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RESEARCH ARTICLE

REVISED *Efficacy of Lycium barbarum* (Goji berry) mouthwash for managing periodontitis: a randomized clinical trial

[version 4; peer review: 2 approved, 3 approved with reservations]

Previously titled: *Lycium barbarum* (Goji berry) mouthwash is a viable alternative to 0.2% chlorhexidine gluconate for managing chronic periodontitis: a randomized clinical trialAmees Sanghavi¹, Laasya Shettigar¹, Aditi Chopra ², Ashmeet Shah¹, Richard Lobo ³, Padmaja A Shenoy ⁴, ShivaPrasada Gadag⁵, Usha Y Nayak ⁵, Mangalore Shravya S⁶, Shobha Ullas Kamath⁶, Prajna P Nayak⁷¹Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India²Periodontology, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India³Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India⁴Microbiology, Kasturba Medical College and Hospital, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India⁵Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India⁶Biochemistry, Kasturba Medical College and Hospital, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India⁷Public Health Dentistry, Manipal College of Dental Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, 576104, India**v4** First published: 20 Mar 2023, 12:302
<https://doi.org/10.12688/f1000research.129891.1>Second version: 16 Nov 2023, 12:302
<https://doi.org/10.12688/f1000research.129891.2>Third version: 05 Jul 2024, 12:302
<https://doi.org/10.12688/f1000research.129891.3>Latest published: 26 Nov 2024, 12:302
<https://doi.org/10.12688/f1000research.129891.4>**Abstract****Background**

Removal of the microbial deposits (plaque and calculus) by performing effective scaling and root planing (SRP) is the fundamental step for managing periodontal disease (gingivitis and periodontitis). Various adjuncts in the form of mouthwash, gels, and toothpaste are also being used as adjuncts to SRP for managing periodontitis. Recently, *Lycium barbarum* (*L. barbarum*), commonly known as goji berry or wolfberry, has gained popularity for managing chronic inflammatory and infectious diseases. However, its efficacy in managing periodontitis has never been explored. Hence the present study aims to evaluate the efficacy of goji berry mouthwash compared

Open Peer Review**Approval Status** ? ✓ ✓ ? ?

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version 4					
(revision)			✓	?	?
26 Nov 2024			view	view	view
version 3					
(revision)		✓	✗		
05 Jul 2024		view	view		
version 2					
(revision)		?			
16 Nov 2023		view			
version 1					
20 Mar 2023	?	✗			
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to chlorhexidine mouthwash for managing periodontitis.

Methods

60 adult participants were divided randomly using computer-generated random sequences into two groups (case group: *L. barbarum* mouthwash (Males: 16; Female: 14); control group: 0.2% chlorhexidine gluconate mouthwash (Males: 14; Females: 16)). The changes in the gingival index (Gi), plaque index (Pi), bleeding on probing (BOP), clinical attachment loss (CAL), probing pocket depth (PPD), microbial load, and antioxidant levels (protein thiol) in saliva were noted at the baseline, at 15 days and one month.

Results

A significant reduction in the mean PPD, Pi, BOP, and Gi was seen from baseline to one month in both the control (P-value=0.006, 0.027, 0.000, and 0.036 respectively) and test groups (P-value=0.035, 0.000, 0.034, and 0.000 respectively). However, the antioxidant levels (protein thiol) in saliva were significantly increased only in the test group.

Conclusion

Goji berry mouthwash along with SRP reduced the Gi, Pi, BOP, and PPD in patients with periodontitis. However, no statistically significant difference was noted between the use of goji berry and chlorhexidine mouthwash. Goji berry mouthwash was more effective than chlorhexidine in increasing the antioxidant levels in saliva.

Keywords

Periodontitis, Periodontal disease, Oral health, Dental Hygiene, Lycium Barbarum, Goji berry, Mouthwash, Chlorhexidine, Herbal, Antioxidants

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Competing interests: No competing interests were disclosed.

Grant information: The author(s) declared that no grants were involved in supporting this work.

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How to cite this article: Sanghavi A, Shettigar L, Chopra A *et al.* *Efficacy of Lycium barbarum (Goji berry) mouthwash for managing periodontitis: a randomized clinical trial* [version 4; peer review: 2 approved, 3 approved with reservations] F1000Research 2024, 12:302 <https://doi.org/10.12688/f1000research.129891.4>

First published: 20 Mar 2023, 12:302 <https://doi.org/10.12688/f1000research.129891.1>

REVISED Amendments from Version 3

The following changes have been made:

1. The microbiology data has been added to the study.
2. The new data on the prevalence of periodontitis globally has been added to the introduction.
3. The Figure 1 has been revised.
4. The intragroup and intergroup changes in BOP have been added to the text.
5. The method of plaque collection has been added in more detail in the methodology section.
6. The results on the stability of the mouthwash are added to the results section.
7. The grammatical and typographical errors have been corrected.

Any further responses from the reviewers can be found at the end of the article

Introduction

Periodontitis is defined as a chronic immuno-inflammatory multifactorial disease that affects the soft tissue around the teeth.¹ Severe periodontitis and edentulism represent a significant public health challenge, with the number of affected individuals projected to increase considerably in the coming decades. In 2021, over one billion people were affected by severe periodontitis, with a global age-standardised prevalence of 12.50%. South Asia had by far the highest prevalence rate, at 17.57%. It may be reasonably extrapolated that severe periodontitis will be among the most impactful level 4 diseases/conditions affecting years lived with disability worldwide by 2050.² It is more prevalent in males compared to females. It is primarily caused by the interaction of the oral microorganisms with the host (soft tissues surrounding the teeth. This host-microbial interaction triggers a massive influx of various pro-inflammatory mediators, microbial by-products, proteolytic enzymes, and free reactive oxygen species (ROS) resulting in increased periodontal hard and soft tissue destruction and inflammation.^{3–6} Due to increased ROS during periodontal disease, it is also referred to as free radical-mediated tissue injury.⁷ Some of the common putative periodontal microbes associated with periodontitis are *Porphyromonas gingivalis*, *Tanarella forsythia*, *Treponema denticola*, *Campylobacter rectus*, and *Fusobacterium nucleatum*.⁷ Apart from the microbial etiology, other risk factors such as smoking, diabetes mellitus, HIV, nutritional deficiency, medications, poor oral hygiene, and genetics influence the severity of periodontal inflammation.⁸

To control periodontal inflammation the primary and most vital step is the removal of oral biofilm formed around the teeth by performing effective mechanical debridement with either hand or machine-driven instruments.^{9,10} Effective scaling and root planing (SRP) can reduce gingival inflammation, thereby preventing disease progression and restoring gingival health.¹¹ However, studies have shown that complete debridement of calculus is technically demanding, as many times removal of hard and soft tissue deposits from deep pockets (>5 mm) and interdental regions becomes challenging.^{11,12} Additionally, it is difficult to completely remove the smooth or burnished calculus from deep and circuitous periodontal pockets, furcation areas, root concavities, and irregular roots owing to a lack of good visibility and accessibility to such areas.^{12,13} Hence for the management of biofilm from deep and tortuous pockets additional periodontal therapy and use of adjuncts are needed.^{12–15}

It is also noted that the efficacy of SRP is dependent on the patient's compliance and motivation to maintain a meticulous oral care regime and effective plaque control at home.¹⁶ SRP alone may not be sufficient to maintain the required plaque control if patient compliance is poor and the patient does not effectively follow oral hygiene instructions. Additionally, studies have shown that even after a good plaque control regime; posterior, palatal, and lingual surfaces of the teeth retain some amount of plaque. Thus, for many patients, adjuncts such as mouthwash and gels along with regular toothbrushing are indicated.^{11,17–24} Various chemical plaque control agents with antimicrobial and anti-inflammatory are being used in the form of mouthwash, gels, gum paints, fibers, varnishes, microspheres, chips, tablets, powder, and capsules for managing gingivitis and periodontitis. Among these agents, chlorhexidine gluconate is the most popular and routinely used agent for managing periodontitis. Additionally, recent reports have shown no additional benefits of using chlorhexidine and the development of antimicrobial resistance among many oral bacteria to chlorhexidine molecules.^{25–29} Prolonged use of chlorhexidine is contraindicated owing to various side effects like altered taste sensation, staining of the teeth and soft tissues such as tongue and mucosa, increased calculus formation, and parotid gland swellings.²⁵ Chlorhexidine is also known to have cytotoxic effects on the gingival fibroblasts,²⁶ periodontal ligament,²⁷ and osteoblastic cells.²⁸

Thus, there is an emerging trend to use natural and herbal extracts with antioxidant and antimicrobial properties to treat periodontal diseases. Herbal extracts from neem, Tulsi, guava, green tea, turmeric, curcumin, pomegranate, and many more plants have been tried to effectively treat gingivitis and periodontitis.^{30–34} Since periodontal disease causes a

massive release of ROS and increases oxidative stress, adjuncts with antioxidant potential are often used with SRP to control the oxidative stress present locally in the gingival and periodontal tissues. Recently, goji berry, commonly known as Wolfberry, Himalayan goji, or Tibetan goji, has gained a lot of popularity due to its strong antioxidant and anti-inflammatory properties.^{35–46}

Goji berry, scientifically known as *Lycium barbarum* (*L. barbarum*), is a fruit native to southeast Europe, China, and Asia.³⁵ The fruit belongs to the family of Solanaceae and is consumed in both fresh and dried forms. It has powerful antioxidant, antimicrobial, immuno-modulating, and anticancer properties.^{36–46} It is used for the treatment of many inflammatory and infectious diseases, including oral diseases.^{36–46} Soesanto et al. (2021) showed that ethanolic extract of *L. barbarum* is effective against oral bacteria (*Streptococcus mutans* and *P. gingivalis*) at 100 µg/mL.⁴⁷ Previous *in-vitro* studies have also reported that the minimal inhibitory concentration (MIC) of *L. barbarum* was comparable to chlorhexidine for inhibiting oral bacteria, however, its efficacy was less as compared to the antibiotic doxycycline. At 50 µg/mL, ethanolic extract of goji berry could inhibit most of the periodontal pathogens.^{48,49} However, no clinical study has yet assessed the effectiveness of goji berry mouthwash as an adjunct to SRP for the management of periodontitis. Therefore, this clinical study aims to evaluate the efficacy of *L. barbarum* mouthwash along with SRP for patients with periodontitis compared to chlorhexidine for the first time.

