

# Effect of Pudilan Keyanning antibacterial mouthwash on dental plaque and gingival inflammation in patients during periodontal maintenance phase: study protocol for double-blind, randomised clinical trial

Jianru Liu,<sup>1</sup> Yan Huang,<sup>1</sup> Xinzhe Lou,<sup>1</sup> Bei Liu,<sup>1</sup> Wenyi Liu,<sup>1</sup> Na An,<sup>2</sup> Rong Wu,<sup>1</sup> Xiangying Ouyang <sup>1</sup>

**To cite:** Liu J, Huang Y, Lou X, et al. Effect of Pudilan Keyanning antibacterial mouthwash on dental plaque and gingival inflammation in patients during periodontal maintenance phase: study protocol for double-blind, randomised clinical trial. *BMJ Open* 2021;0:e048992. doi:10.1136/bmjopen-2021-048992

► Prepublication history for this paper is available online. To view these files, please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2021-048992>).

JL and YH contributed equally.

Received 13 January 2021  
Accepted 13 September 2021



© Author(s) (or their employer(s)) 2021. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

<sup>1</sup>Department of Periodontology, Peking University, School of Stomatology, Beijing, China

<sup>2</sup>Department of General Dentistry II, Peking University, School of Stomatology, Beijing, China

**Correspondence to**  
Professor Xiangying Ouyang;  
kqouyangxy@bjmu.edu.cn

## ABSTRACT

**Introduction** Plaque control plays a critical role in the prevention and treatment of periodontitis. Antibacterial mouthwash is one of the most important tools for plaque control. Pudilan, including extracts of *Scutellaria baicalensis* root, *Taraxacum mongolicum*, Bunge corydalis herb and *Isatis indigotica*, was reported playing the role of anti-inflammatory and anti-bacterial. However, its effect on dental plaque and periodontal inflammation remains unknown. We aimed to assess the efficacy of Pudilan Keyanning antibacterial mouthwash which contains the active essence of Pudilan and 0.03%–0.06% cetylpyridinium chloride, as well as Pudilan active essence for plaque control and gingival anti-inflammation in patients during periodontal maintenance phase.

**Methods and analysis** In this double-blind, randomised, placebo-controlled clinical trial, a total of 120 participants during periodontal maintenance phase will be enrolled. After supragingival scaling, they will be randomly assigned into three groups in a 1:1:1 ratio: the Pudilan Keyanning antibacterial mouthwash group, a chlorhexidine acetate mouthwash (0.12%) group or a placebo group with mouthwash containing the same components as the Pudilan Keyanning mouthwash except for Pudilan active ingredients. They will rinse with mouthwash, respectively, two times per day for 6 weeks.

Clinical parameters (such as plaque index, bleeding index) and the level of volatile sulfide in the breath will be measured and analysed. The subgingival plaque will be collected and analysed microbiologically. Questionnaire feedback will be analysed.

**Ethics and dissemination** The study protocol (V.4) was reviewed and approved by the Medical Ethical Committee of Peking University School and Hospital of Stomatology (Ethics Approval No. PKUSSIRB-201950153b). All participants signed a written consent form.

**Trial registration number** ChiCTR2000041253.

## INTRODUCTION

Periodontitis is one of the most common chronic oral inflammatory diseases in China.<sup>1</sup> Its primary aetiological factor is the

## Strengths and limitations of this study

- This is a double-blind, randomised, placebo-controlled clinical trial to evaluate the effect and safety of the Pudilan Keyanning antibacterial mouthwash for plaque control and preventing gingival inflammation.
- The study aims to generate evidence for the efficacy of the Pudilan Keyanning antibacterial mouthwash, which uses Chinese traditional drugs as its main constituents, for dental plaque control in patients during periodontal maintenance phase.
- Two control groups will be assigned to this study. The aim is to determine the unique antibacterial and anti-inflammatory effects of the Pudilan Keyanning antibacterial mouthwash and Pudilan active ingredients.
- Intergroup differences in patient compliance may have an impact on the reliability of the experiment results.
- The study is limited by its ability to detect adverse events due to the relatively small sample size.

