

Antimicrobial photodynamic therapy as an adjunct for management of deep caries lesions - Study protocol for a randomized, controlled clinical trial

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Title

Antimicrobial photodynamic therapy as an adjunct for management of deep caries lesions - Study protocol for a randomized, controlled clinical trial

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Abstract

- **Background:** Alternatively to conventional treatments, chemo-mechanical caries removal agents can be used. A modality of treatment that has been increasing in dentistry is antimicrobial photodynamic therapy (aPDT). *Bixa orellana*, is being researched for application in aPDT. This protocol aims to determine the effectiveness of aPDT with *Bixa orellana* extract in deep caries lesions.
- **Methods:** A total of 160 teeth with deep occlusal dental caries will be selected and divided in 3 groups: G1 - control group (Caries removal with a lowspeed drill); G2 - Partial Caries Removal with Papacarie; G3 - Partial Caries Removal with Papacarie and application Bixa orellana extract (20%); G4 - Partial Caries Removal with Papacarie and application Bixa orellana extract (20%) with LED (aPDT). After treatment, all the teeth will be restored with glass ionomer cement and followed up clinically and radiographically, with evaluations at immediately, 1 week, 1, 3, 6, and 12 months. Dentin samples before and after treatment will be analyzed microbiologically. The data will be submitted to descriptive statistical analysis of the association between the categorical variables using the chi-square test and Fisher exact test. The Student t test and analysis of variance will be used for the comparison of mean signs and symptoms of reversible pulpitis. Pearson correlation coefficients will be calculated for the analysis of correlations among the continuous variables.
- **Discussion:** Procedures using aPDT have been developed for the treatment of dental caries, but, there are few controlled clinical trials in the literature confirming its efficacy.

- **Trial registration:** This protocol is registered at ClinicalTrials.gov under the number NCT05236205 and it was first posted on 01/21/2022 and last updated on 05/10/2022,
<https://clinicaltrials.gov/ct2/show/NCT05236205?term=NCT05236205&draw=2&rank=1>.

Keywords

Photodynamic therapy, Dental caries, Bixa Orellana, LED, aPDT

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see <http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/>).

Title {1}	Antimicrobial photodynamic therapy as an adjunct for management of deep caries lesions - Study protocol for a randomized, controlled clinical trial
Trial registration {2a and 2b}.	This protocol is registered at ClinicalTrials.gov under the number NCT05236205. The register used for registration collects all items from the World Health Organization Trial Registration Data Set.
Protocol version {3}	This is the first version and it was first posted on 01/21/2022 and last updated on 05/10/2022, https://clinicaltrials.gov/ct2/show/NCT05236205?term=NCT05236205&draw=2&rank=1 .
Funding {4}	One of the authors has received a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), under the number 306577/2020-8.
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Name and contact information for the trial sponsor {5b}	*Corresponding author – Sandra Kalil Bussadori. Professor of the Postgraduate Program on Biophotonics Applied to Health Sciences, Nove de Julho University, Vergueiro Street, 235/249 – Liberdade - ZIP 01504-001 - São Paulo- SP- Brazil. Phone No.: +55 11 3385-9088. E-mail address: sandra.skb@gmail.com
Role of sponsor {5c}	Collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities.

Introduction

Background and rationale {6a}

Antimicrobial photodynamic therapy (aPDT) is a promising new approach that aims to promote the reduction of microorganisms and can be an innovative treatment to control oral biofilm microorganisms [1,2]. Furthermore, it has been used in the treatment of dental caries [3]. Many studies have been conducted to confirm the effectiveness of this treatment modality [3–6].

The minimally invasive clinical treatment is considered an approach to the treatment of tooth decay, which acts on detection, diagnosis, interception and treatment at the microscopic level. The partial removal of dental caries in order to maintain the integrity of the pulp is currently considered the treatment of choice for deep carious lesions, provided that some diagnostic principles are respected [7].