The objectives of the study include

1. To evaluate the effect of *L. barbarum* (goji berry) mouthwash on the Plaque index (Pi), Gingival index (Gi), Bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) at baseline, 15 days and one month compared to 0.2% chlorhexidine gluconate mouthwash.
2. To evaluate and compare the change in the protein thiol levels in saliva at 15 days, and one month compared to baseline in participants using goji berry and chlorhexidine mouthwash.
3. To evaluate and compare the reduction in the microbial count at the end of one month compared to baseline in participants using *L. barbarum* mouthwash compared to chlorhexidine mouthwash.

Methods

Trial design

The study was designed as a randomized, double-blind single-centered parallel arm clinical trial with an allocation ratio of 1:1. The study was conducted at Manipal from 2019 to 2020 following the “Helsinki Declaration of 1975 (as revised in 2000)”. The trial was initiated after receiving ethical clearance from the Kasturba Medical Hospital Institutional Ethics Committee with IEC no 117/2019. The trial was registered at the ‘Clinical Trial Registry (CTRI/2019/05/019042)’ and followed the CONSORT and SAGER guidelines (Figures 1 and 2).⁴⁸ The steps in the clinical trial are explained as follows:

Formulation of mouthwash

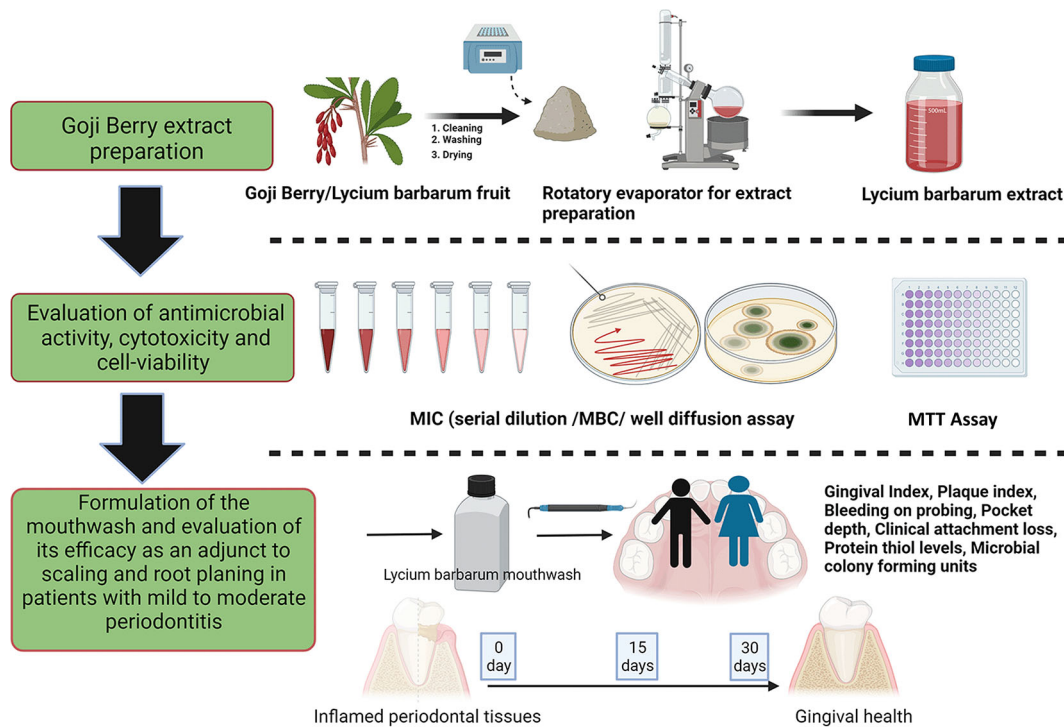
The mouthwash was prepared at the “Manipal College of Pharmaceutical Sciences, Manipal”. *L. barbarum* mouthwash was prepared by preparing an ethanolic extract of dried goji berry using Soxhlet’s method and dissolving the 50 µg/ml of weighed goji berry extract for formulating the mouthwash as described previously.⁴⁹ A concentration of 50 µg/ml was mixed in 1.15 of 100% alcohol (ethanol) and then homogenized using 15% glycerol. To this around 15% propylene glycol and 1% Tween solution was added to formulate the mouthwash. 0.1% menthol was added to adjust the taste of the mouthwash. The volume was adjusted to 100% by using distilled water. 100% alcohol was added to serve both as a preservative and a dissolvent.

Assessment of stability of mouthwash

The shelf life of the mouthwash was measured as per the ICH guidelines and the samples were tested for three months under three different conditions: “35°±2% C with a relative humidity of 60% ± 5%; 25°C± 2°C and relative humidity: 60% ± 5%; and 40°C ± 2°C with a relative humidity of 75% ± 5%”. The mouthwash was kept in opaque plastic bottles and one of the bottles was kept in the stability compartment (Thermo lab, India). The samples were analyzed at baseline, one, two, and three months by visual observation and UV spectrum analysis (Shimadzu UV-1601PC, Japan) and analyzed for their physical parameters and stability.

Clinical study design

Ninety subjects aged 20 to 50 years (both male/females) visiting the outpatient department were screened for the presence of localized/generalized periodontitis according to the 2017 classification of periodontal disease and selected based on the following exclusion and inclusion criteria⁵⁰:



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Figure 1. Schematic representation of the study design (Created in Biorender.com).

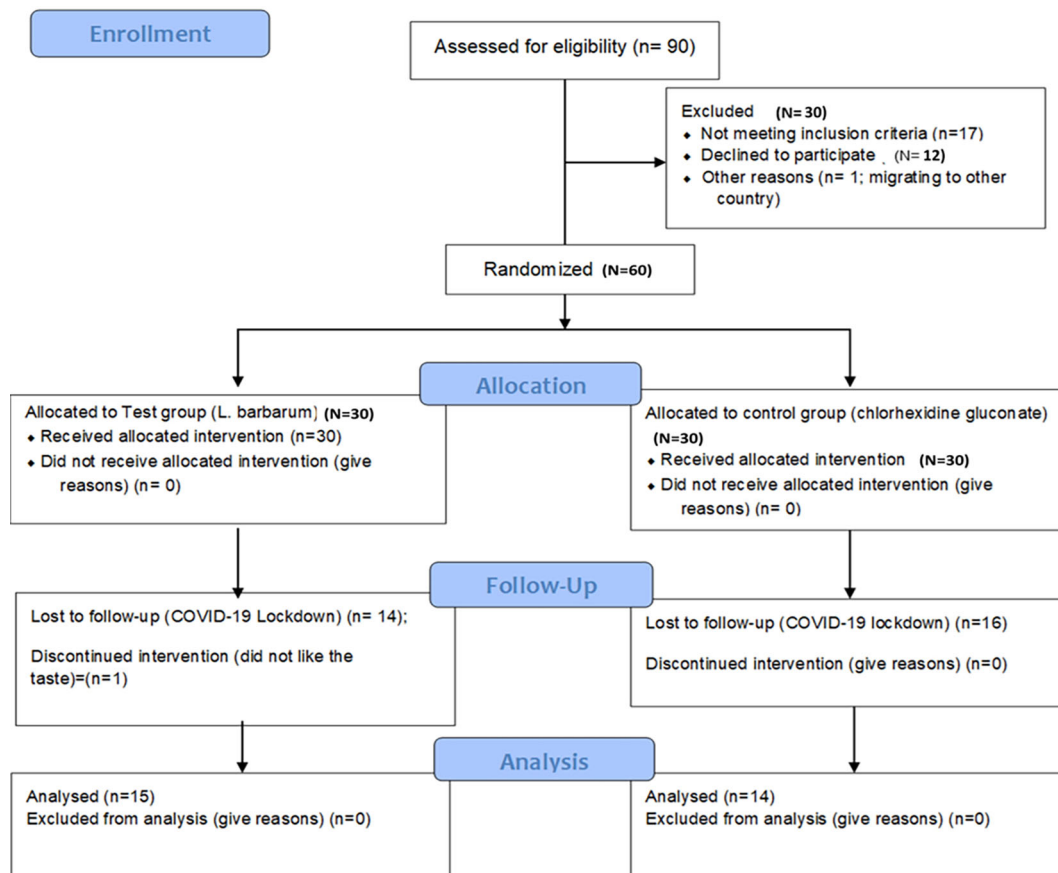


Figure 2. Participant flow diagram.

Inclusion criteria:

1. Participants in the age group of 20-50 years diagnosed with stage I to stage II localized/generalized periodontitis (Grades A to B).
2. Participants with a minimum of 24 functional teeth.

Exclusion criteria:

1. Participants with any allergic reactions to chlorhexidine.
2. Participants with any systemic diseases such as hypertension, diabetes mellitus, cardiovascular, renal, and neurological diseases.
3. Participants who were on any anti-inflammatory, antibiotics, or analgesics in the last six months.
4. Pregnant or lactating mothers.
5. Participants with any oral abusive habits such as smoking, alcohol, betel nut chewing, gutka, paan, supari, and areca nut.
6. Participants who had undergone any periodontal treatment/surgery in the last six months.
7. Participants undergoing orthodontic treatment.
8. Participants using any other oral hygiene agents (mouthwash or gels) were excluded to remove confounding bias arising due to the difference in plaque control measures.

All participants who satisfied the above-mentioned criteria were recruited after obtaining both oral and written informed consent.