accumulation of pathogenic plaque biofilms, although this disease is caused by complex interactions of many factors, such as host immunity and the environment. Evidence has shown that plaque control is the key step in treatment of this disease.<sup>2</sup> Mechanical methods, including supragingival and subgingival scaling, air-polishing and tooth-brushing, are the most effective strategies for biofilm removal. Mechanical plaque control is restricted in some specific circumstances, for example, after oral surgery. In this situation, antibacterial mouthwash as chemical plaque control has become the most important tool. Chlorhexidine (CHX) mouthwash is



frequently recommended post-surgically, due to its efficiency against gram-negative and gram-positive bacteria.<sup>2</sup> Evidence has shown that CHX not only exhibits a bactericidal effect but also penetrates biofilms and interferes with biofilm formation.<sup>3 4</sup> A significant reduction in plaque (means of 29%–86% after 1 week) and bleeding (up to 73%) was reported with the use of CHX (concentrations of 0.12%–2% after surgery).<sup>3</sup> However, CHX mouthwash showed several side effects,<sup>5</sup> such as taste stimulation and alteration, and staining on tooth surface and tongue, especially after a long period of time. Some patients did not accept the CHX mouthwash. Therefore, a new alternative for clinical use is warranted.

Pudilan Keyanning antibacterial mouthwash is an anti-bacterial herbal mouthwash, containing the active essence of Pudilan, 0.03%–0.06% cetylpyridinium chloride (CPC) and other excipients commonly used in mouthwash preparation.

It is reported that CPC, as a cationic surfactant of quaternary ammonium salts, could induce a significant shift of the structure of dental plaque, reduce the viability of oral biofilms and inhibit biofilm formation.<sup>6 7</sup> The use of CPC-containing mouthwash does not cause any irritations to the oral mucosa, tooth surface staining or toxic side effects. CPC mouthwash is widely used in oral healthcare. Its bactericidal effect on periodontal pathogens has been confirmed in clinical trials.<sup>7 8</sup> However, it is well recognised that CPC mouthwash (0.05%) is not as effective as CHX mouthwash (0.12%) for plaque control.<sup>9</sup>

Pudilan is another active ingredient of Pudilan Keyanning antibacterial mouthwash. It includes *Scutellaria baicalensis* root extract, *Taraxacum mongolicum* extract, Bunge corydalis herb extract and *Isatis indigofera* extract, that are obtained by extracting and concentrating methods.<sup>10</sup> *T. mongolicum* belongs to the Asteraceae (Compositae) family. It is a non-toxic herb that has garnered attention for its antioxidant, antirheumatic and anti-inflammatory properties.<sup>11 12</sup> *S. baicalensis* belongs to the genus *Scutellaria* (Lamiaceae), one of the most popular herbs used in traditional Chinese medicine (TCM). *S. baicalensis* extracts have anti-inflammatory, antiviral, antimicrobial and anti-oxidative properties.<sup>13</sup> Bunge corydalis herb is traditionally used in ancient Chinese medicine and possesses various biological effects, such as anti-inflammatory and antibacterial properties, and inhibition of the immune functions of the host.<sup>14</sup> *I. indigofera* extract (Banlangen) is a TCM famous for its broad antiviral, anti-inflammatory, antioxidant and antipyretic properties.<sup>15–17</sup> Accumulative evidence has shown the extracts from these four drugs can all decrease the production of inflammatory mediators nitric oxide (NO) and prostaglandin E2 (PGE2), and proinflammatory cytokines including tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin 1.<sup>14 15</sup>

Moreover, Pudilan is well known to be efficacious against fever, inflammation and swelling.<sup>18 19</sup> This drug is available in many different dosage forms and different routes of administration. Its use is indicated for a variety of inflammatory conditions. Jin *et al*<sup>20</sup> reported that patients

with mild recurrent aphthous ulcers recovered faster, had reduced pain and experienced no adverse effects when Pudilan Keyanning oral liquid was used.

According to China Inspection Body and Laboratory Mandatory Approval (CMA) inspection reports, it was identified that *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, *Candida albicans*, *Staphylococcus aureus* and *Escherichia coli* were inhibited by Pudilan Keyanning antibacterial mouthwash. The bacteriostatic rate is higher than 95% after applying with the mouthwash for 2 min.