Currently, the treatment of these lesions is done with the removal of carious tissue using burs, hand excavators, or other techniques. Alternatively, chemo-mechanical caries removal agents can be used instead [8]. Papacarie™ is considered a minimally invasive clinical treatment approach to treating dental caries with proven efficacy [3,7–10]. However, it is necessary to improve its antimicrobial action. Clinical studies conducted to evaluate the antimicrobial potential of Papacarie™ attribute its efficacy to its physical chemical properties [8]. Partial caries removal by the Papacarie™ is capable of effectively dissolve the structure of caries-infected dentin through cysteine protease enzymatic action with additional bactericidal and anti-inflammatory properties [10].

This approach has been increasing in dentistry, being associated with antimicrobial photodynamic therapy (aPDT) [4–6]. The use of red/yellowish dyes, is being researched for their application in aPDT. Bixa orellana, popularly known as “urucum”, is a plant native to Brazil. This dye has important antioxidant and antimicrobial activities, and recent studies have shown its potential as a photosensitizer in aPDT [3,11–13]. Due to its positive characteristics, especially regarding the lack of mutagenic and cytotoxic activity and antimicrobial effect, the Bixa orellana extract could be considered a suitable candidate as a photosensitizer. Using a dye with these characteristics would make aPDT more accessible [6,14].

There are no controlled clinical trials in the literature that confirm the effectiveness of this type of therapy on deep caries-affected dentin associating Papacarie™ and aPDT with Bixa orellana extract, especially in the deep caries lesion. The justification for performing the treatment with Papacarie™ associated with aPDT using the Bixa Orellana extract is to reduce the risk of pulp exposure in permanent teeth and increase the antimicrobial activity.

Objectives {7}

General objectives

To investigate the use of Papacarie™ and aPDT with Bixa orellana extract on the caries-affected dentin of permanent teeth through a controlled clinical trial.

Specific objectives

- To verify the efficacy of photodynamic therapy with Bixa Orellana and Papacarie™ with regard to microbiological, radiographic, and clinical aspects;

- To evaluate the antimicrobial effect of aPDT on caries-affected dentin in permanent teeth;
- To perform a radiographic evaluation of the remaining dentin and radiographic density at 6 evaluation times, over a 12-month period.

Hypothesis

Hypothesis (H1): The administration of Papacarie™ and aPDT with Bixa orellana extract is effective in the treatment of caries-affected dentin in permanent teeth.;

Hypothesis (H0): The administration of Papacarie™ and aPDT with Bixa orellana extract reduces the number of viable bacteria but is not effective in the treatment of caries-affected dentin in permanent teeth.

Trial design {8}

This is protocol for a randomized and blind controlled clinical trial. It is in accordance with the criteria for designing a clinical Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT statement). Figure 1 is the SPIRIT figure.

Methods: Participants, interventions and outcomes

Study setting {9}

Participants will be selected from male and female children (with no restrictions regarding race or ethnicity) enrolled for treatment at the pediatric clinic of the dentistry course of Paulo Picanço School of Dentistry (Fortaleza-Ceará, Brazil).

Eligibility criteria {10}

Inclusion criteria

- Adequate health with no systemic conditions;
- Adequate cooperation;
- Clinically presenting at least 1 permanent molar with an acute, active caries on the dentin not surpassing 2/3 and only involving the occlusal face, with direct view and access as well as no clinical or radiographic signs of pulp involvement.

Exclusion criteria

- Systemic adverse health condition;
- Uncooperative behavior;
- Class II, III, IV, or V carious lesion based on Black classification;
- Clinically: caries involving enamel, deficient restorations, small carious lesions on dentin with no access for manual scalers, hidden caries, sign or symptom of pulp involvement, clinical impossibility of restoration;
- Radiographically: evidence of pulp involvement, carious lesion extending beyond 2/3 of dentin.