Randomization, allocation concealment, and blinding

After screening the participants, 60 participants were recruited. The participants were randomly allocated to the test and control group using the computer-generated random sequence as follows: the test group (*L. barbarum*, n = 30) or the control group (0.2% chlorhexidine gluconate mouthwash (Hexidine, ICPA Health Products, India) n = 30). This sample size was based on p-value <0.05, alpha value = 0.05 two-tailed, power = 0.8, and the effect size = 0.7 for CAL. The allocation of participants into two groups was done by an investigator who was not part of either biological sample collection, analysis, periodontal therapy (SRP), or clinical or periodontal examination. The allocation concealment was done by dispensing both mouthwash in similar-sized opaque amber-colored bottles to all participants. The investigators giving the bottles to the patients did not know the content and nature of the mouthwash. The participants were also blinded about their group about the content of the mouthwash, although the taste of the mouthwash could not be matched. The investigator and statistician analyzing the clinical data were not aware of the grouping. Following grouping, the following biological samples were collected at baseline:

a. Microbiology plaque sample collection

“The plaque from the subgingival region was collected from the pocket with the greatest probing depth in each quadrant using a sterile, site-specific Gracey curette. Following collection, the curette was promptly immersed in a reduced transport medium (Thioglycolate both supplemented with hemin and vitamin K) for microbiological analysis. Each sample underwent vortexing, and quantitative cultures were performed across various dilutions (1:10, 1:100, 1:1000, and 1:10,000). A volume of 10 µl from each dilution was plated onto pre-reduced 5% sheep blood agar (BD, Becton Dickinson, Heidelberg, Germany), with plates incubated at 37°C for 48 hours in an anaerobic chamber (Whitley A35 Anaerobic workstation, Don Whitley Scientific, Shipley, UK). The colony-forming units were analyzed for each sample using the following formula: CFU/mL = (number of colonies × dilution factor)/volume of the culture plate.”

b. Saliva Collection

Following plaque collection, a stringent method for saliva collection was followed. All participants were requested to sit comfortably in an upright position. Following this, around 2ml of saliva was collected by the 'spitting method' and without any stimulation.⁵¹ All participants were requested to spit saliva into an Eppendorf vial. The collected saliva was then stored immediately in a refrigerator at -80 degrees Celsius. The Eppendorf tubes used to store saliva samples were numerically marked according to the participant number. The saliva collected was sent for biochemical analysis for evaluation of the protein thiol levels using Ellman's Reagent. The absorbance of the agent was measured after incubation at room temperature at 412 nm for around five minutes and the concentration of protein thiol was determined with the standard curve of glutathione.

c. Examination of the clinical periodontal parameters

Following saliva sample collection, a periodontal examination was done for all the sextants for all participants. The following periodontal parameters were recorded: Gi by Loe & Silness, 1963; percentage of sites with BOP; Pi by Silness & Loe 1964; PD and CAL. All the clinical evaluations were done by an investigator who was blinded about the patient's grouping. The BOP, PPD, and CAL were recorded by the Williams periodontal probe (Hu-Friedy, USA). The sites with BOP were checked by noting the presence and absence of bleeding on all four surfaces (buccal, lingual, mesial, and distal) for all the teeth. The percentage of sites with BOP was considered by the percentage of the teeth with BOP to the total teeth present. The PPD and CAL were calculated at the buccal, mesial, distal, and lingual sides. At the interdental region (mesial and distal), probing was done from both the buccal and lingual sides. The deepest pocket depth at each surface was recorded. The average or mean of the interdental pocket on each side along with buccal and lingual probing depth was considered as the final PD for that tooth. The mean PPD and CAL were assessed by adding the reading from each tooth and dividing it by the total number of teeth.

A thorough SRP was initiated for all the participants after periodontal examination and sample collection. A single trained investigator examined the baseline and follow-up visits for all the patients. The supervisor assessed the scaling to ensure the complete removal of plaque and calculus was done. All patients were educated to brush their/teeth in modified bass technique for two minutes twice daily, nullifying any confounding effects arising from differences in the oral hygiene measures. All participants were given the opaque amber color bottles which were coded (AX or BX). This was done to blind the patients and investigators regarding the type of mouthwash given to the participants. All patients were instructed to use 10 ml mouthwash (goji berry mouthwash) diluted with 10 ml of water for 30-45 seconds twice a day for a month. For chlorhexidine (Hexidine mouthwash, India), participants were requested to use the undiluted mouthwash. Patients were recalled after 15 days and one month for reevaluation. At each recall visit Pi, Gi, percentage of sites with BOP, CAL, and PPD were noted clinically. The plaque from the same site and saliva samples were also collected at each recall visit. The investigation for each participant, at each recall visit, was done by the same investigator.

Statistical analysis

Data obtained was analyzed by the 'SPSS version 26.0, IBM'. For descriptive data frequency and categorical data mean and standard deviation for all the numerical data were analysed using the 'Kolmogorov-Smirnov test'. The normality of the distribution was checked for all variables. The inter-group comparisons of all the assessed outcomes were done using an 'independent sample t-test'. The comparisons between the goji berry mouthwash and chlorhexidine mouthwash were done to measure any significant increase/reduction from baseline to 15 days and one month using Repeated measures ANOVA with Geisser correction' followed by a 'post-hoc analysis with Bonferroni adjustment'. Inter-group comparison of reduction of all the variables at follow-up was done using ANCOVA after adjusting the respective baseline scores. The p-value of less than 0.05 was considered to be significant.

Results

The results of the stability of the mouthwash showed no change in color, odor, consistency, or phase separation at three months. The sample showed no change when observed under the ultraviolet analysis at 274 nm. This indicated that the mouthwash was stable till the end of three months (Table 1).

Results from the clinical trial: Of the 90 participants assessed for eligibility, 30 participants were excluded as they did not meet the eligibility criteria. The rest 60 participants were allocated into test (n=30) and control (n=30) groups. The mean age of participants in the test group was 35.42 ± 11.79 years and in the controls was 32.12 ± 12.85 . The gender-wise distribution in the test group was males: 16; females: 14 and the control group was males: 14; females: 16 (Table 2). Out of the 30 patients in each group, 14 patients in the test group and 16 patients in the control did not come for the follow-up visit due to the sudden lockdown imposed by COVID-19. Additionally, one patient in the test group reported the bitter taste of the goji berry mouthwash and discontinued the mouthwash. Thus, for analysis 15 patients in the test and 14 patients in the

Table 1. Physical properties and evaluation of the stability of the mouthwash.

Temperature	Evaluation parameters	Observation (in months)			
		Baseline	1	2	3
Room temperature (3 – 5 ± 2°C)	Visual appearance	Slight yellowish-brown	Slight yellowish-brown	Slight yellowish-brown	Slight yellowish-brown
	Precipitation/ Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room temperature (25°C ± 2°C) Relative humidity (60% ± 5%)	Visual appearance	Slight yellowish-brown	Slight yellowish-brown	Slight yellowish-brown	Slight yellowish-brown
	Precipitation/ Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good
Room temperature 40°C ± 2°C Relative humidity (75% ± 5%)	Visual appearance	Slight yellowish-brown	Slight yellowish-brown	Slight yellowish-brown	Slight yellowish-brown
	Precipitation/ Phase separation	Nil	Nil	Nil	Nil
	Homogeneity	Good	Good	Good	Good

Table 2. Demographic data of the groups.

Groups	Goji berry group (case group)	Chlorhexidine group (Control group)	p-value
Age (in Years)	35.42 ± 11.79	32.12 ± 12.85	0.29 ^a
	Male-Female	Male-Female	
Gender	16-14	13-14	0.11 ^b

Based on: One-way ANOVA/Chi Square test.

control were included in the analysis. The comparison between goji berry mouthwash and chlorhexidine mouthwash showed no significant differences in the mean values for Pi (p = 0.470), Gi (p = 0.239), BOP (p = 0.450), PPD (p = 0.216), CAL (p = 0.220), and Microbial level (p = 0.251) (Table 3).

The intra-group comparison showed a significant reduction in the mean PPD, Pi, BOP, and Gi, and antioxidant capacity from baseline to one month in both the control and test groups. The CAL reduced significantly only in the chlorhexidine group compared to the goji berry group (Table 3, Table 4). The percentage of sites with BOP reduced from 84.8 ± 0.32 at

Table 3. Inter-group and intra-group comparison of baseline, 15 days, and 1-month follow-up scores.

	Group	Baseline	15 days	1 month	P-value ¥		
		Mean ± SD	Mean ± SD	Mean ± SD	Baseline vs 15 days	Baseline vs 1 month	15 days vs 1 month
Plaque index	Control	1.45 ± 0.42	1.03 ± 0.50	0.92 ± 0.43	0.104	0.027	0.334
	Test	1.6 ± 0.38	1.04 ± 0.36	0.89 ± 0.17	0.000	0.000	0.076
	P-value #	0.470					
Gingival index	Control	1.4 ± 0.64	0.87 ± 0.47	0.81 ± 0.43	0.001	0.036	0.214
	Test	1.35 ± 0.38	0.85 ± 0.31	0.75 ± 0.24	0.000	0.000	0.092
	P-value #	0.239					

Table 3. Continued

	Group	Baseline	15 days	1 month	P-value ^Y		
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Baseline vs 15 days	Baseline vs 1 month	15 days vs 1 month
Percentage of sites with bleeding on probing	Control	80.7 $\pm$ 0.42	42.7 $\pm$ 0.49	10.7 $\pm$ 0.42	0.001	0.034	0.210
	Test	84.8 $\pm$ 0.32	40.8 $\pm$ 0.29	09.0 $\pm$ 0.32	0.000	0.000	0.090
	P-value [#]	0.450					
Probing pocket depth	Control	2.35 $\pm$ 0.56	1.62 $\pm$ 0.56	1.78 $\pm$ 0.35	0.004	0.006	0.186
	Test	2.76 $\pm$ 1.06	1.73 $\pm$ 0.45	2.15 $\pm$ 0.68	0.002	0.035	0.327
	P-value [#]	0.216					
Clinical attachment level	Control	2.03 $\pm$ 0.90	1.52 $\pm$ 0.65	1.33 $\pm$ 0.43	0.020	0.005	0.174
	Test	2.62 $\pm$ 1.16	2.29 $\pm$ 0.99	2.02 $\pm$ 0.61	0.103	0.248	0.260
	P-value [#]	0.220					
Biochemical analysis	Control	233.06 $\pm$ 144.49	235.48 $\pm$ 103.39	225.06 $\pm$ 72.11	0.874	0.899	0.760
	Test	132.68 $\pm$ 56.17	227.05 $\pm$ 72.21	248.30 $\pm$ 68.31	0.000	0.000	0.272
	P-value [#]	0.021					
Microbial level	Control	7.44 $\pm$ 0.43	7.35 $\pm$ 0.584	7.33 $\pm$ 0.567	0.183	0.130	0.756
	Test	7.57 $\pm$ 0.77	7.19 $\pm$ 0.74	7.04 $\pm$ 0.85	0.222	0.564	0.264
	P-value [#]	0.251					

Test: L. barbarum mouthwash; Control: chlorhexidine gluconate mouthwash.

N for Case group = 15; Control group = 14. Values in bold indicate significant difference.

