1.000
S. aureus						
Natural	5	53.926	10.785	33985.620	<0.0001	0.999
Light	4	1193.569	298.392	940281.907	<0.0001	0.999
Natural*Light	20	270.422	13.521	42607.197	<0.0001	1.000
S. mutans						
Natural	5	98.826	19.765	67209.937	<0.0001	0.999
Light	4	458.872	114.718	390090.696	<0.0001	1.000
Natural*Light	20	534.762	26.738	90921.034	<0.0001	1.000

df= degrees of freedom; SS= Sum of squares; MS= mean square; F= MS factor/ MS residual; p= probabil significance, α= 0.050; ; *interaction between variables analyses

For *C. acnes* a reduction of 1.30 log CFU / mL was observed after PDT with *S. macranthera*. Total bacterial reduction (7.98 log CFU / mL) was achieved after PDT with *S. splendida* and PDT with *S. reticulate*. For *S. aureus*, a reduction of 1.06 log CFU / mL and 2.24 log CFU / mL were observed after PDT with *C. arabica* or *S. reticulata*. Total bacterial reduction (6.92 log CFU / mL) was achieved after PDT with *E. uniflora*, *S. macranthera* or *S. splendida*. For *S. mutans*, a bacterial reduction of 3.19 log CFU / mL was observed after PDT with *S. macranthera*. Total bacterial reduction (8.02 log CFU / mL) was achieved after PDT with *S. splendida* or *S. reticulata* (Figure 1).