In addition, the safety of Pudilan Keyanning antibacterial mouthwash has been inspected with CMA. It was shown that Pudilan Keyanning antibacterial mouthwash has no irritation in an acute eye irritation test in a rabbit. The components of Pudilan Keyanning antibacterial mouthwash include the active essence of Pudilan, 0.03%–0.06% CPC and other excipients. The active essence of Pudilan in the studied mouthwash is the same as Pudilan antiphlogistic oral liquid, which has been widely used in clinic with high safety.<sup>21–23</sup> As an active ingredient, CPC has been added to a variety of commercial mouthwash products, and 0.03%–0.06% CPC in mouthwash shows satisfactory safety.<sup>24–26</sup> Other excipients are added according to national standards or industrial standards, which have been commonly used in mouthwash preparation.

Bioactive chemical constituents of the studied mouthwash are the active essence of Pudilan and 0.03%–0.06% CPC. Pudilan Keyanning antibacterial mouthwash contains the same active essence of Pudilan as Pudilan antiphlogistic oral liquid, such as baicalin, wogonoside, cichoric acid, caffeic acid, dehydrocheilanthifoline, dihydrosanguinarine and indirubin.<sup>27</sup>

The therapeutic effect of Pudilan Keyanning antibacterial mouthwash on gingivitis and periodontal pathogens has not been reported. Whether this mouthwash is as effective as CHX mouthwash (0.12%) in plaque and periodontal inflammation is unknown. Therefore, this double-blind, randomised clinical trial aimed to determine the efficacy and safety of Pudilan Keyanning antibacterial mouthwash on the inhibition of plaque and gingival inflammation. By comparing it with the CHX group (control 1 group), the antibacterial effect of the mouthwash and its influence on periodontal pathogenic microorganisms will be evaluated. In order to further evaluate the anti-inflammatory effect of Pudilan ingredients alone on the gingiva, the control 2 group will be set up. The mouthwash used in control 2 group contained the same components as the Pudilan mouthwash but without the Pudilan components.

## METHODS AND ANALYSIS

### Materials

Pudilan Keyanning antibacterial mouthwash used in this study is a commercial product that has been widely used among the people. It is prepared by water extraction first, alcohol extraction second, and then concentration of *S.*

*baicalensis* root, *T. mongolicum*, Bunge corydalis herb and *I. indigotica*. After that, 0.03%–0.06% CPC and other excipients were added to dissolve and filter. Each component is added in strict accordance with the content described in the patent for quality control. For quality control, 30 samples were selected randomly from each production batch and 10 of them were inspected according to the criteria of quality control and preparation of the mouthwash (Q/PDLRH001-2018).

### Study design

A double-blind, randomised, placebo-controlled clinical trial is designed to evaluate the effect of Pudilan mouthwash on plaque and gingival inflammation in patients during periodontal maintenance phase.

### Eligibility criteria

Inclusion criteria:

1. Aged 18–65 years.
2. Systemically healthy.
3. Non-smoker.
4. During supportive periodontal therapy.
5. The number of sites with probing depth  $\geq 6$  mm is no more than 5.
6. The percentage of sites with bleeding on probing (BOP)  $\geq 10\%$ .
7. More than 20 natural permanent teeth (excluding third molars).
8. Having mastered the Bass toothbrushing method.

Exclusion criteria:

1. Oral mucosal damage at baseline.
2. Acute gingival inflammation at baseline.
3. Open dental caries at baseline.

4. Removable dentures or fixed orthodontic devices at baseline.
5. Having taken antibiotics in the past 3 months.
6. Having used any mouthwash in the past 1 month.
7. Pregnant or lactating.

### Recruitment, setting and ethics

Patients are being recruited from the Department of Periodontology, Peking University, Hospital of Stomatology, from January 2021. Two weeks after periodontal maintenance treatment (supra/subgingival scaling and polishing), patients are screened by two periodontists (JL, NA) and a postgraduate in periodontology (BL) to determine whether to be enrolled or not.

Informed consent was given to the subjects. The purpose, process, risks and benefits of the study are also verbally explained to the subjects by the researchers with ethical training. Only those who signed informed consent are enrolled in this study. Participants are made aware that they have the right to withdraw from the study at any time.