[#]P-value for inter-group comparisons (independent sample *t*-test);

^YP-value for intra-group comparisons (Repeated measures ANOVA).

baseline in the test group to 09.0 $\pm$ 0.32 at the end of six months. These results were comparable to the control group with no statistically significant difference between the test and control. A significant difference was noted in the antioxidant levels (protein thiol) in saliva in the goji berry group alone at the end of one month. No change in the salivary antioxidant level was noted in the chlorhexidine group. No significant differences were reported in the log-transformed microbial CFU counts in both groups at any given time. In the case of the test group, the mean Pi reduced from 1.6 $\pm$ 0.38 at baseline and was 0.89 $\pm$ 0.17 at one month. However, in the case of the goji berry mouthwash, the mean PPD was reduced during the 15-day follow-up (1.73 $\pm$ 0.45) compared to the baseline (2.76 $\pm$ 1.06).

Intergroup comparison at one-month follow-up (15 days and one month) was done using ANCOVA after adjusting the respective baseline scores. There were no differences seen in the mean values for Pi ($p = 0.791$), Gi ($p = 0.594$), PPD ($p = 0.134$), protein thiol levels ($p = 0.211$), and microbial levels ($p = 0.188$) between the two groups (Table 4). No harms were reported by any patient.

Table 4. Inter-group comparisons at one month follow-up after adjusting for baseline values.

Outcomes	Adjusted baseline	One-month		p-value
		Control Mean $\pm$ SE	Test Mean $\pm$ SE	
Plaque index	1.61	0.92 $\pm$ 0.092	0.88 $\pm$ 0.09	0.791
Gingival index	1.46	0.82 $\pm$ 0.100	0.75 $\pm$ 0.10	0.594
Percentage of sites with BOP	78.4	12.9 $\pm$ 0.56	13.1 $\pm$ 0.42	0.494
Probing pocket depth	2.06	1.77 $\pm$ 0.254	2.15 $\pm$ 0.26	0.134
Clinical attachment level	2.33	1.33 $\pm$ 1.22	2.02 $\pm$ 0.11	0.001
Biochemical analysis	182.77	225.06 $\pm$ 16.83	248.30 $\pm$ 16.83	0.211
Microbial level	7.45	7.33 $\pm$ 0.17	7.05 $\pm$ 0.19	0.188

Values in bold indicate significant difference.

Discussion

The present study is the first clinical trial to evaluate the role of goji berry mouthwash in managing periodontitis. The study assessed the efficacy of goji berry mouthwash compared to 0.2% chlorhexidine mouthwash in controlling the gingival inflammation, plaque formation, BOP, PPD, CAL, microbial profile, and antioxidant levels (protein thiols) in the saliva. We found that the mean reduction in Gi, Pi, BOP, and PPD with goji berry mouthwash was comparable to chlorhexidine mouthwash. The reduction in CAL was better for chlorhexidine mouthwash compared to goji berry mouthwash. However, we did not find any statistically significant difference between the goji berry mouth and chlorhexidine mouthwash for Gi, Pi, BOP, and PPD at the end of one month. We compared the goji berry mouthwash to chlorhexidine since chlorhexidine is considered the gold standard mouthwash for managing patients with periodontitis. However in the current times when oral bacteria are becoming resistant to chlorhexidine, there is a need to look for viable alternatives to chlorhexidine for managing periodontitis.^{51–54} Additionally, it is known that since prolonged use of chlorhexidine is not advised for more than one month and oral bacteria may develop resistance within 10 days, this preliminary study was done with only a small pilot sample size with recall visits for one month.^{55,56} Prolonged and continuous use of chlorhexidine is contraindicated to prevent the onset of various side effects such as alterations in taste, staining, and increased calculus, alternatives to chlorhexidine become important. Costa et al. (2017) in a systematic review and meta-analysis also concluded that adjunctive use of chlorhexidine with SRP provides only a minor PPD reduction compared to SRP alone. Therefore, clinicians should consider the small additional gain in PPD reduction, negligible effect on CAL, and potential for adverse effects when prescribing chlorhexidine to their patients.^{52–54}

Based on our findings, goji berry mouthwash increased the protein thiol levels in saliva, unlike chlorhexidine. This is a positive finding that goji berry would be advantageous compared to chlorhexidine in increasing the antioxidant levels in saliva in patients with periodontal disease. Since periodontitis is linked with increased oxidative stress and free radical-mediated tissue injury, the use of compounds with antioxidant properties like goji berry would be beneficial to control to reduce oxidative stress and control the periodontal inflammation. The increase in the antioxidant levels in saliva can be attributed to the presence of *Lycium barbarum* polysaccharides (LBPs), catechin, epicatechin, quercetin, chlorogenic acid, citric acid, coumaric acid, scopoletin, linoleic acid, kaempferol, and coumaric acid in goji berry.^{51–58} The flavonoids in goji berry are proven to have good antimicrobial effects and this justifies the reduction in the Pi and control of gingival inflammation with goji berry mouthwash.⁵⁹ The reduction in gingival inflammation can also be attributed to the ability of goji berry to inhibit proinflammatory cytokines, radical scavenging activities, and interactions with other antioxidants. Previous studies have shown that goji berries can improve fibroblast healing and increase collagen formation, which could be beneficial for the healing of periodontal pockets and improving clinical attachment levels.^{41,60–63}

Another study by Sanghavi et al. (2022) also noted that goji berry has good antimicrobial, anti-adhesion, and anti-biofilm properties against periodontal pathogens (*P. gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *Prevotella intermedia*, *Tannerella forsythia*). Goji berry extract could inhibit the growth of all these key periodontal pathogens, however, the zone of inhibition was less when compared to doxycycline and chlorhexidine. The anti-adhesion properties of goji berry (96%) were comparable compared to chlorhexidine (96.3%). However, the anti-biofilm activity of chlorhexidine (96%) was found to be slightly better than that of goji berry extract (91.6%).⁴⁹

Studies by Du et al. (2014) and Ren et al. (2012) also found that goji berry supplementation enhances the maturation and increases the recruitment of neutrophils and monocytes to sites of infection.^{64,65} This ability of goji berry to modulate the immune cells also proves its ability to control inflammation, which would be beneficial in lowering the microbial load and controlling periodontal inflammation. Lai et al. (2022) conducted an *in-vitro* and animal study to assess the alkaline phosphatase activity and osteogenic potential of human periodontal ligament stem cells upon treatment with goji berry and found that goji berry can enhance the proliferation and migration of stem cells. This could facilitate superior healing and control of gingival inflammation. The study also noted a reduction in alveolar bone resorption.⁶⁶

Hence based on our results and existing evidence, it can be stated that goji berry mouthwash has good antimicrobial and antioxidants for managing periodontitis and can be used as an adjunct to SRP for managing periodontitis. However, one should also note that the present study evaluated the role of goji berry for only a short recall time of one month and prolonged exposure to chlorhexidine mouthwash was avoided to prevent side effects. One should also note that although the patients were blinded by using coded amber-colored bottles to mask the color of the mouthwash, the taste of each mouthwash was different. The calibration of mouthwash has not been done. The study was conducted on patients with mild to moderate periodontitis. Therefore, future studies should assess the efficacy of goji berry mouthwash for managing periodontitis with no longer follow-up. Future studies should also evaluate the effect of goji berry on specific periodontal pathogens or local and systemic inflammatory markers for periodontitis.

Conclusion

Goji berry mouthwash along with SRP was effective in reducing the Gi Pi, BOP, and PPD in patients with periodontitis. No statistically significant difference was noted between the use of goji berry mouthwash and chlorhexidine mouthwash. However, goji berry mouthwash was more effective than chlorhexidine in increasing the antioxidant levels (protein thiol) in saliva.

Ethical statement

The study was conducted after receiving ethical approval from the Kasturba Medical College and Kasturba Hospital Ethic Committee with IEC no: 117/2019. The trial has been registered at the 'Clinical Trial Registry (CTRI/2019/05/019042)'.

Data availability

Figshare: Data on study titled: Lycium barbarum (Goji berry) mouthwash is a viable alternative to 0.2% chlorhexidine gluconate for managing chronic periodontitis: a randomized clinical trial; doi: [10.6084/m9.figshare.21834939](https://doi.org/10.6084/m9.figshare.21834939).⁶⁷

This project contains the following data:

- CONSORT checklist
- CONSORT flow diagram

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/) (CC-BY 4.0).

The datasets related to our study is also available with the corresponding author and can be shared on reasonable request via email to aditi.chopra@manipal.edu.

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Version 4

Reviewer Report 06 March 2025

<https://doi.org/10.5256/f1000research.174704.r356385>

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The study and methodology have been well designed, mainly the figures 1 and 2 explain it well. However, the microbial analysis during the stability test trial of the mouthwash has been shown in the figure 1 but not explained in the methodology text.

Sample size can be increased to evaluate the effects of the mouthwash better, so that or before, it can be commercially used as an alternative to the Chlorhexidine mouthwash.

Methods: The inclusion and exclusion criteria have been well formatted. But as per point 8 of exclusion criteria, even the different types of dentifrice used by each participant varies. This can also add to the bias in the results. It has not been mentioned whether all participants were given the same dentifrice during the study duration.

Methods:

c. Examination of the clinical periodontal parameters:

Why were the participants of Goji berry mouthwash instructed to dilute it and the participants of chlorhexidine mouthwash requested to use undiluted mouthwash?

During follow up, Substantively of the mouthwash can be tested, if a follow up is included for 7 or 15 days after washout period, i.e. after stopping the use of mouthwash, for both the groups.

Also, a Placebo group could have been included to compare the results.

Results have been explained well.

Discussion: A certain grammatical errors and repetition of words have been found.

In the article: (Prolonged and continuous use of chlorhexidine is contraindicated to prevent the onset of various side effects such as alterations in taste, staining, and increased calculus, alternatives to chlorhexidine become important.)

Can be corrected as: Prolonged and continuous use of chlorhexidine is contraindicated to prevent the onset of various side effects such as alterations in taste, staining, and increased calculus. **Hence** alternatives to chlorhexidine become important.

In the article: The increase in the antioxidant levels in saliva can be attributed to the presence of *Lycium barbarum* polysaccharides (LBPs), catechin, epicatechin, quercetin, chlorogenic acid, citric acid, **coumaric acid**, scopoletin, linoleic acid, kaempferol, and **coumaric acid** in goji berry.⁵¹⁻⁵⁸
Coumaric acid has been typed twice.