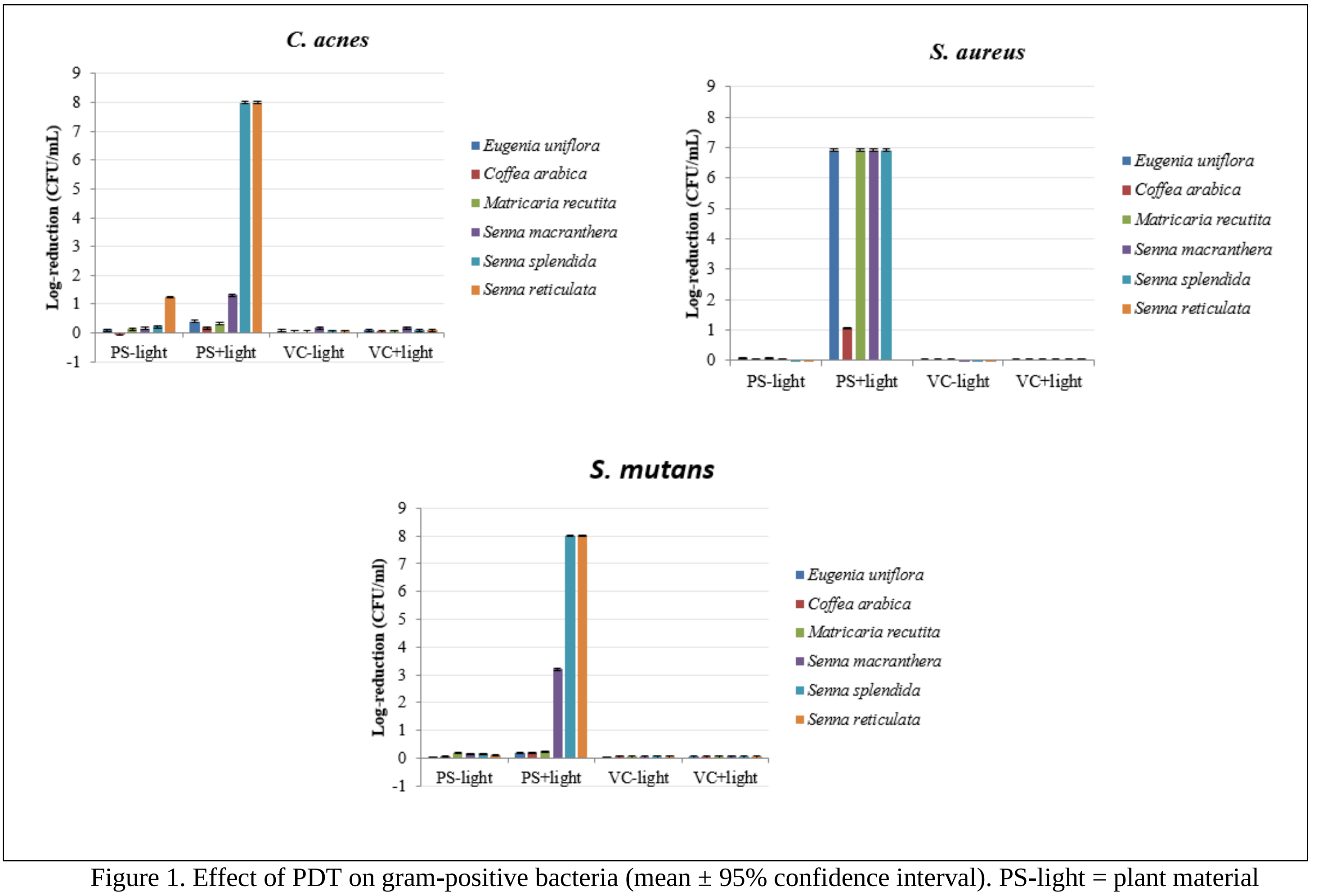

Figure 1. Effect of PDT on gram-positive bacteria (mean ± 95% confidence interval). PS-light = plant material without exposure to light; PS+light= plant material with exposure to light; VC-light= vehicle control without exposure to light; VC+light= vehicle control with exposure to light.

For *E. coli* a reduction of 1.08 log CFU/mL was observed after PDT with *E. uniflora*. Total bacterial reduction (8.90 log CFU/mL) was achieved after PDT with *M. recutita* (Figure 2).

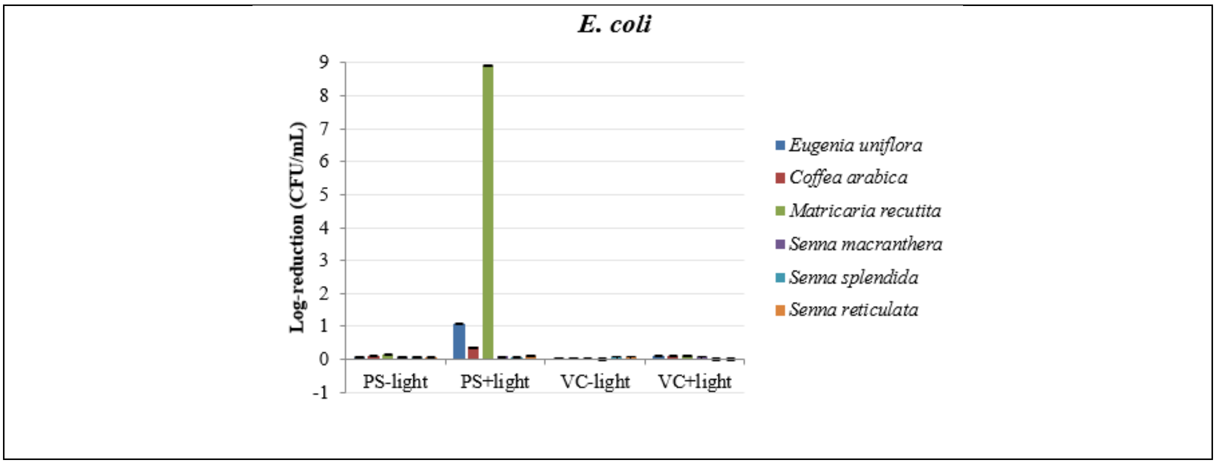

Figure 2. Effect of PDT on gram-negative bacteria (mean ± 95% confidence interval). PS-light = plant material without exposure to light; PS+light= plant material with exposure to light; VC-light= vehicle control without exposure to light; VC+light= vehicle control with exposure to light.

For *C. albicans* a reduction of 2.32 log CFU/mL was observed after PDT with *E. uniflora*. Total reduction (5.93 CFU/mL) was achieved after PDT with *M. recutita, S. splendida* or *S. reticulata* as can be seen in figure 3.