### Treatment and assessment schedule

The study spans 6 weeks. At baseline, all participants will undergo a full-mouth periodontal examination by a periodontist (JL), using Williams probe (HuFriedy, USA). The parameters included in the periodontal examination are the modified Quigley-Hein plaque index (PLI, Turesky *et al*),<sup>28</sup> gingival index (GI, Loe and Silness),<sup>29</sup> periodontal pocket depth (PD) and gingival sulcus bleeding index (BI, Mazza *et al*).<sup>30</sup> The breath test is conducted using the Helimeter (INTERSCAN, USA)

Baseline		Date: _____	Day1	Day2	Day3	Day4	Day5	Day6	Day7
Rinse method: 10ml mouthwash to rinse for 1 minute, twice per day		Morning							
		Evening							
Teeth brushing: twice per day		Morning							
		Evening							
Taste evaluation	Score 0 = Comfortable								
	Score 1 = Slightly bitter and irritating								
	Score 2 = Moderately bitter and irritating, but tolerable								
	Score 3 = Obviously bitter or irritating, intolerable								
Taste alteration		Yes							
		No							
Staining		Yes							
		No							
Other symptoms									

**Figure 1** Participant diary card. Participants recorded every item in the diary card every day. In ‘Teeth brushing’ column, participants filled in the brushing time. In ‘Other symptoms’ column, participants filled in other symptoms not listed in the card. In other columns in the card, participants ticked selectively.



and samples of subgingival plaque of the representative teeth are collected. All participants will receive supragingival scaling by a well-trained postgraduate (YH) after the examination.

On the day of the baseline, after periodontal examination and supragingival scaling were performed, all the participants will review the Bass toothbrushing method by watching a video and oral instruction. Afterward, they will be instructed to brush their teeth using the Bass method in the morning and evening. The same type of toothbrush and toothpaste (without bacteriostatic substances) provided by the sponsor will be used by each patient. Each brushing time will be recorded in the participant diary card (PDC).

After brushing their teeth, the participants will be asked to use 10mL mouthwash to rinse for 1 min, two times per day, and fill in the PDC (figure 1). Participants in the experimental group will rinse with Pudilan Keyanning antibacterial mouthwash containing the active essence of Pudilan and 0.03%–0.06% CPC. Participants in control group 1 will rinse with 0.12% CHX acetate mouthwash. Participants in control group 2 will rinse with mouthwash containing the same components as Pudilan Keyanning antibacterial mouthwash but without the Pudilan active ingredients.

During the trial, dental floss, interdental brush and other tools will not be used by the participants. A periodontal examination will be performed by the same periodontist at 2, 4 and 6 weeks. Subsequent samples of subgingival plaque will be collected at 4 and 6 weeks. Any staining on the tooth surfaces or dorsum of tongue will be recorded on clinical research form (CRF) (figure 2).

## Parameters and methods of periodontal assessment

*Modified Quigley-Hein PLI*

### *Examination method*

All the teeth of the participants will be stained with a disclosing agent and then examined. Six regional sites of all teeth except the third molars (distal buccal, central buccal, mesial buccal, mesial lingual, central lingual and distal lingual sites) will be scored. The sum of all the site scores is divided by the total number of sites to obtain the average PLI for each participant.

### *Scoring standards*

0=no dental plaque.

1=only separate flecks of dental plaque at the cervical margin of the tooth.

2=a thin continuous band of dental plaques (no more than 1 mm) at the cervical margin of the tooth.

3=the band of dental plaques is wider than 1 mm but covers less than one-third of the crown of the tooth.

4=dental plaque covers more than one-third but less than two-thirds of the crown of the tooth.

5=dental plaque covers at least two-thirds or more of the crown of the tooth.