In the article: The anti-adhesion properties of goji berry (96%) were comparable compared to chlorhexidine (96.3%).

Can be corrected as: The anti-adhesion properties of goji berry (96%) were comparable to that of chlorhexidine (96.3%).

In the article: Hence based on our results and existing evidence, it can be stated that **goji berry goji berry** mouthwash has good antimicrobial and antioxidants for managing periodontitis
Goji berry has been typed twice

In the article: Therefore, future studies should assess the efficacy of goji berry mouthwash for managing periodontitis with **no** longer follow-up.

Is the word **NO**, typed by mistake?

References:

There have been past studies in the MCODS, Manipal, where other Alternatives of Herbal mouthwashes like Triphala, Aloe vera, Tea Tree oil, etc. have been used in comparison to Chlorhexidine mouthwash.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Prevention of Caries, in Pediatric age group, so that quality of life is improved during the earliest part of life itself.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 07 January 2025

<https://doi.org/10.5256/f1000research.174704.r351776>

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Gaetano Isola 

University of Catania, Catania, Italy

In the manuscript entitled: "Efficacy of Lycium barbarum (Goji berry) mouthwash for managing periodontitis: a randomized clinical trial" the authors aimed to analyze the efficacy of goji berry mouthwash compared to chlorhexidine mouthwash for managing periodontitis.

The authors concluded that Goji berry mouthwash along with SRP reduced the Gi, Pi, BOP, and PPD in patients with periodontitis. However, no statistically significant difference was noted between the use of goji berry and chlorhexidine mouthwash. Goji berry mouthwash was more effective than chlorhexidine in increasing the antioxidant levels in saliva.

Major comments:

In general, the idea and innovation of this study regards the analysis of periodontitis and related effects and mediators is interesting and novel because the role these aspects in medicine are validated but further studies on this topic could be an innovative issue in this field could be open a creative matter of debate in literature by adding new information.

The study was well conducted by the authors; However, there are some concerns to revise that are described below.

The introduction section resumes the existing knowledge regarding the important factor linked with periodontal treatment and related mediators.

However, as the importance of the topic, the reviewer recommends to update the literature through read, discuss some recent interesting articles, that helps the authors to better introduce and discuss the role of periodontal treatment approaches and related mediators that could impact the development of periodontitis evolution especially about CVD risk 1) Polizzi A.et.al 2024 (Ref 1) 2.) Isola G.et.al 2024 (Ref 2)

The authors should be better specified, at the end of the introduction section, the rationale of the study and the aim of the study. In the central section, should better clarify inclusions and exclusions criteria of the selected sample.

Please better state the results obtained in the abstract.

The discussion section appears well organized with the relevant paper that support the conclusions, even if the authors should better discuss the relationship regarding by periodontal treatment approaches that could improve the quality of life in periodontitis patients. The conclusion should reinforce in light of the discussions.

In conclusion, I am sure that the authors are fine clinicians who achieve very nice results with their

adopted protocol.

Minor Comments:

Abstract: Better formulate the abstract section by better describing the aim of the study

Introduction: Please refer to major comments

Discussion: Please add a specific sentence that clarifies the results obtained in the first part of the discussion

References

1. Polizzi A, Nibali L, Tartaglia GM, Isola G: Impact of nonsurgical periodontal treatment on arterial stiffness outcomes related to endothelial dysfunction: A systematic review and meta-analysis. *J Periodontol*. 2024. [PubMed Abstract](#) | [Publisher Full Text](#)
2. Isola G, Polizzi A, Santonocito S, Alibrandi A, et al.: Effect of quadrantwise versus full-mouth subgingival instrumentation on clinical and microbiological parameters in periodontitis patients: A randomized clinical trial. *J Periodontol Res*. 2024; **59** (4): 647-656 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

No

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: dentistry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 04 December 2024

<https://doi.org/10.5256/f1000research.174704.r343647>

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Paulo José Lima Juiz

Federal University of Recôncavo da Bahia, Feira de Santana, Brazil

The authors provided a comprehensive review of the work, which enhances the clarity and understanding of the results. This thoughtful approach helps to highlight the key findings.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 3

Reviewer Report 10 October 2024

<https://doi.org/10.5256/f1000research.168737.r324931>

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Paulo José Lima Juiz

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The article is pertinent and highlights the extract's therapeutic potential. However, the sample size was insufficient to conclusively support its use in periodontal therapy. Furthermore, to validate the results, it is essential to assess groups that only underwent scaling and root planning (SRP) and groups treated with the vehicle used in the formulation without the extract. Therefore, I recommend further research to strengthen the findings before presenting the article with more substantial data in the future.

Below are some compelling observations:

1) Written in the text: Periodontitis is considered the 6th most common disease, affecting approximately 20–50% of the adult population worldwide.

Please ensure that the epidemiological data specified is from 2017 and provide more recent data, if available.

2) Rewrite the sentence: It is primarily caused by the **IMMUNE RESPONDE DUE TO THE**

interaction of the gingival and periodontal tissues with the microorganisms in the oral biofilm

3) Regarding Figure 1, should it be labeled as MMT instead of MTT?

4) **Written in the text:** ...mixed in 1.15 of 100% alcohol and then homogenized using 15% glycerol. Kindly specify the alcohol concentration in Methods. Did the authors use methanol and ethanol?

5) Without the extract in the formulation, could you confirm if this formulation is considered active?

A concentration of 50 ug/ml was mixed in 1.15 of 100% alcohol and then homogenized using 15% glycerol. To this around 15% propylene glycol and 1% Tween solution was added to formulate the mouthwash. 0.1% menthol was added to adjust the taste of the mouthwash. The volume was adjusted to 100% by using distilled water. 100% alcohol was added to serve both as a preservative and a dissolvent.

6) Shouldn't Table 1 be classified as a result?

7) Please provide the reference for the classification presented.

Written in the text: Ninety subjects aged 20 to 50 years (both male/females) visiting the outpatient department were screened for the presence of localized/generalized periodontitis according to the 2017 classification of periodontal disease

8) The description of the microbiological method appears to be insufficient. Can you furnish details such as the reference, growing conditions, anaerobiosis, and the culture media used?

Written in the text: The plaque from the subgingival region was collected from the pocket with the maximum probing depth in each quadrant using a sterile site-specific Gracey curette. After collection, the curette was gently submerged in a reduced transport medium (Thioglycolate bath) for testing the total colony Forming Units (CFU). The number of colonies was calculated on the plated blood agar and then converted to CFU/mL using the following formula: $CFU/mL = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of the culture plate}$.

9) Is it a thioglycolate bath or broth?

10) Figure 1, which depicts a schematic representation of the study design, notes that MTT was conducted between tests, yet the method and results were not described. Was the test cytotoxic?

11) BOP needed to be delineated in the results text.

12) **Written in the text:** Since goji berry mouthwash showed comparable results to chlorhexidine, it can be used along with SRP for managing periodontitis, **especially in the current times when oral bacteria are becoming resistant to chlorhexidine**

After one month, does the bacteria become resistant?

13) I did not observe the microbiological results in RESULTS and DISCUSSION.

14) Can we confirm the veracity of the following statement?

Written in the text: It can be stated that goji berry has a promising role in managing periodontitis.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Biotechnology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 11 Nov 2024

Aditi Chopra

Firstly, we would like to thank the reviewers for their time and provide valuable insights for improving our work. We are truly thankful for the same. We have revised the manuscript as suggested (in track change mode). However, we are truly grateful for the valuable suggestion and will plan for further research based on the valuable recommendations. We agree with the reviewers and their suggestions and responses to each comment are as follows. The changes in the manuscript are highlighted in track change mode. We would like to humbly request to note that the study was designed to test the effect of a new mouthwash. Hence it was conducted as a pilot randomized study with a small sample size to check the effect of goji berry mouthwash on patients with periodontitis for the first time.

Comment 1: Written in the text: Periodontitis is considered the 6th most common disease, affecting approximately 20–50% of the adult population worldwide. Please ensure that the epidemiological data specified is from 2017 and provide more recent data, if available.

Response: We have revised the data and added recent data on the prevalence of

periodontitis as follows from articles from Nascimento et al (2024): "In 2021, over 1 billion people were affected by severe periodontitis, with a global age-standardized prevalence of 12.50%. South Asia had the highest prevalence rate, at 17.57%."

Reference: Nascimento GG, Alves-Costa S, Romandini M. Burden of severe periodontitis and edentulism in 2021, with projections up to 2050: The Global Burden of Disease 2021 study. J Periodontal Res. 2024 Oct;59(5):823-867. doi: 10.1111/jre.13337.

Comment 2: Rewrite the sentence: It is primarily caused by the Immune response due to the interaction of the gingival and periodontal tissues with the microorganisms in the oral biofilm

Response: We thank the reviewers for the valuable insights. We have revised the sentence as follows: "It is primarily caused by the interaction of the oral microorganisms with the host (soft tissues surrounding the teeth. This host-microbial interaction triggers a massive influx of various pro-inflammatory mediators, microbial by-products, proteolytic enzymes, and free reactive oxygen species (ROS) leading to periodontal tissue destruction and inflammation"

Comment 3: Regarding Figure 1, should it be labeled as MMT instead of MTT?

Response: We have revised the figure as Figure 1 and corrected the Typo error.

Comment 4: Written in the text: ...mixed in 1.15 of 100% alcohol and then homogenized using 15% glycerol. Kindly specify the alcohol concentration in Methods. Did the authors use methanol and ethanol?

Response: We thank the reviewer for pointing out this inadequacy in content. We have used ethanol, and the same has been added to the text.

Comment 5: Without the extract in the formulation, could you confirm if this formulation is considered active? A concentration of 50 ug/ml was mixed in 1.15 of 100% alcohol and then homogenized using 15% glycerol. To this around 15% propylene glycol and 1% Tween solution was added to formulate the mouthwash. 0.1% menthol was added to adjust the taste of the mouthwash. The volume was adjusted to 100% by using distilled water. 100% alcohol was added to serve both as a preservative and a dissolvent.