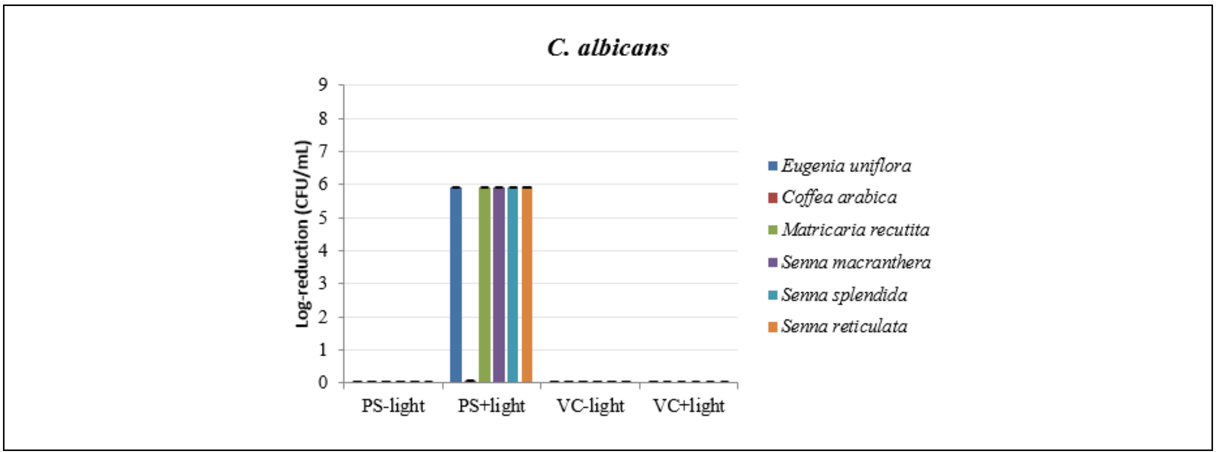

Figure 3. Effect of PDT on yeast (mean ± 95% confidence interval). PS-light = plant material without exposure to light; PS+light= plant material with exposure to light; VC-light= vehicle control without exposure to light; VC+light= vehicle control with exposure to light.

A summary of the results found in the present study, showing which plant materials reduced the viability of microbial suspensions by 100% are found in Table 4.

Table 4: Summary of the results found, showing which plant materials reduced the viability of microbial suspensions by 100% (indicated with "X")

	C. acnes	*C. albicans*	*E. coli*	*S. aureus*	*S. mutans*
C. arabica					
E. uniflora				X	
M. recutita		X	X	X	
S. macranthera		X		X	
S. reticulata	X	X			X
S. splendida	X	X		X	X

3.2 ROS detection

ROS detection was performed in all plant material, except *C. arabica* that did not show CFU reduction against microbial suspensions. Significant interaction was observed between the type of plant material and the type of fluorescent probe used (Table 5).

Table 5. Summary of two-way ANOVA results for ROS detection and natural products. The variables analyses were: type of fluorescence probe ("Probe") and natural substances ("Natural")

Source	**Df**	**SS**	**MS**	**F**	**p**	**Partial Eta-Square**
Probe	1	443454379201.389	443454379201..389	228.893	<0.001	0.792
Natural	5	126125468739.611	25225093747.922	13.020	<0.001	0.520
Probe*Natural	5	32430327625.778	6486065525.156	3.348	0.010	0.218

df= degrees of freedom; SS= Sum of squares; MS= mean square; F= MS factor/ MS residual; p= probability of significance, α= 0.050; ; *interaction between variables analyses

Figure 4 shows that there are significant difference bewteen *S. macranthera*, *S. splendida* and *S. reticulata* against *M. recutita*. Additionally, *S. macranthera* and *S. reticulata* were the natural products that generated the greatest amount of •OH radical. The levels of O_2 were bellow detection limit.

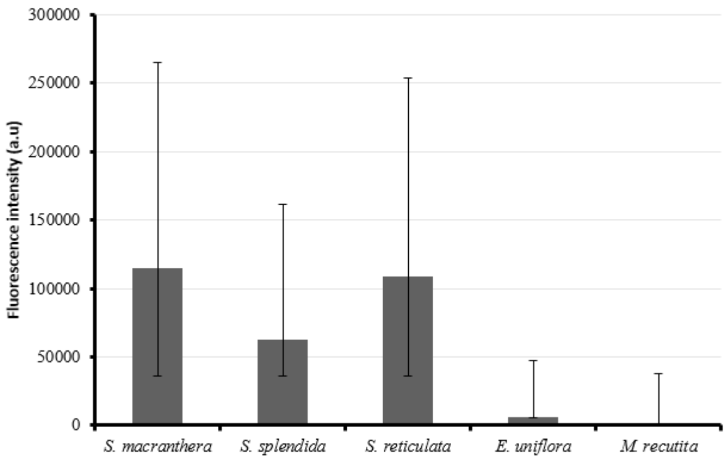

Figure 4. Production of •OH radical in the natural substances after light exposure (mean ± 95% confidence interval)