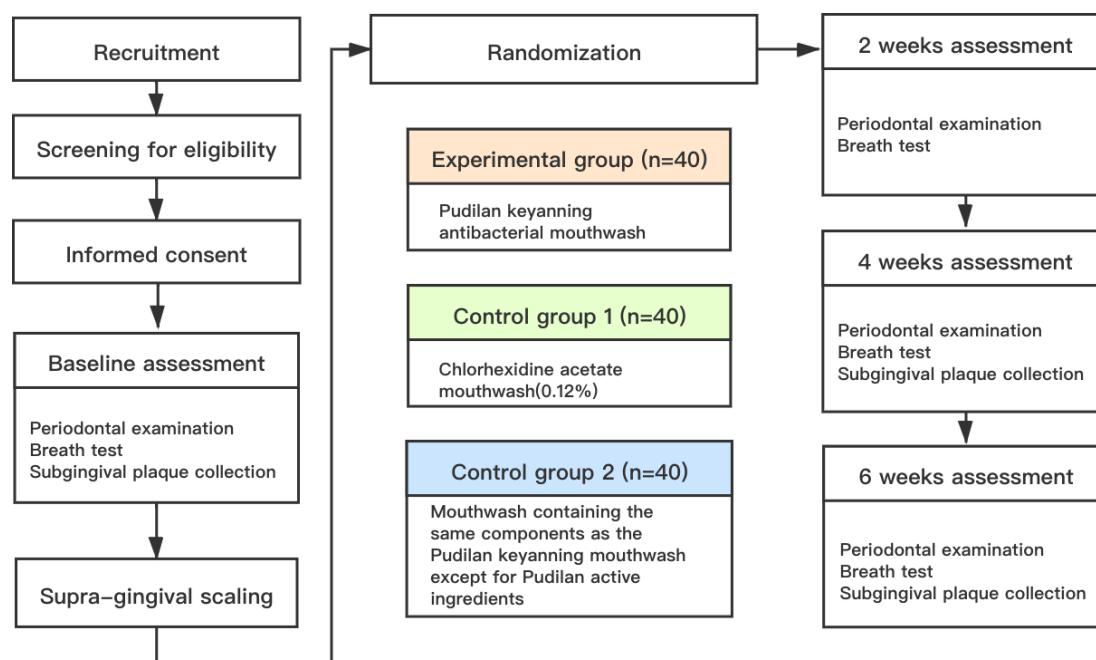
9=unable to be recorded (assessment prevented due to the presence of a lot of dental calculus covering the tooth surface, residual roots, or bad restorations, etc).

X=missing.

## Gingival index

### *Examination methods*

Each gingival unit (labial/buccal, lingual/palatal, mesial, distal) of each tooth except the third molar will be given a score from 0 to 3, called the GI for the area. For the buccal (labial) and lingual/palatal surfaces, the measurements



**Figure 2** Flow chart of this clinical trial.

will be taken at the middle of the surface. For the mesial and distal surfaces, measurements will be taken buccally (labially) to the contact points. Before examination for GI, the gingiva will be dried either by a blast of air and/or cotton rolls. The periodontal probe will be gently placed into the gingival margin, and the gingival condition including bleeding is observed for 30s after the probe was removed. The scores of each tooth will be divided by the four tooth surfaces to determine the mean score. The GI for the patient is thus a mean score for the teeth examined.

#### *Scoring criteria*

0=no inflammation.

1=inflammation is mild: slight change in colour and little change in texture.

2=inflammation is moderate: moderate glazing, redness, oedema and hypertrophy. Bleeding on probing.

3=inflammation is severe: marked redness and hypertrophy of gingiva, which has the tendency to spontaneous bleeding.

9=unable to be recorded (assessment prevented due to the presence of a lot of dental calculus covering the tooth surface, residual roots, or bad restorations, etc).

X=missing.

#### **Periodontal PD**

The periodontal probe will be inserted to the bottom of the periodontal pocket with a strength of 20g. The distance from the bottom of the periodontal pocket to the gingival margin will be measured. Six sites will be recorded for each tooth.

#### **Bleeding index**

##### *Measure method*

The periodontal probe will be inserted to the bottom of the periodontal pocket with a strength of 20 N. The gingival BI will be recorded. Six points will be recorded for each tooth. The score of each tooth will be divided by the 6 points to determine the mean. The BI for the patient is thus an average score for the teeth examined.

#### *Scoring criteria*

0=healthy gingiva, which appears normal.

1=colour changes because of inflammation, without bleeding on probing.

2=slight bleeding that remains at the point of probing.

3=bleeding extending from the point of probing and flowing around the gingival margin.

4=profuse bleeding on probing that overflows the gingival margin.

5=spontaneous bleeding.

##### *Periodontal examination at baseline:*

The modified Quigley-Hein PLI, GI, PD and BI will be examined at baseline. The methods and scoring criteria are the same as detailed above.

#### **Periodontal examination at 2, 4 and 6 weeks**

At the 2-week, 4-week and 6-week follow-up, the periodontal examination indexes—modified Quigley-Hein

PLI, GI and BI—will be recorded. The examination methods and scoring criteria will be the same as at baseline.