Response: The active ingredient is an extract at a concentration of 50 µg/ml. Since alcohol is present at 1.15%, it is not an active antimicrobial agent. Alcohol must be present in the final product at a concentration of around 15-20% for it to contribute as an active antimicrobial ingredient. Anything below this level will primarily act as a preservative or solvent aid. The other components, such as glycerol, propylene glycol, and tween, are inactive and act as a solvent and stabilizer. Menthol is considered inactive in terms of antimicrobial activity at this concentration and is used to provide taste and a cooling sensation.

Ref: Jones, C. G. (1997). Chlorhexidine: Is it still the gold standard? Periodontology 2000, 15(1), 55-62.

Comment 6: Shouldn't Table 1 be classified as a result?

Response: We thank the reviewer for their valuable insight, we have revised the section and now included it in the result section as suggested.

Comment 7: Please provide the reference for the classification presented. Written in the

text: Ninety subjects aged 20 to 50 years (both male/females) visiting the outpatient department were screened for the presence of localized/generalized periodontitis according to the 2017 classification of periodontal disease.

Response: We thank the reviewer for their valuable insights. We have added the following reference for the classification system for periodontal disease.

Papapanou PN, Sanz M, Buduneli N, Dietrich T, Feres M, Fine DH, Flemmig TF, Garcia R, Giannobile WV, Graziani F, Greenwell H, Herrera D, Kao RT, Kebschull M, Kinane DF, Kirkwood KL, Kocher T, Kornman KS, Kumar PS, Loos BG, Machtei E, Meng H, Mombelli A, Needleman I, Offenbacher S, Seymour GJ, Teles R, Tonetti MS. Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018 Jun;89 Suppl 1:S173-S182. doi: 10.1002/JPER.17-0721.

Comment 8: The description of the microbiological method appears to be insufficient. Can you furnish details such as the reference, growing conditions, anaerobiosis, and the culture media used? Written in the text: The plaque from the subgingival region was collected from the pocket with the maximum probing depth in each quadrant using a sterile site-specific Gracey curette. After collection, the curette was gently submerged in a reduced transport medium (Thioglycolate bath) for testing the total colony Forming Units (CFU). The number of colonies was calculated on the plated blood agar and then converted to CFU/mL using the following formula: $\text{CFU/mL} = (\text{no. of colonies} \times \text{dilution factor}) / \text{volume of the culture plate}$.

Response: We thank the reviewer for the insight. We have revised the text in the document as follows:

"The plaque from the subgingival region was collected from the pocket with the greatest probing depth in each quadrant using a sterile, site-specific Gracey curette. Following collection, the curette was promptly immersed in a reduced transport medium (Thioglycolate broth supplemented with hemin and vitamin K) for microbiological analysis. Each sample underwent vortexing, and quantitative cultures were performed across various dilutions (1:10, 1:100, 1:1000, and 1:10,000). A volume of 10 μL from each dilution was plated onto pre-reduced 5% sheep blood agar (BD, Becton Dickinson, Heidelberg, Germany), with plates incubated at 37°C for 48 hours in an anaerobic chamber (Whitley A35 Anaerobic workstation, Don Whitley Scientific, Shipley, UK). The colony-forming units were analyzed for each sample using the following formula: $\text{CFU/mL} = (\text{number of colonies} \times \text{dilution factor}) / \text{volume of the culture plate}$."

Comment 9: Is it a thioglycolate bath or broth?

Response: We apologize for the Typo error, We have changed the word to "broth".

Comment 10: Figure 1, which depicts a schematic representation of the study design, notes that MTT was conducted between tests, yet the method and results were not described. Was the test cytotoxic?

Response: We have modified the figure as suggested.

Comment 11: BOP needed to be delineated in the results text.

Response: We have revised the results section and added data on BOP as follows: "The percentage of sites with BOP reduced from 84.8 ± 0.32 at baseline in the test group to 09.0 ± 0.32 at the end of six months. These results were comparable to the control group with no statistically significant difference between the test and control".

Comment 12: Written in the text: Since goji berry mouthwash showed comparable results to chlorhexidine, it can be used along with SRP for managing periodontitis, especially in the current times when oral bacteria are becoming resistant to chlorhexidine. After one month, does the bacteria become resistant?

Response: Studies have found that oral bacteria can become resistant to chlorhexidine and time and amount of resistance develop depending upon the concentration and duration of chlorhexidine used, and the nature of the microorganism. In an article by Fröh et al 2022, the authors investigated the adaptation of oral bacteria to chlorhexidine digluconate (CHX) and potential cross-adaptation to antibiotics after repeated exposure of supragingival plaque samples to subinhibitory concentrations of CHX. Plaque samples from six healthy donors were passaged for 10 days in subinhibitory concentrations of CHX while passaging of plaque samples without CHX served as a control. Subsequently, the minimum inhibitory concentrations (MIC) of these isolates toward CHX were determined using a broth-microdilution method, and phenotypic antibiotic resistance was evaluated using the epsilometer test. Repeated exposure of supragingival plaque samples to subinhibitory concentrations of CHX led to the selection of oral bacteria with 2-fold up to 4-fold increased MICs toward CHX. Furthermore, these isolates showed up to 12-fold increased MICs towards some antibiotics such as erythromycin and clindamycin. Conversely, biofilm-forming capacity was decreased. Wang et al. (2017a) evaluated changes in MIC in eight common oral bacterial species over 10 passages of CHX-challenge and re-growth in CHX-free medium and reported adaptation in *Streptococcus gordonii*, *E. faecalis*, *Fusobacterium nucleatum*, and *P. gingivalis*. Further analysis of the adapted *S. gordonii* strain showed delayed growth with prolonged log-phase and decelerated growth rate as compared to its parental strain indicating reduced metabolic activity that may be responsible for the reduced susceptibility toward CHX (Wang et al., 2017a).

Ref: Fröh R, Anderson A, Cieplik F, Hellwig E, Wittmer A, Vach K, Al-Ahmad A. Antibiotic Resistance of Selected Bacteria after Treatment of the Supragingival Biofilm with Subinhibitory Chlorhexidine Concentrations. *Antibiotics (Basel)*. 2022 Oct 17;11(10):1420. doi: 10.3390/antibiotics11101420. PMID: 36290078; PMCID: PMC9598507.

Comment 13: I did not observe the microbiological results in RESULTS and DISCUSSION.

Response: We thank the reviewers for their valuable insights. We have now added the microbiological section to the results section in both the abstract and text.

Comment 14: Can we confirm the veracity of the following statement? Written in the text: It can be stated that goji berry has a promising role in managing periodontitis.

Response: We have modified the sentence as follows: It can be stated that goji berry can be used as an adjunct to SRP for managing periodontitis. Based on the previous pieces of evidence (in-vitro and in-vivo studies) that confirm the antioxidant and antimicrobial properties of goji berry and previously published reports on the efficacy of oral bacteria on periodontal pathogens, we can conclude that goji berry is a good source of antimicrobial and antioxidants for managing periodontitis.

Competing Interests: Nil

Reviewer Report 17 July 2024

<https://doi.org/10.5256/f1000research.168737.r299638>

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Francisco Wilker Mustafa Gomes Muniz 

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No further comments.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Periodontology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 2

Reviewer Report 14 December 2023

<https://doi.org/10.5256/f1000research.158544.r223446>

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Francisco Wilker Mustafa Gomes Muniz 

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³ Graduate Program in Dentistry, Department of Periodontology, Universidade Federal de Pelotas, Pelotas, State of Rio Grande do Sul, Brazil

This is the second round of peer review of this referee. Overall, the quality of the manuscript increased significantly, and authors have answered most of my queries properly. However, some issues still remain.

Methods

- Remove the following sentence: "1. Individuals with severe periodontitis (localized or generalized stage III and stage IV) were excluded." Authors have included only individuals with periodontitis stage I and II. Therefore, it is not possible to exclude stage III and IV.
- For periodontal assessment, authors wrote the following: "The deepest pocket depth at each surface was recorded. The average of each surface was considered as the reading for that tooth." It is clear now that six sites were measured, but only four were record. However, was is the deepest one or a mean of both interproximal surfaces?
- It is still unclear what commercial brand, if any, was used for chlorhexidine.
- Chlorhexidine was diluted in water. This is not appropriate and must be discussed. In fact, a very lower concentration of the product was achieved by this protocol.
- The lack of calibration process is worrisome and must be discussed.

Discussion

- Remove the following sentence: "In such situations, the use of herbal mouthwash like goji berry would be a viable alternative.⁵¹" Firstly, the very low additional effect of chlorhexidine is not a justification for the use of herbal mouthwash. In addition, reference 51 do not advise for the use of goji berry.
- Italicize "in vitro" in the whole manuscript, as previously requested.
- Correct the following sentence to: "The study was conducted on patients with mild to moderate periodontitis. Therefore, future studies should assess the efficacy of goji berry mouthwash on severe cases of periodontitis with longer follow-up."

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Periodontology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 02 Jul 2024

Aditi Chopra

Firstly, we would like to thank the reviewers for their time and provide valuable insights for improving our work. We are truly thankful for the same. We have revised the manuscript as suggested (in track change mode). The responses to each comment are as follows:

Comment 1: Remove the following sentence: "1. Individuals with severe periodontitis (localized or generalized stage III and stage IV) were excluded." Authors have included only individuals with periodontitis stage I and II. Therefore, it is not possible to exclude stages III and IV.

Response: We have removed the line from the exclusion criteria as suggested

Comment 2: For periodontal assessment, authors wrote the following: "The deepest pocket depth at each surface was recorded. The average of each surface was considered as the reading for that tooth." It is clear now that six sites were measured, but only four were

recorded. However, was it the deepest one or a mean of both interproximal surfaces?
Response: We have revised the paper and added the line that means the interdental area was recorded to take the overall measurement for the interdental site.

Comment 3: It is still unclear what commercial brand, if any, was used for chlorhexidine.
Response: We added the name of the brand used for chlorhexidine

Comments 4: Chlorhexidine was diluted in water. This is not appropriate and must be discussed. A very low concentration of the product was achieved by this protocol.
Response: We have revised the ambiguity in the paper. We would like to highlight that Hexidine mouthwash (brand name was given to all the patients) and the patients were asked not to dilute the mouthwash. For the goji berry mouthwash, 10 ml of water was used to dilute.

Comment 5: The lack of a calibration process is worrisome and must be discussed.
Response: we have discussed the lack of calibration process in the limitations of the study in the discussion.