The correlation study was conducted for evaluated whether the log-reduction can be explained by the •OH radical generation. A statistically significant correlation between •OH detection and log-reduction was found for *S. macranthera* (p=0.0164 / r=0.9425 / r^2=0.8882) and *S. reticulata* (p=0.0104 / r = 0.9575/ r^2 = 0.9169). For *E. uniflora*, the p-value calculated was at the significance limit (p=0.0539/ r=0.8720/ r^2 = 0.7604). For other natural substances (*M. recutita* and *S. splendida*) a statistically significant correlation was was not (p>0.05). The results are illustrated in figure 5.

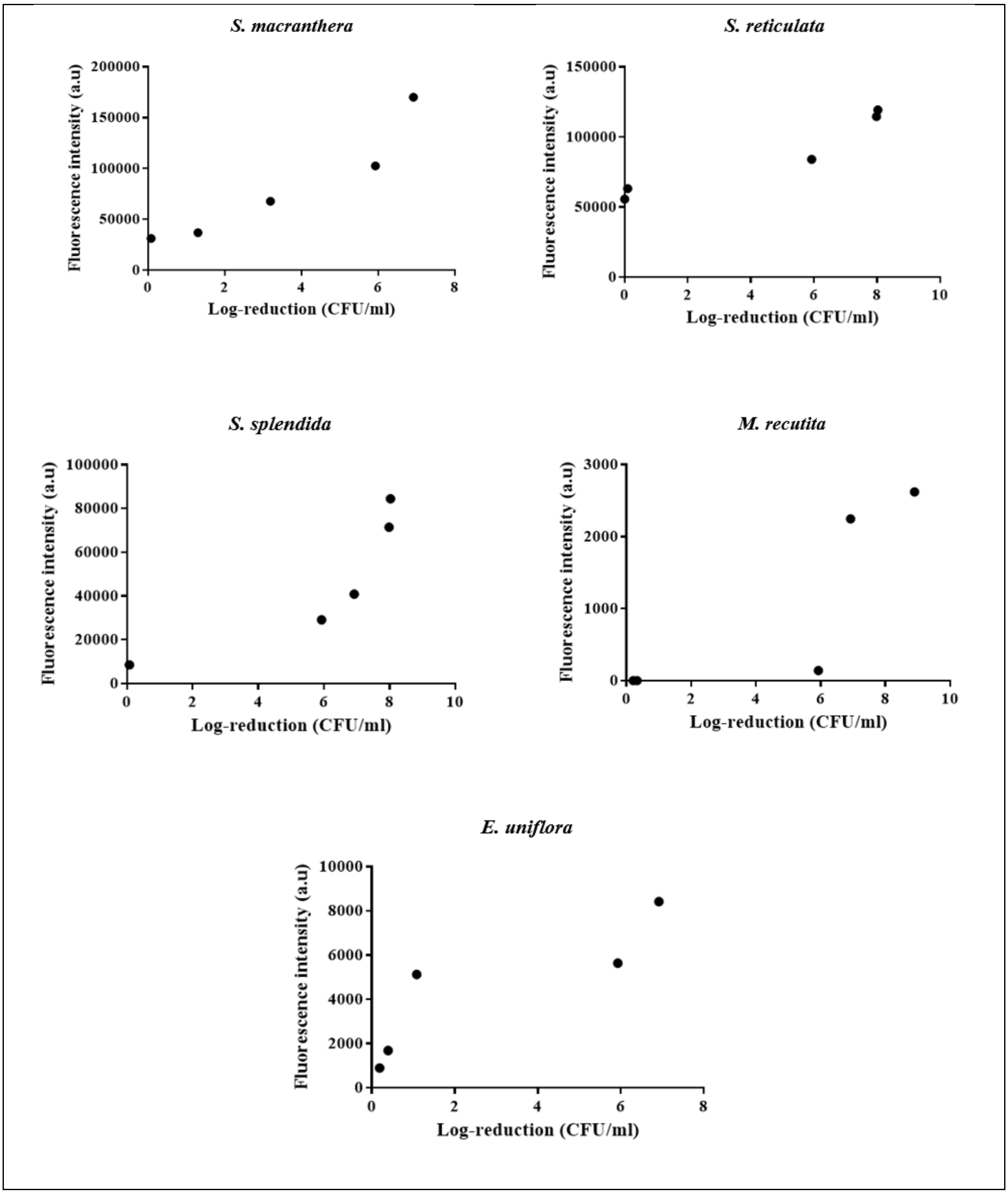

Figure 5. Correlation study between microorganism's log-reduction and amount of OH radical for each natural substance.