Who will take informed consent? {26a}

The same researcher responsible for the procedures will be in charge of obtaining the consent form. The parents or guardians who agree to allow the minors they are responsible for to participate, will sign a written statement of informed consent. The children will sign a written assent term.

Additional consent provisions for collection and use of participant data and biological specimens {26b}

Not applicable.

Interventions

Explanation for the choice of comparators {6b}

The conventional method (drill) will be compared with the use of Papacarie™ with or without the Bixa Orellana extract, and with or without the irradiation for aPDT. This will show which of these treatments will be more efficient with regard to microbiological, radiographic, and clinical aspects.

Intervention description {11a}

Group 1 – Caries removal with a lowspeed drill (control group):

1. Initial periapical and interproximal radiographs;
2. Prophylaxis with toothbrush and fluoride toothpaste;
3. Relative isolation with lip bumper, cotton roll, and aspirator;
4. Microbiological sampling with ear curette for standardization of volume of carious tissue;
5. Removal of carious dentin with carbide burs and manual instruments;
6. Additional microbiological sampling;
7. Clinical inspection of texture of remaining dentin with an exploratory probe;
8. Restoration with glass ionomer cement (Ketac Molar EasyMix – 3M ESPE);
9. Clinical and radiographic follow-up immediately, 1 week, 1, 3, 6, and 12 months after treatment.

Group 2 – Partial removal of carious tissue with the administration of Papacarie™:

1. Initial periapical and interproximal radiographs;
2. Relative isolation with lip bumper, cotton roll, and aspirator;
3. Microbiological sample with otoscope curette to standardize volume of carious tissue;
4. Application of Papacarie™ for 5 minutes, removal of carious tissue around lateral walls of the cavity with noncutting curette and no removal of carious tissue on pulp floor;
5. Second microbiological sample of remaining dentin with curette;
6. Clinical evaluation by inspection of texture of remaining dentin with exploratory probe;
7. Restoration with glass ionomer cement (Ketac Molar EasyMix 3M ESPE);
8. Clinical and radiographic follow-up immediately, 1 week, 1, 3, 6, and 12 months after treatment.

Group 3 – Partial removal of carious tissue with the administration of Papacarie™ and application of Bixa Orellana extract (20%):

1. Initial periapical and interproximal radiographs;
2. Relative isolation with lip bumper, cotton roll, and aspirator;
3. Microbiological sample with otoscope curette to standardize volume of carious tissue;
4. Application of Papacarie™ with Bixa Orellana extract (20%) for 5 minutes, removal of carious tissue around lateral walls of the cavity with noncutting curette and no removal of carious tissue on pulp floor;
5. Irradiation of dental tissue for 1 minute on a single point;
6. Second microbiological sample of remaining dentin with curette;
7. Clinical evaluation by inspection of texture of remaining dentin with exploratory probe;
8. Restoration with glass ionomer cement (Ketac Molar EasyMix 3M ESPE);
9. Clinical and radiographic follow-up immediately, 1 week, 1, 3, 6, and 12 months after treatment.

Group 4 – Partial removal of carious tissue with the administration of Papacarie™, application of Bixa Orellana extract (20%) and LED (aPDT):

1. Initial periapical and interproximal radiographs;
2. Relative isolation with lip bumper, cotton roll, and aspirator;
3. Microbiological sample with otoscope curette to standardize volume of carious tissue;
4. Application of Papacarie™ with Bixa Orellana extract (20%) for 5 minutes and the light-emitting diode (LED) light curing device (Valo Cordless Ultradent®), an office appliance, with a coupled radiometer, and a spectrum of 440–480 nm will be used. Both the volunteer to be treated and the professional will be using specific eye protection glasses. The active end of the LED will be coated with clear disposable plastic (PVC), thus avoiding cross contamination. Removal of carious tissue around lateral walls of the cavity with noncutting curette and no removal of carious tissue on pulp floor;
5. Irradiation of dental tissue for 1 minute on a single point;
6. Second microbiological sample of remaining dentin with curette;
7. Clinical evaluation by inspection of texture of remaining dentin with exploratory probe;
8. Restoration with glass ionomer cement (Ketac Molar EasyMix 3M ESPE);
9. Clinical and radiographic follow-up immediately, 1 week, 1, 3, 6, and 12 months after treatment.