#### **Breath examination**

At baseline, the 2nd, 4th and 6th week of the study, the Halimeter breath analyser will be applied according to the manufacturer's instructions to measure the level of volatile sulfide (VSA) (unit: ppb) in the breath. The method is as follows: after turning on the instrument and preheating it, the 'zero' key is pressed. This is followed by a period of waiting as the screen number drops to 20. Then, the patient will be instructed to wrap the straw around his or her mouth and breathe with the nose instead of blowing into the straw. When the second light is on, the straw is removed. The screen number decreases from 180 to reach the final value. The above procedures will be repeated three times. After each examination, the VSA test value displayed by the instrument will be recorded.

#### **Subgingival plaque sample**

Subgingival plaque samples were collected from the mesial buccal and mesial lingual sites of 16, 26, 36, 46, 11, and 31 at baseline, and 4 and 6 weeks. After isolating the area with cotton rolls and gently air-drying it, supragingival deposits were carefully removed with a Gracey curette. A new sterilised Gracey curette will be inserted into the subgingival area to collect subgingival plaque samples. Twelve samples from each participant will be pooled and transferred into a sterilised Eppendorf tube and stored at -80°C until used. DNA from subgingival plaque samples will be extracted and the bacterial composition will be analysed by sequencing the V3-V4 variable region of the 16S rRNA gene at Allwegen Technology (Allwegen Technology Co, Beijing, China).

#### **Randomisation**

Random allocation to Pudilan group, control group 1 or control group 2 occurred through the use of blocked randomisation, with block sizes of 6. The allocation sequence was generated by a study author (WL) using computer-generated random numbers. Allocations were concealed in opaque study envelopes which were opened once the participant had signed the consent form. All mouthwashes used in this study had no tag and were put into white boxes that could not be opened during the visit.

#### **Participant safety**

In this study, an adverse event will be defined as any unfavourable or unintended sign or symptom temporally associated with the use of the allocated mouthwash.

All adverse events will be documented in the CRF, as well as in the diary card. Serious adverse events will be reported to the Medical Ethical Committee of Peking University School and Hospital of Stomatology.



## Quality monitoring

The clinicians conducting the periodontal examination and collecting subgingival plaque had received training on the assessment procedures, scoring criteria and data storing, and passed the self-consistency test. A well-trained periodontist will carry out supragingival scaling. All the staff in this study demonstrated an independent understanding of the correct study procedures. Important protocol modifications were discussed and followed up by the Principal Investigator (PI) (XO) and changes were reported to the ethics committee.

## Sample size

It has been reported that the supragingival plaque was reduced by 42% using CHX mouthwash (0.12%),<sup>31</sup> and by 13.3% using CPC mouthwash (0.075%) for 4 weeks.<sup>32</sup> The supragingival plaque reduction by using Pudilan Keyanning antibacterial mouthwash has not been reported. According to the pharmacological action, it was hypothesised that the plaque may reduce by approximately 18% using Pudilan mouthwash for 4 weeks. With respect to power calculations, we designed this study to have a power of 80% at a two-sided significance level of 0.05. Based on these data, the sample size is calculated by X<sup>2</sup> test, and at least 37 patients per study group were required. Considering a dropout rate of 10%, we enrolled 120 patients (40 per group). The ratio of the three groups was 1:1:1.

## Data management and confidentiality

All periodontal examination forms will be kept by an authorised study research staff. The data will be imported into a specific computer. After data entry and verification, the original data will be filed and saved in the order of numbering. Electronic data files will be stored via CD and an encrypted mobile hard disk, and kept by the authorised study research staff. Access to patient data in both paper and digital forms, under any academic and other conditions, was restricted to the authorised study research staff only.

## Statistical analysis

Full analysis set with the intention-to-treat principle and per-protocol set will be both used for analysis in the study. Descriptive statistical analysis of PLI, GI and sulfide values in the breath will be performed using mean (SD) or median (range) based on data distribution. Clinical parameters among the three groups will be compared using the independent t-test, Mann-Whitney test or X<sup>2</sup> test according to the data type. Intragroup clinical parameters will be assessed by the paired t-test, Mann-Whitney test or X<sup>2</sup> test according to the data type.

Species' diversity analysis based on operational taxonomic units (OTUs) will be performed with QIIME software. The distance matrix between different sequences will be obtained and transformed into similarity, and 97% gene similarity is taken as the threshold value (that is, 3% sequence difference is considered as different OTU).

The community OTU index, species' richness estimation index and species' diversity index scores will be calculated.