4 DISCUSSION

According to Brad Spellberg (2014) the development of new antimicrobial substances today is a complex, long and expensive task. In this context, PDT has been studied as a therapeutic alternative for the treatment of diseases caused by microorganisms, aiming to minimize the side effects of commonly used therapies (Kashef and Hamblin, 2017).

The present study aimed to evaluate the effect of PDT on different microorganisms of medical-dental interest (Table 1) in suspension, using as photosensitizers the essential oils from *C. arabica, E. uniflora* and *M. recutita* and plant extracts from *S. macranthera, S. splendida* and *S. reticulata*. To the best of our knowledge, this is the first study to evaluate the efficacy of PDT mediated by these natural substances.

All plant materials, except *C. arabica*, showed a microbial reduction greater than 3 log CFU/mL for at least one of the microorganisms studied. These results shows that the use of these plant materials as photosensitizers is promising because reduction levels equal to or greater than 3 log CFU/mL is one of the factors that predict whether an antimicrobial compound will be of clinical significance (Pankey and Sabath 2004). Moreover, it is important to stress that the concentration of plant material used is below the maximum recommended dose for plant material use (Freires et al., 2015).

There is no consensus in the literature about the optimal light dose to be used in PDT (Oliveira et al., 2019). Studies in the literature have shown light doses ranging from 30 to 105 J/cm^2 yielding reductions of 3 to 6 log CFU/mL in gram-positive microorganism suspensions. It is important to note that the light dose (J/cm^2) must be adjusted for each microorganism and for each photosensitizer studied. The light dose used in the present study was based on the study of De Annunzio (2017). There are many scientific reports about the use of natural photosensitizers in antimicrobial photodynamic therapy (aPDT) (Manoil et al., 2014; Azizi et al., 2016; Sampaio et al., 2016; Yang et al., 2018; Agel et al., 2019; Azizi et al., 2019; Daliri et al., 2019; Dos Santos et al., 2019; Freitas et al., 2019; Silva et al., 2019). However, the present study was motivated by the fact that these plant materials has shown a series of limitations, such as low cell permeability, absorption band different from the desired therapeutic window, high permanence in some tissues, especially the skin, leaving the patient photosensitive for weeks, formation of toxic secondary metabolites after irradiation and limited antimicrobial capacity.

In addition, total microbial reduction was observed after PDT with some plant materials. For the plant materials studied, *S. splendida*, *S. reticulata* and *M. recutita* promoted a total microbial reduction in a greater amount of microorganisms (Table 4). A maximum reduction obtained in the present study was 8.9 log CFU/mL. Similar results were not previously reported in the literature for *C. acnes*, *C. albicans*, *E. coli* and *S. mutans*. For *S. aureus*, there is only one study in the literature that reported similar results (reduction of 6 log CFU / mL compared to negative control) after aPDT with *Myciaria cauliflora* extract. Thus, the results of the present work are promising for future approaches in aPDT. Interestingly, the greater microbial reduced observed has direct relation with the effect dimension (Table 5; partial-eta square) found in the statistical analysis, which is one measure related with biological significance. This parameter is related with the biological significance, and in the present study the effect dimension is classified as very high (Tabachnick and Fidell, 1996).

Senna spp. extracts produced a great amount of •OH radical. The •OH radical is the most harmful and deleterious reactive oxygen species, because it can cause oxidative damage to various cells, including carbohydrates, lipids, proteins and nucleic acids, which can lead to cell death. Due to their very short half-life, organisms exposed to it cannot develop ways to inhibit its action (Drzeżdżon et al., 2018; Gligorovski et al., 2018).