Criteria for discontinuing or modifying allocated interventions {11b}

Seeing as the treatment is immediate, there will be no criteria for discontinuing or modifying allocated interventions.

Strategies to improve adherence to interventions {11c}

Participants will be enrolled for treatment at the pediatric clinic of the dentistry course of Paulo Picanço School of Dentistry (Fortaleza-Ceará, Brazil). As they will already be in treatment, we believe this will improve adherence.

Relevant concomitant care permitted or prohibited during the trial {11d}

There are no specific procedures that are permitted or prohibited, as treatments will be performed in a single session.

Provisions for post-trial care {30}

No harms are expected, and ulterior assistance participants may need, will be provided.

Outcomes {12}

Microbiological evaluation

This is the primary outcome of the study. A sample of caries-affected dentin will be taken from each selected tooth before the removal of the carious tissue. The samples will be standardized with the use of a Meyhoefer auricular curette n° 2 and placed into test tubes containing 3.8 ml of transport medium (phosphate buffered saline). The dental tissue will be dispersed in the transport tube containing glass pearls through agitation at maximum speed in a vortex device for 30 seconds to homogenize the biological material. The biofilm will be diluted in series on the order of 10^1 to 10^6 in peptone water and inoculated in culture media in Petri dishes. Aliquots of dilutions 10^4 , 10^5 , and 10^6 will be sewn on the surface of Brucella agar (Difco Laboratories, Detroit, Michigan) containing defibrinated sheep blood (50ml/L), hemin (5 mg/ml), and menadione (10mg/ml) for the determination of the total number of viable microorganisms (VM). Aliquots of dilutions 10^3 and 10^4 will be sewn on Mitis Salivarius agar (Difco Laboratories, Detroit, Michigan) for the determination of the total number of streptococcus (S). Aliquots of dilutions 10^1 and 10^2 will be sewn on Mitis Salivarius agar with the addition of debacitracin for the determination of the population of streptococcus of the mutans group (SM).

Aliquots (100ml) from each dilution will be sewn onto the surface of agar and spread with the aid of a Drigalski spatula. Undiluted aliquots and aliquots from dilution 10^2 (100ml) will be pour plated on Rogosa SL agar (Difco Laboratories, Detroit, Michigan) for the determination of lactobacilli (LB). The Brucella agar dishes will be incubated in an anaerobic chamber (PLAS by LABS, Lansing, MI) at 37°C for 7 days. The Mitis Salivarius agar and Mitis Salivarius Bacitracin dishes will be incubated in a 10% CO₂ atmosphere (CO₂ greenhouse, Shel Lab, mod. 2123, Oregon) at 37°C for 48 hours. The dishes containing Rogosa agar will be incubated in a 10% CO₂ atmosphere at 37°C for 72 hours. After incubation, the characteristic colonies in each dish will be counted with the aid of a stereomicroscope at a magnification of 10 times in dilutions with 30 to 300 colonies per dish. All procedures will be performed in duplicate, and the mean of the counts will be calculated. The results will be expressed in CFU of SM and LB as well as in proportion of streptococcus (% S/VM), SM group (% SM/VM) and lactobacilli (% LB/VM) in relation to the total of VM. For SM, the proportion in relation to the total of streptococci (% SM/S) will also be calculated. Immediately after the removal of the carious tissue, samples of the remaining dentin will be taken with a Meyhoefer auricular n° 2 curette and the procedures will be repeated [15].