Comment 6: Remove the following sentence: "In such situations, the use of herbal mouthwash like goji berry would be a viable alternative.⁵¹" Firstly, chlorhexidine's very low additional effect is not a justification for the use of herbal mouthwash. In addition, reference 51 does not advise for the use of goji berries.
Response: We have removed the abovementioned sentence as suggested.

Comment 7: Italicize "in vitro" in the whole manuscript, as previously requested.
Response: We have Italicized the word 'in vitro' throughout the manuscript.

Comment 8: Correct the following sentence to: "The study was conducted on patients with mild to moderate periodontitis. Therefore, future studies should assess the efficacy of goji berry mouthwash on severe cases of periodontitis with longer follow-up."
Response: We have corrected the following lines as suggested.

Competing Interests: Nil

Version 1

Reviewer Report 09 October 2023

<https://doi.org/10.5256/f1000research.142608.r205506>

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**Francisco Wilker Mustafa Gomes Muniz**

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This is the first round of peer review of this referee. Several queries were raised as stated below:

Overall

- A very short follow-up period was followed for the chosen outcomes, such as BOP, CAL and PPD. A longer follow-up period, to detect any important change, is necessary. The antiplaque and antigingivitis efficacy may be important to the literature, though.
- Throughout the manuscript the term “periodontal disease” is used, maybe, referring to periodontitis. The term periodontal disease is very broad, and do not mean only periodontitis. Authors must revise it.

Title

- Chlorhexidine is an excellent mouthwash to control biofilm and gingival inflammation. However, literature does not state that this mouthwash is useful for the treatment of periodontitis. Other treatments are more efficacious for this periodontal disease. Therefore, title must be revised, as it may be misleading in its current format. Please see Sanz, M., Herrera, D., Kebschull, M., Chapple, I., Jepsen, S., Berglundh, T., ... & Wennström, J. (2020). Treatment of stage I-III periodontitis—The EFP S3 level clinical practice guideline. *Journal of clinical periodontology*, 47, 4-60.
- Moreover, the term “chronic periodontitis” should not be used since 2018. Please see Caton, J. G., Armitage, G., Berglundh, T., Chapple, I. L., Jepsen, S., Kornman, K. S., ... & Tonetti, M. S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions—Introduction and key changes from the 1999 classification. *Journal of periodontology*, 89, S1-S8. Prefer the term “periodontitis” whenever appropriate, and this must be applied to all manuscript.
- Additionally, lower gain of CAL was detected in the test group. Title must not state that similar results were detected between groups.

Abstract

- Authors wrote: “A statistically significant difference was noted in the Pi (P-value = 0.791), Gi (P-value= 0.594), PPD (with P-value= 0.134), and microbial levels (P-value = 0.188) in both groups from the baseline.” However, none of the showed p-values mean statistical significance. The sentence is not understandable. Does the comparison mean within or between comparisons?
- Conclusion may also be misleading. *L. barbarum* mouthwash presented similar clinical efficacy, when compared to chlorhexidine. The SRP performed may be the main reason to

the so-called “effective in managing periodontal disease”. Please provide a interpretation aligned with your aims.

Introduction

- The term “exaggerate” in the first paragraph should be revised, as may be not be the most appropriate term in an epidemiological point of view. In addition, authors have cited both risk factors and indicator factors associated with periodontitis.
- Reference is necessary for the following sentence: “However, mechanical debridement is technically demanding, as many times removal of hard and soft tissue deposits from pockets greater than five mm becomes challenging.” In well-trained clinicians, this may not be a problem.
- Provide citation for the following sentences:
 - “Recently, goji berry, commonly known as Wolfberry, Himalayan goji, or Tibetan goji, has gained a lot of popularity due to its strong antioxidant and anti-inflammatory properties.”
 - “The stems and berries of goji berry plant was effective against many various Gram-negative bacteria and Gram-positive bacteria.”
- Italicize the word “in vitro.”
- Reference #47 may not be necessary in the following sentence: “(...) efficacy of *L. barbarum* mouthwash along with SRP for patients with chronic periodontitis compared to chlorhexidine for the first time.⁴⁷”
- Why salivary levels of protein thiol were assessed in the present study? What is the relevance for the present study. This is not properly explained in this section.

Objective

- Pi, Gi, BOP, PPD, and CAL must be explained, as this is the first time their appear in the text.

Methods

- Figure 1 do not represent the flowchart of the study, and do not follow what CONSORT recommends. Figure 1 is illustrative and important, but the sentence must be revised.
- Figure 2, in fact, must be placed in the Results section, as recommended by CONSORT.
- Do not use Stage 1, use “stage I.” Provide reference for this inclusion criterion
- Do not mistake exclusion criteria as the opposite of inclusion criteria. For instance, if only generalized periodontitis was included. It is not necessary to exclude those with localized periodontitis.
- What is “chlorhexidineg”?
- Revise the following sentence: “Participants currently using anti-inflammatory, antibiotics, or analgesics in the last six months.” It seems that patients that used antibiotics in the last six months were included.
- Revise exclusion criteria #10, as the sentence is in the future.

- Sample size calculation is not properly described. Readers to not know the necessary number of patients to detect important measures in the primary outcome. For instance, the effect size of 0.7 is referring to what outcome?
- Why allocation stated uneven (30:27) at baseline (Figure 2), as a 1:1 allocation ratio was established in the Methods?
- In fact, allocation concealment is not properly explained.
- Both mouthwashes may be very distinct in the general characteristics, such as taste, color and visual aspects. Therefore, this referee does not understand the following sentence: "The participants were also blinded about their group."
- It is well-established that the best periodontal examination must be performed in six sites per tooth. It is not clear why authors have performed it only in four.
- Further information regarding chlorhexidine is necessary. Was it manufactured by the authors? What percentage was used? Provide details.
- Chlorhexidine, such as those commercially available, must not be diluted in water. The lower efficacy of chlorhexidine must not be ruled out in the present study.
- It is not clear for how long (who many seconds) volunteers should rinse.
- Calibration, if any, is not reported.

Results

- In Table 3, why p-values for the comparison between groups are reported only for baseline?
- The following sentence should be better placed in the Methods: "(...) repeated measures of ANOVA with Greenhouse Geisser correction' followed by a 'post-hoc analysis with Bonferroni adjustment'."
- Figure 3 and Table 3 are repetitive results. Both of them are not necessary.
- Authors wrote: "One patient reported bitter taste of the mouthwash and discontinued the mouthwash." What mouthwash?

Discussion

- Effectiveness was not tested. Do not mistake it with efficacy.
- Only mild periodontitis patients were included in the present study. Do not generalize it. This type of patient is not discussed.
- The following sentences are not correct: "Based on the results, it was noted that goji berry mouthwash can control the gingival inflammation, BOP, and plaque formation at 15 days following SRP with statistically significant difference compared to chlorhexidine group. A significant in the Gi, Pi, BOP, PPD was noted in participants using goji berry mouthwash." No differences between groups were detected.

- Again, chlorhexidine is not largely used for the management of periodontitis. In fact, its use as adjuvant to periodontitis treatment is not well-established, and the previously mentioned clinical practice guideline do not recommend it.
- A broader discussion regarding the *L. barbarum* on periodontal tissues is warranted.
- There is no discussion regarding the very short follow-up period.

References

1. Sanz M, Herrera D, Kebschull M, Chapple I, et al.: Treatment of stage I-III periodontitis-The EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2020; **47 Suppl 22** (Suppl 22): 4-60 [PubMed Abstract](#) | [Publisher Full Text](#)
2. Caton JG, Armitage G, Berglundh T, Chapple ILC, et al.: A new classification scheme for periodontal and peri-implant diseases and conditions - Introduction and key changes from the 1999 classification. *J Clin Periodontol*. 2018; **45 Suppl 20**: S1-S8 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No

Are the conclusions drawn adequately supported by the results?

No

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Periodontology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 04 Nov 2023

Aditi Chopra

Firstly, we would like to thank all the reviewers and the editor for spending their valuable time and providing us with their valuable comments and suggestions. We truly appreciate your guidance in improving our work. We have tried to address the comments and revised the manuscript accordingly. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

Reviewer 2

- A very short follow-up period was followed for the chosen outcomes, such as BOP, CAL, and PPD. A longer follow-up period, to detect any important change is necessary. The antiplaque and antigingivitis efficacy may be important to the literature, though. Throughout the manuscript the term “periodontal disease” is used, maybe, referring to periodontitis. The term periodontal disease is very broad, and do not mean only periodontitis. The authors must revise it.

Response: We thank the reviewers for their valuable insight. We agree with the reviewer that a long-term follow-up is necessary, however, due to various side effects of chlorhexidine it is not advised to use for a long time. Additionally, there are studies that have found the development of antimicrobial resistance in periodontal pathogens against Chlorhexidine, hence the use of herbal mouthwashes is constantly being researched. We have also revised the term periodontal disease to periodontitis as suggested. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

- Chlorhexidine is an excellent mouthwash to control biofilm and gingival inflammation. However, literature does not state that this mouthwash is useful for the treatment of periodontitis. Other treatments are more efficacious for this periodontal disease. Therefore, title must be revised, as it may be misleading in its current format. Please see Sanz, M., Herrera, D., Kebschull, M., Chapple, I., Jepsen, S., Berglundh, T., ... & Wennström, J. (2020). Treatment of stage I–III periodontitis—The EFP S3 level clinical practice guideline. *Journal of Clinical Periodontology*, 47, 4-60. Moreover, the term “chronic periodontitis” should not be used since 2018. Please see Caton, J. G., Armitage, G., Berglundh, T., Chapple, I. L., Jepsen, S., Kornman, K. S., ... & Tonetti, M. S. (2018). A new classification scheme for periodontal and peri-implant diseases and conditions—Introduction and key changes from the 1999 classification. *Journal of periodontology*, 89, S1-S8. Prefer the term “periodontitis” whenever appropriate, and this must be applied to all manuscript.

Response: we thank the reviewers for sharing the article. Based on your suggestion we have now revised the title as follows: Efficacy of Lycium barbarum (Goji berry) mouthwash for managing periodontitis: a randomized clinical trial. We have now revised the terminology chronic periodontitis to periodontitis as requested. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

- Additionally, lower gain of CAL was detected in the test group. Title must not state that similar results were detected between groups.

Response: we have now revised the interpretation as requested both in abstract and result/discussion section.