Correlation between log reduction and OH radical detection were extremely strong for PDT woth *S. macranthera* or *S. reticulata*. Additionally, r^2 values show that the association between log-reduction and •OH radical detection is explained in 88.87% for *S. macranthera* and in 91.69% for *S. reticulata*. Other mechanisms of action may be associated with the log reduction found for plant materials E. uniflora and M recutita, such as indirect PDT photosensitization mechanisms, type III and type IV reactions (Scherer et al., 2017), which should be evaluated in the future.

In a recent systematic review about natural photosensitizers and their application in medicine, 100 substances with potential use in aPDT were found (Siwert and Stuppner, 2019). These compounds have been divided into ten classes of photosensitizers comprising furanocoumarins, polyacetylenes, thiophenes, curcumin, xanthenoids, alkaloids, anthraquinones, phenalenones, porphyrins and chlorines. *Senna* spp., included in the present study, are known to have anthraquinones in their chemical constitution (Macedo et al., 2016). This class of molecules absorbs light in the 300-450 nm wavelength region and are involved in the enzymatic photodynamic generation of reactive species of oxygen (Rajendran, 2016).

The essential oil of *M. recutita* has a blue color due to its major component, chamazulene, which is a chemical compound derived from azulene (Sebai et al., 2015). The use of azulene dye as a photosensitizer has already been reported in the literature against *Prevotella* spp., *Fusobacterium* spp. and beta-hemolytic *Staphylococcus* spp. (Garcez et al., 2007). The essential oil of *E. uniflora* is mainly composed by sesquiterpenes, such as curzerene (Sobeh et al., 2016). Curzerene is also found isolated in the rhizomes of curcuma longa, which has been used as PS in PDT (Dosoky et al., 2019). Curzerene is probably the chemical compound responsible for the photoactivity of these substances. Future phytochemical studies to unravel the chemical composition of the extracts and the essential oils should be carried out in the future.

The discovery of products of plant origin for use as photosensitizers in PDT is of great value, since the development of new photosensitizers is important, especially to increase the amount of therapeutic resources available for the treatment of infectious diseases. In this context, the plant materials studied here have great potential for use in PDT, as they are popular plant species in the world, easily acquired, and the preparation of oils and extracts are simple and require little financial resources.

3 CONCLUSION

According to the methodology employed in this *in vitro* study, PDT mediated by plant extracts of *S. macranthera, S. splendida* and *S. reticulata* and essential oils *of E. uniflora and M. recutita* are an effective alternative to eliminate *C. acnes, C. albicans, E. coli, S. aureus and S. mutans*. This study is the first to report the use of some plant materials as a photosensitizer, so further investigation is needed to evaluate the cytotoxicity and to validate the results of this study.