Radiographic evaluation

Periapical and interproximal radiographs will be taken initially and immediately after the procedure. Subsequently, follow up will be performed immediately, 1 week, 1, 3, 6, and 12 months after for the evaluation of optical density on the radiographs and the visual clinical interpretation of the remaining dentin as well as the evaluation using the radiographic subtraction method. The radiographic images from the different evaluation

times will be scanned for the analysis of differences in density. For such, an specific program will be used. The density of the remaining dentin will be based on the changes in optical density. The teeth submitted to restorative treatment in all groups will be reevaluated after 3, 6, and 12 months. This method will consist of analyzing the standardized and digitized periapical and interproximal radiographs of to determine quantitatively the gray tones in the affected dentin region immediately below the restoration in class ionomer cement, the radiographic control of which for the visualization of sound dentin will allow the clinical examiner to compare the density of the remaining dentin in the different groups. The statistical analysis of optical density will be performed using the mixed-effects model [16].

Evaluation of time required for procedure

The time required for each procedure will be measured using a digital stopwatch (Kenko, Hong Kong) in minutes and seconds from the onset of treatment until the complete removal of the carious tissue. The time will be recorded on a specific chart for analysis. The need or non-need for anesthesia will also be recorded.

Evaluation of need for local anesthesia during intervention and degree of pain/discomfort of children during procedure

All interventions will be initiated without the prior administration of local anesthesia. The children will be told that anesthesia could be administered at any time during the intervention. A face scale with different expressions will be used to evaluate the need for local anesthesia and the child will be asked to point to the expression that most corresponds to his/her degree of pain/discomfort.

Interpretation of face scale:

1. No pain.
2. Mild pain.
3. Moderate pain.
4. A little worse pain.
5. Strong pain.
6. Worst pain.

Clinical evaluation

The clinical evaluation will be performed by a researcher blinded to the different treatment groups. The criteria used for the evaluation will be the retention of the restorative material in the cavity and the occurrence of secondary caries. The evaluation scores will be based on the results of previous studies. Digital photographs of the restorations will also be taken and serve to complement the clinical and radiographic findings. The visual demonstration will contribute to any necessary clarifications and facilitate the discussion and documentation of the cases. Thus, digital photographs will be taken of all teeth in the different groups before and after the interventions. We believe participants will be present for the follow-ups due to concern and other possible treatments in clinic.

0 = present; no defects;

1 = present; small marginal defects measuring less than 0.5mm in depth; no need for repair;

2 = present; small marginal defects measuring 0.5mm to 1mm in depth; need for repair;

3 = present; large marginal defects measuring 1 or more mm in depth; need for repair;

4 = absent; restoration nearly or completely lost; need for treatment;

5 = absent; additional treatment having been performed for some reason;

6 = tooth absent for any reason;

7 = present; surface wear measuring less than 0.5mm in depth; no need for replacement;

8 = present; surface wear greater than 0.5mm in depth; need for replacement;

9 = impossible to diagnose.

Participant timeline {13}

			STUDY PERIOD							
	Enrolment	Allocation	Post-allocation							Close-out
TIMEPOINT**	30 May 2022 – 30 April 2023	0	t_1 Baseline	t_2 Immediately after	t_3 1 week	t_4 1 month	t_5 3 months	t_6 6 months	t_7 12 months	01 May 2023 – 01 June 2023
ENROLMENT:										

Eligibility screen	X									
Informed consent	X									
Allocation		X								
INTERVENTIONS:										
<i>Caries removal with a lowspeed drill</i>			X							
<i>Papacarie</i>			X							
<i>Papacarie and Bixa orellana extract</i>			X							
<i>Papacarie and aPDT (Bixa orellana extract +LED)</i>			X							
ASSESSMENTS:										
<i>Microbiological Evaluation</i>										
<i>Radiographic evaluation</i>			X	X	X	X	X	X	X	
<i>Clinical Evaluation</i>			X	X	X	X	X	X	X	
<i>Time required for procedure</i>				X						
<i>Need for local anesthesia during intervention</i>				X						
<i>Degree of pain/discomfort of children during procedure</i>				X						
<i>Statistical Analysis</i>										X

Figure 1: SPIRIT figure as recommended by 2013 SPIRIT Statement.