Neither patients nor the public will be involved in the process of the study design, implementation and reporting.

## Patient and public involvement

Neither patients nor the public were involved in study design, or enrolment, or conduct, or reporting of this search. The results of this trial will be disseminated to study participants via publication of academic articles about this trial.

**Contributors** XO is the PI of this project and was responsible for designing and supervising the trial. JL is in charge of participant recruitment and oral examinations. YH is involved in supragingival scaling, data collection and analysis. BL is involved in participant recruitment, data collection and analysis. NA is in charge of participant recruitment. RW is in charge of patient appointment and data record. XL is responsible for breath testing and correspondence with the ethics committee. WL is in charge of the sample size calculations, mouthwash preparation, allocation and randomisation.

**Funding** This study was sponsored by Jiangsu Pudilan Daily Chemical Company. The company provided the research funds, Pudilan Keyanning antibacterial mouthwash, toothpaste, toothbrushes and other materials required for the study.

**Disclaimer** The company was not involved in the design, operation, data acquisition and analysis.

**Competing interests** None declared.

**Patient and public involvement** Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

## ORCID iD

Xiangying Ouyang <http://orcid.org/0000-0003-3703-0847>

## REFERENCES

- 1 Sun HY, Jiang H, Du MQ, et al. The prevalence and associated factors of periodontal disease among 35 to 44-year-old Chinese adults in the 4th national oral health survey. *Chin J Dent Res* 2018;21:241–7.
- 2 Villa O, Ramberg P, Fukui H, et al. Interaction between chlorhexidine and fluoride in a mouthrinse solution-a 4-day and 6-week randomized clinical pilot study. *Clin Oral Investig* 2018;22:1439–48.
- 3 Solderer A, Kaufmann M, Hofer D, et al. Efficacy of chlorhexidine rinses after periodontal or implant surgery: a systematic review. *Clin Oral Investig* 2019;23:21–32.
- 4 Blanc V, Isabel S, Sánchez MC, et al. Characterization and application of a flow system for in vitro multispecies oral biofilm formation. *J Periodontal Res* 2014;49:323–32.
- 5 Jose A, Butler A, Payne D, et al. A randomised clinical study to evaluate the efficacy of alcohol-free or alcohol-containing mouthrinses with chlorhexidine on gingival bleeding. *Br Dent J* 2015;219:125–30.
- 6 Latimer J, Munday JL, Buzzia KM, et al. Antibacterial and anti-biofilm activity of mouthrinses containing cetylpyridinium chloride and sodium fluoride. *BMC Microbiol* 2015;15:169.
- 7 Teng F, He T, Huang S, et al. Cetylpyridinium chloride mouth rinses alleviate experimental gingivitis by inhibiting dental plaque maturation. *Int J Oral Sci* 2016;8:182–90.
- 8 Herrera D, Escudero N, Pérez L, et al. Clinical and microbiological effects of the use of a cetylpyridinium chloride dentifrice and mouth