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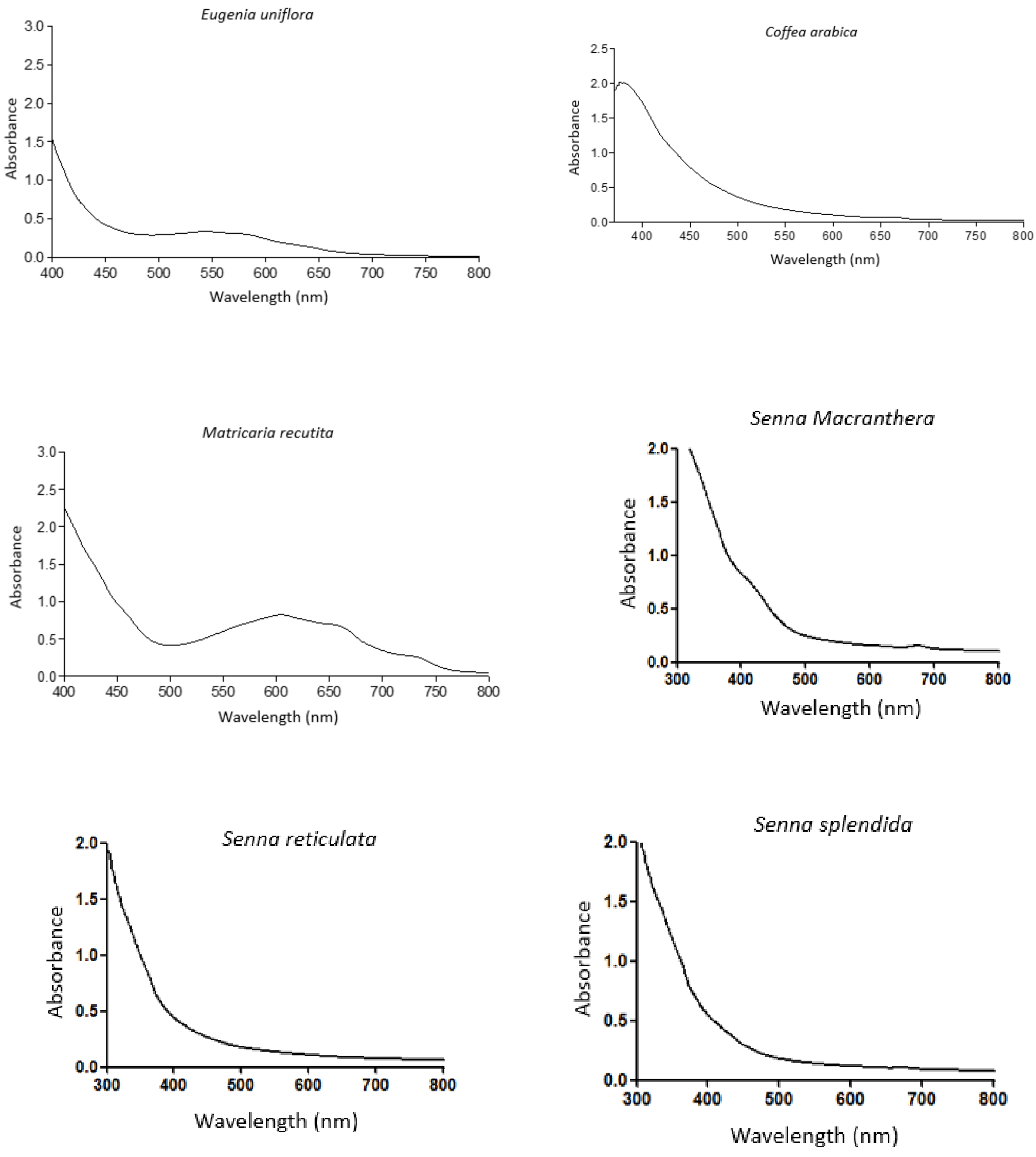
Supplementary data 1. Absorption spectrum graphs of plant materials. Essential oils: diluted in Tween 80; plant extracts: diluted in DMSO



Supplementary data 2. Summary of results from ANOVA TWO-WAY followed by confidence interval estimation to each microorganism and studied natural substance.

	Study groups Mean ± Confidence Interval (95%)					
Characteristics	**Control**	**PS-Light**	**PS+Light**	**VC-Light**	**VC+Light**	**P**
C.albicans						
Eugenia Uniflora	5.932±0.023	5.772±0.018	3.617±0.018	5.903±0.025	5.901±0.025	
Coffea Arabica	5.932±0.023	5.898±0.018	5.869±0.018	5.903±0.025	5.901±0.025	
Matricaria recutita	5.932±0.023	5.903±0.018	0.000±0.018	5.903±0.025	5.901±0.025	
Senna Macrantera	5.932±0.023	5.899±0.018	0.000±0.018	5.891±0.025	5.884±0.025	<0.001
Senna Esplendida	5.932±0.023	5.903±0.018	0.000±0.018	5.891±0.025	5.884±0.025	
Senna Reticulata	5.931±0.025	5.906±0.017	0.000±0.018	5.891±0.025	5.884±0.025	
C.acnes						
Eugenia Uniflora	8.096±0.038	8.009±0.038	7.702±0.038	8.026±0.038	8.007±0.038	
Coffea Arabica	8.066±0.038	8.090±0.038	7.883±0.038	8.026±0.038	8.007±0.038	
Matricaria recutita	8.066±0.038	7.927±0.038	7.741±0.038	8.026±0.038	8.007±0.038	
Senna Macrantera	8.100±0.040	7.941±0.040	6.783±0.040	7.932±0.040	7.921±0.040	<0.001
Senna Esplendida	7.988±0.040	7.767±0.038	0.000±0.038	7.932±0.038	7.903±0.038	
Senna Reticulata	7.988±0.040	6.746±0.038	0.000±0.038	7.932±0.038	7.903±0.038	

Legend. PS-Light: photosensitizer without light; PS+Light: photosensitizer with light; VC-Light: vehicle control without light; VC+Light: vehicle control with light; p<0.05 means significant statistical difference

Supplementary data 2. Continue. Summary of results from ANOVA TWO-WAY followed by confidence interval estimation to each microorganism and studied natural substance.