Sample size {14}

The sample size was calculated based on a previous study in the literature [17,18], considering the expected difference and standard deviation and weighting colony forming units (CFU) of bacteria. For the statistical calculation, paired samples were considered, with $\alpha=5\%$ and an 80% test power. The minimum number in this clinical trial was determined to be 34 teeth per group. Therefore, 40 teeth per group will be recruited to compensate possible dropout during the experimental period. G*Power 3.1 was used to perform the calculations.

Recruitment {15}

As participants will already be in pediatric dentistry treatment, we believe recruitment will be feasible. We believe participants will be present for the follow-ups due to concern and other possible treatments in clinic.

Assignment of interventions: allocation

Sequence generation {16a}

Randomization will be performed using the website randomizer.org, the numbers will be placed in a brown envelope and the participant will remove the envelope at the time of treatment.

Concealment mechanism {16b}

Numbers according to treatments will be kept in opaque envelopes:

Number 1 – Traditional caries removal with a low-speed drill (Group 1);

Number 2 – Partial removal of carious tissue with the administration of Papacarie™ (Group 2);

Number 3 – Partial removal of carious tissue with the administration of Papacarie™ and application of Bixa Orellana extract (20%) (Group 3);

Number 4 - Partial removal of carious tissue with the administration of Papacarie™, application of Bixa Orellana extract (20%) and LED (aPDT) (Group 4).

Implementation {16c}

The researchers responsible for the treatment application will generate the allocation sequence, enrol participants, and assign participants to interventions.

Assignment of interventions: Blinding

Who will be blinded {17a}

The clinical evaluations of the carious tissue removal, as well as the microbiological and radiographic analyses, will be performed by examiners blinded to the treatments performed on each tooth. Only the researcher in charge of procedures will know to which group the participants belong.

Procedure for unblinding if needed {17b}

Unblinding is not permitted.

Data collection and management

Plans for assessment and collection of outcomes {18a}

Researchers will be previously trained to collect data and perform evaluations, according to the parameters described in outcome measures.

Plans to promote participant retention and complete follow-up {18b}

As participants will already be in pediatric dentistry treatment, we believe recruitment will be feasible. We believe participants will be present for the follow-ups due to concern and other possible treatments in clinic.

Data management {19}

All data will be entered electronically. The participants' files will be stored in numerical order in a safeplace, accessible only to the authors of this study.

Confidentiality {27}

The data sets generated and analyzed during the study will be available from the corresponding author at reasonable request. After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it. The authors also intend to publish the results.

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Not applicable.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The data will be stored in Excel datasets analyzed statistically employing different tests and considering a 5% level of significance. Only the researchers involved in the study will have access to the data, even if the study is discontinued. The results will be submitted to descriptive analysis for the association of categorical variables in age and gender using the chi-square test and Fisher exact test. The student t test and analysis of variance will be used for the comparison of means. Pearson correlation coefficients will be calculated to determine correlations among the continuous variables.

Interim analyses {21b}

The corresponding author make the final decision to terminate the trial, if necessary. The authors will have access to these interim results.

Methods for additional analyses (e.g. subgroup analyses) {20b}

Not applicable.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

No losses greater than 5% of the sample are expected.

Plans to give access to the full protocol, participant level-data and statistical code {31c}

The final datasets will be available from the corresponding author, at a reasonable request. The authors have the intention of publishing the results, in articles and conferences.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee {5d}

The authors themselves will coordinate and steer the trial.

Composition of the data monitoring committee, its role and reporting structure {21a}

Changes in the protocol will be reported to the Ethics Committee of Faculdade Paulo Picanço, that approved and will monitor the study when it comes to ethics.