- rinse in orthodontic patients: a 3-month randomized clinical trial. *Eur J Orthod* 2018;40:465–74.
- 9 Pizzo G, Guiglia R, Imburgia M, et al. The effects of antimicrobial sprays and mouthrinses on supragingival plaque regrowth: a comparative study. *J Periodontol* 2006;77:248–56.
- 10 Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China 2020:1787–8.
- 11 González-Castejón M, Visioli F, Rodriguez-Casado A. Diverse biological activities of dandelion. *Nutr Rev* 2012;70:534–47.
- 12 You Y, Yoo S, Yoon H-G, et al. In vitro and in vivo hepatoprotective effects of the aqueous extract from *Taraxacum officinale* (dandelion) root against alcohol-induced oxidative stress. *Food Chem Toxicol* 2010;48:1632–7.
- 13 Zhao T, Tang H, Xie L, et al. *Scutellaria baicalensis* Georgi. (Lamiaceae): a review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. *J Pharm Pharmacol* 2019;71:1353–69.
- 14 Zhai X-T, Chen J-Q, Jiang C-H, et al. *Corydalis bungeana* Turcz. attenuates LPS-induced inflammatory responses via the suppression of NF-κB signaling pathway in vitro and in vivo. *J Ethnopharmacol* 2016;194:153–61.
- 15 Xiao P, Huang H, Chen J, et al. In vitro antioxidant and anti-inflammatory activities of *Radix Isatidis* extract and bioaccessibility of six bioactive compounds after simulated gastro-intestinal digestion. *J Ethnopharmacol* 2014;157:55–61.
- 16 Wang T, Wang X, Zhuo Y, et al. Antiviral activity of a polysaccharide from *Radix Isatidis* (*Isatis indigotica* fortune) against hepatitis B virus (HBV) in vitro via activation of JAK/STAT signal pathway. *J Ethnopharmacol* 2020;257:112782.
- 17 Xiao P, Huang H, Li X, et al. Characterization, evaluation of nutritional parameters of *Radix isatidis* protein and its antioxidant activity in D-galactose induced ageing mice. *BMC Complement Altern Med* 2019;19:297.
- 18 Kong Q, Wu Y, Gu Y, et al. Analysis of the molecular mechanism of Pudilan (PDL) treatment for COVID-19 by network pharmacology tools. *Biomed Pharmacother* 2020;128:110316.
- 19 Feng L, Yang N, Li C, et al. Pudilan xiaoyan oral liquid alleviates LPS-induced respiratory injury through decreasing nitrooxidative stress and blocking TLR4 activation along with NF-κB phosphorylation in mice. *J Ethnopharmacol* 2018;214:292–300.
- 20 Jin Y, Lin X, Song L, et al. The effect of Pudilan anti-inflammatory oral liquid on the treatment of mild recurrent aphthous ulcers. *Evid Based Complement Alternat Med* 2017;2017:1–6.
- 21 Bian L-L, Wang F-W, Yang Y, et al. [Systematic review of effectiveness and safety of pudilan Xiaoyan oral liquid in treating pediatric suppurative tonsillitis]. *Zhongguo Zhong Yao Za Zhi* 2017;42:1482–8.
- 22 Bai Y, Li Y-X, Shi Y-J, et al. [Meta-analysis on effectiveness and safety of Pudilan Xiaoyan Oral Liquid on child upper respiratory infection]. *Zhongguo Zhong Yao Za Zhi* 2020;45:2203–9.
- 23 Rong P, Pan G, Wang X. Meta-Analysis on incidence of ADR induced by Pudilan Xiaoyan oral liquid. *Pharmacology and Clinics of Chinese Materia Medica* 2020;36:206–14.
- 24 Guerra F, Pasqualotto D, Rinaldo F, et al. Therapeutic efficacy of chlorhexidine-based mouthwashes and its adverse events: Performance-related evaluation of mouthwashes added with Anti-Discoloration system and cetylpyridinium chloride. *Int J Dent Hyg* 2019;17:229–36.
- 25 Eduardo FdeP, Corrêa L, Heller D, et al. Salivary SARS-CoV-2 load reduction with mouthwash use: a randomized pilot clinical trial. *Heliyon* 2021;7:e07346.
- 26 Rajendiran M, Trivedi HM, Chen D, et al. Recent development of active ingredients in mouthwashes and Toothpastes for periodontal diseases. *Molecules* 2021;26. doi:10.3390/molecules26072001. [Epub ahead of print: 01 Apr 2021].
- 27 Zhao L-J, Gao W-Y, Gu X-R, et al. [Identification and attribution of chemical compounds of Pudilan Antiphlogistic Oral Liquid]. *Zhongguo Zhong Yao Za Zhi* 2019;44:1573–87.
- 28 Turesky S, Gilmore ND, Glickman I. Reduced plaque formation by the chloromethyl analogue of victamine C. *J Periodontol* 1970;41:41–3.
- 29 Loe H, Silness J. Periodontal disease in pregnancy. I. prevalence and severity. *Acta Odontol Scand* 1963;21:533–51.
- 30 Mazza JE, Newman MG, Sims TN. Clinical and antimicrobial effect of stannous fluoride on periodontitis. *J Clin Periodontol* 1981;8:203–12.
- 31 Leyes Borrajo JL, Garcia VL, Lopez CG, et al. Efficacy of chlorhexidine mouthrinses with and without alcohol: a clinical study. *J Periodontol* 2002;73:317–21.
- 32 Rawlinson A, Pollington S, Walsh TF, et al. Efficacy of two alcohol-free cetylpyridinium chloride mouthwashes - a randomized double-blind crossover study. *J Clin Periodontol* 2008;35:230–5.