	Study groups Mean ± Confidence Interval (95%)					
Characteristics	**Control**	**PS-Light**	**PS+Light**	**VC-Light**	**VC+Light**	**P**
E.coli						
Eugenia Uniflora	8.903±0.025	8.848±0.020	7.841±0.020	8.827±0.028	8.801±0.028	<0.001
Coffea Arabica	8.903±0.025	8.791±0.020	8.535±0.020	8.887±0.028	8.801±0.028	
Matricaria recutita	8.903±0.025	8.758±0.020	0.000±0.020	8.887±0.028	8.801±0.028	
Senna Macrantera	8.908±0.025	8.828±0.020	8.825±0.020	8.856±0.028	8.902±0.028	
Senna Esplendida	8.908±0.025	8.851±0.020	8.836±0.020	8.856±0.028	8.902±0.028	
Senna Reticulata	8.908±0.025	8.839±0.020	8.810±0.020	8.856±0.028	8.902±0.028	
S.aureus						
Eugenia Uniflora	6.922±0.029	6.831±0.022	0.000±0.022	6.902±0.031	6.914±0.031	<0.001
Coffea Arabica	6.922±0.029	6.902±0.022	5.858±0.022	6.905±0.031	6.914±0.031	
Matricaria recutita	6.922±0.029	6.854±0.022	0.000±0.022	6.905±0.031	6.914±0.031	
Senna Macrantera	6.922±0.029	6.870±0.022	0.000±0.022	6.952±0.031	6.914±0.031	
Senna Esplendida	6.922±0.029	7.569±0.022	0.000±0.022	6.952±0.031	6.914±0.031	
Senna Reticulata	6.922±0.029	7.708±0.022	4.675±0.022	6.952±0.031	6.914±0.031	

Legend. PS-Light: photosensitizer without light; PS+Light: photosensitizer with light; VC-Light: vehicle control without light; VC+Light: vehicle control with light; $p<0.05$ means significant statistical difference.

Supplementary data 2. Continue. Summary of results from ANOVA TWO-WAY followed by confidence interval estimation to each microorganism and studied natural substance.

	Study groups Mean ± Confidence Interval (95%)					
Characteristics	**Control**	**PS-Light**	**PS+Light**	**VC-Light**	**VC+Light**	**P**
S.mutans						
Eugenia Uniflora	8.027±0.028	7.988±0.021	7.837±0.021	7.999±0.030	7.966±0.030	<0.001
Coffea Arabica	8.028±0.039	7.959±0.021	7.833±0.021	7.999±0.030	7.966±0.030	
Matricaria recutita	8.027±0.028	7.860±0.021	7.817±0.021	7.999±0.030	7.966±0.030	
Senna Macrantera	8.027±0.021	7.872±0.021	4.834±0.021	7.987±0.030	7.989±0.030	
Senna Esplendida	8.028±0.023	7.900±0.021	0.000±0.021	7.987±0.030	7.989±0.030	
Senna Reticulata	8.028±0.023	7.926±0.021	0.000±0.021	7.987±0.030	7.989±0.030	

Legend. PS-Light: photosensitizer without light; PS+Light: photosensitizer with light; VC-Light: vehicle control without light; VC+Light: vehicle control with light; p<0.05 means significant statistical difference.

4 CONCLUSÃO

A terapia fotodinâmica tem se mostrado uma modalidade promissora para o tratamento de doenças causadas por micro-organismos, principalmente na área da Odontologia. A descoberta de produtos de origem vegetal para a utilização como fotossensibilizadores em TFD é grande valor, pois o desenvolvimento de novos fotossensibilizadores é importante para aumentar a quantidade de recursos terapêuticos disponíveis para o tratamento de doenças infecciosas.

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2. Lin, S.-K. *Editorial:* Non-Open Access and Its Adverse Impact on Molecules. *Molecules* **2007**, *12*, 1436-1437 (PDF format 16 K, HTML format).
3. Recently a research paper of 30 pages has been published: ***Molecules*** **2008, *13*(5), 1081-1110**.
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