Adverse event reporting and harms {22}

No harms are expected, and ulterior assistance participants may need, will be provided.

Frequency and plans for auditing trial conduct {23}

Not applicable.

Plans for communicating important protocol amendments to relevant parties (e.g. trial participants, ethical committees) {25}

Changes in the protocol will be reported to the Ethics Committee of Faculdade Paulo Picanço and altered in ClinicalTrials.gov.

Dissemination plans {31a}

After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it. The authors also intend to publish the results.

Discussion

Studies have shown that the isolated action of aPDT is not completely efficient for the microbial reduction and painful symptoms of patients [19], highlighting the importance of dental professionals in both the treatment and prevention of deep caries-affected dentin of permanent teeth [20]. Procedures using aPDT have also been developed to the treatment of dental caries [3,4,20], however, there are few controlled clinical trials in the literature confirming its efficacy. Papacarie™ is a gel composed of papain and chloramine [8,9] employed for the partial removal of carious tissue, effective against bacteria due to its properties, but some studies show that the antibacterial action is not so evident. Bixa Orellana is a Brazilian plant, and its seeds produce one of the most frequently used worldwide dyes, which is called annatto [6,21]. Its leaves and seeds were reported to have antimicrobial, antifungal, anticonvulsant, analgesic and anti-inflammatory activities, and showed important activity against Gram-positive and Gram-negative bacteria [22,23]. The Bixa Orellana extract proposed with this study could enhance the antimicrobial action through aPDT [6] and improve the effectiveness of conservative treatments using the selective removal of carious tissue and the use of

Papacarie™. In addition, there is a tendency for patients to value new treatment technologies, which can increase their satisfaction with this therapy.

Trial status

This protocol is registered at ClinicalTrials.gov under the number NCT05236205 and it was first posted on 01/21/2022 and last updated on 05/10/2022, <https://clinicaltrials.gov/ct2/show/NCT05236205?term=NCT05236205&draw=2&rank=1>. Recruitment will take place from 30 May 2022 to 30 April 2023.

Abbreviations

aPDT: antimicrobial photodynamic therapy;
CFU: colony forming units;
LB: lactobacilli;
LED: light-emitting diode;
S: streptococcus;
SM: streptococcus mutans;
SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials;
VM: viable microorganisms.

Declarations

Acknowledgements

Not applicable.

Authors' contributions {31b}

LFBM, LRS, DMP, VPF, SKB participated in the conception and design of the study. LFBM, LRS, DMP, VPF will participate in the data collection and drafting of the present protocol. LJM will perform statistical analysis. MLLG, KPSF, RAMF, ACRT and SKB critically reviewed the manuscript for intellectual content. LFBM and SKB coordinated the study. All authors read and approved the final protocol.

Funding {4}

One of the authors has received a grant from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), under the number 306577/2020-8.

Availability of data and materials {29}

The data sets generated and analyzed during the study will be available from the corresponding author at reasonable request. After the analysis of the data, volunteers will be invited to a meeting and the results will be shared, in case they wish to attend it. The authors also intend to publish the results.

Ethics approval and consent to participate {24}

The study will be conducted in accordance with the ethical percepts stipulated in the Declaration of Helsinki (World Medical Association Declaration of Helsinki, 2008). The protocol was approved by the Ethics Committee of Faculdade Paulo Picanço, under the number 55612821.6.0000.9267, report number 5.260.733. Changes in the protocol will be reported to this same committee. Individuals deemed eligible will receive clarifications regarding the objectives and procedures of the study. The parents or guardians who agree to allow the minors they are responsible for to participate, will sign a written statement of informed consent. The children will sign a written assent term. The identity of all individuals will be preserved throughout all stages of the research.

Consent for publication {32}

Participant will be told that the results of the study will be published, but maintaining their individual identities and privacy. This will also be stated in the consent term.

Competing interests {28}

The authors declare that they have no competing interests.

Authors' information (optional)

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