Abstract

- Authors wrote: "A statistically significant difference was noted in the Pi (P-value = 0.791), Gi (P-value= 0.594), PPD (with P-value= 0.134), and microbial levels (P-value = 0.188) in both groups from the baseline." However, none of the showed p-values mean statistical significance. The sentence is not understandable. Does the comparison mean within or between comparisons?

Response: we have now revised the result section and removed the following as they were baseline comparisons: that "statistically significant difference was noted in the Pi (P-value = 0.791), Gi (P-value= 0.594), PPD (with P-value= 0.134), and microbial levels (P-value = 0.188) in both groups." Instead, we have now added the follow-up comparisons. The p-values are for intergroup comparison. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

- Conclusion may also be misleading. L. barbarum mouthwash presented similar clinical efficacy, when compared to chlorhexidine. The SRP performed may be the main reason to the so-called "effective in managing periodontal disease". Please provide a interpretation aligned with your aims.
- Response: We thank the reviewers for their valuable suggestions. We have now revised the conclusion as follows: Goji berry mouthwash along with SRP was effective in reducing the Gi, Pi, BOP, and PPD. Goji berry mouth also increased the antioxidant levels in saliva in patients with periodontitis. Thus, goji berry mouthwash is a viable adjunct to SRP for patients with periodontitis. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

Introduction

- The term "exaggerate" in the first paragraph should be revised, as may be not be the most appropriate term in an epidemiological point of view. In addition, authors have cited both risk factors and indicator factors associated with periodontitis.

Response: We thank the reviewers for their valuable suggestions. We have now revised the term as follows: Apart from the microbial etiology, other risk factors that influence the severity of periodontal inflammation include smoking, diabetes mellitus, HIV, nutritional deficiency, medications, poor oral hygiene, and genetics. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

- Reference is necessary for the following sentence: "However, mechanical debridement is technically demanding, as many times removal of hard and soft tissue deposits from pockets greater than five mm becomes challenging." In well-trained clinicians, this may not be a problem.
- Response: We have now added two references for this sentence as requested. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

Heitz-Mayfield L.J., Trombelli L., Heitz F., Needleman I., Moles D. A systematic review of the effect of surgical debridement vs. non-surgical debridement for the treatment of chronic

periodontitis. J. Clin. Periodontol. 2002;29(Suppl. S3):92–102. doi: 10.1034/j.1600-051X.29.s3.5.x.

Sherman P.R., Hutchens L.H., Jr., Jewson L.G., Moriarty J.M., Greco G.W., McFall W.T., Jr. The effectiveness of subgingival scaling and root planing. I. Clinical detection of residual calculus. J. Periodontol. 1990;61:3–8. doi: 10.1902/jop.1990.61.1.3.

- Provide citation for the following sentences: “Recently, goji berry, commonly known as Wolfberry, Himalayan goji, or Tibetan goji, has gained a lot of popularity due to its strong antioxidant and anti-inflammatory properties.” And “The stems and berries of goji berry plant was effective against many various Gram-negative bacteria and Gram-positive bacteria.”
- Response: We thank the reviewer for their suggestion, We have now added reference no 38-48 , which discusses the antimicrobial and antioxidant properties of the goji berry as suggested. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

- Italicize the word “in vitro.”

Response: We thank you for your suggestion, We have now Italicized the word as requested.

- Reference #47 may not be necessary in the following sentence: “(...) efficacy of L. barbarum mouthwash along with SRP for patients with chronic periodontitis compared to chlorhexidine for the first time.⁴⁷”

Response: We have removed reference no 47 from the sentences mentioned above.-

- Why salivary levels of protein thiol were assessed in the present study? What is the relevance of the present study? This is not properly explained in this section.

Response: we thank the reviewers for their valuable insight and key appraisal. we have now added the importance of assessment of local antioxidant levels in oral cavity as immune defense mechanisms in the discussion and introduction section. Since protein thiol is an important antioxidant during periodontal disease and the ability of goji berry to increase it levels in saliva as additional advantage over chlorhexidine. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

- Objective: Pi, Gi, BOP, PPD, and CAL must be explained, as this is the first time they appear in the text.

Response: We have now added the full forms for each of the abbreviations in the objective section as suggested.

- Methods: Figure 1 does not represent the flowchart of the study, and does not follow what CONSORT recommends. Figure 1 is illustrative and important, but the sentence must be revised.

Response: We thank the reviewers for the recommendation, we have now revised the figure 1.

- Figure 2, in fact, must be placed in the Results section, as recommended by CONSORT.

Response: We thank the reviewer for their suggestion, we have now placed figure 2 in the result section.

- Do not use Stage 1, use “stage I.” Provide reference for this inclusion criterion

Response: We have now revised the term Stage 1 to Stage I as suggested.

- Do not mistake exclusion criteria as the opposite of inclusion criteria. For instance, if only generalized periodontitis was included. It is not necessary to exclude those with localized periodontitis.

Response: We thank the reviewer for their valuable insight. We have now revised the criteria as suggested : Participants in age group of 20-50 years diagnosed with stage I to stage II localized/generalized periodontitis (Grades A to B).

- What is “chlorhexidineg”?

Response: We apologise for the Typo error, we have now corrected it as Chlorhexidine

- Revise the following sentence: “Participants currently using anti-inflammatory, antibiotics, or analgesics in the last six months.” It seems that patients that used antibiotics in the last six months were included.

Response: We have now revised the sentence as follows: Participants who were on any anti-inflammatory, antibiotics, or analgesics in last six months were excluded

- Revise exclusion criteria #10, as the sentence is in the future.

Response: We have now revised the sentence as follows: Participants using any other oral hygiene agents (mouthwash or gels) were excluded to remove confounding bias arising due to the difference in plaque control measures

- Sample size calculation is not properly described. Readers to not know the necessary number of patients to detect important measures in the primary outcome. For instance, the effect size of 0.7 is referring to what outcome?

Response: We thank the reviewer for pointing our mistake and not mentioning the effect size linked outcome. The effect size was calculated based on clinical attachment loss.

- Why allocation stated uneven (30:27) at baseline (Figure 2), as a 1:1 allocation ratio was established in the Methods?

Response: We thank the reviewer for their insight. We apologize for the mistake in numbers mentioned in the concealment. Allocation was done at ratio of 1:1 as mentioned in the method. We have now corrected and uploaded the revised figure as suggested.

- In fact, allocation concealment is not properly explained.

Response: We thank the reviewer for their insight. We have now added an explanation on how the allocation concealment was done using opaque amber-colored bottles to mask the color of the mouthwash.

- Both types of mouthwash may be very distinct in general characteristics, such as taste, color, and visual aspects. Therefore, this referee does not understand the following sentence: "The participants were also blinded about their group."

Response: We agree with the reviewer that both goji berry and chlorhexidine mouthwash, mouthwash were stored in similar amber-colored bottles to mask the color of the mouthwash. However, differences in the taste will remain as a major factor in patient blinding/ concealment

- It is well-established that the best periodontal examination must be performed in six sites per tooth. It is not clear why authors have performed it only in four.

Response: We agree with the reviewers and apologize for the ambiguity in the sentence we have performed periodontal examination on buccal, lingual, mesial, and distal (in the interdental area the probing was done both from the buccal and lingual side of the interdental area. We have now revised the sentence in the manuscript.

- Further information regarding chlorhexidine is necessary. Was it manufactured by the authors? What percentage was used? Provide details.

Response: We have added the percentage of chlorhexidine (0.2%)

- Chlorhexidine, such as those commercially available, must not be diluted in water. The lower efficacy of chlorhexidine must not be ruled out in the present study.

Response: We agree with the reviewers. however, the chlorhexidine used our study 0.2% was prepared and was diluted in 10 ml of water.

- It is not clear for how long (who many seconds) volunteers should rinse.

Response: We have now added the time the mouthwash should be used in manuscript.

- Calibration, if any, is not reported.

Response: Since single trained investigator examined the baseline and follow-up visits, calibration was not done.

Results

- In Table 3, why p-values for the comparison between groups are reported only for baseline?

Response: The p-values for comparison between groups at follow-ups and mentioned in the last columns of the same table.

- The following sentence should be better placed in the Methods: "(...) repeated measures of ANOVA with Greenhouse Geisser correction' followed by a 'post-hoc analysis with Bonferroni adjustment'."

Response: We have now revised the statement and mentioned it in Methods section.

- Figure 3 and Table 3 are repetitive results. Both of them are not necessary.
- Response: We agree with reviewer suggestions. We have now removed the figure 3.

- Authors wrote: "One patient reported bitter taste of the mouthwash and discontinued the mouthwash." What mouthwash?

Response: we have now added which group (Test group) patient reported bitterness.

- Discussion: Effectiveness was not tested. Do not mistake it with efficacy.
- Response: We have modified the title according to the suggestion and instead of effectiveness mentioned it as efficacy
- Only mild periodontitis patients were included in the present study. Do not generalize it. This type of patient is not discussed.
- Response: We have added this in the limitations this points our results
- The following sentences are not correct: "Based on the results, it was noted that goji berry mouthwash can control the gingival inflammation, BOP, and plaque formation at 15 days following SRP with statistically significant difference compared to chlorhexidine group. A significant in the Gi, Pi, BOP, PPD was noted in participants using goji berry mouthwash." No differences between groups were detected.
- Response: We agree with the reviewers, we apologize for the error and interpretation. we have now corrected the results section accordingly.
- Again, chlorhexidine is not largely used for the management of periodontitis. In fact, its use as adjuvant to periodontitis treatment is not well-established, and the previously mentioned clinical practice guideline do not recommend it.

Response: we thank the reviewers for their recommendation, we have now added these points to our introduction and discussion sections

- A broader discussion regarding the *L. barbarum* on periodontal tissues is warranted.

Response: We thank the reviewers for their recommendation. We have now added the role of the *L. barbarum* on periodontal pathogens, periodontal ligament fibroblast, neutrophils and wound healing

References:

- Gan L, Zhang SH, Liu Q, et al.: A polysaccharide-protein complex from *Lycium barbarum* upregulates cytokine expression in human peripheral blood mononuclear cells. *Eur. J. Pharmacol.* 2003;471(3):217–222. 12826241 10.1016/s0014-2999(03)01827-2
- Bo R, Liu Z, Zhang J, et al.: Mechanism of *Lycium barbarum* polysaccharides liposomes on activating murine dendritic cells. *Carbohydr. Polym.* 2019;205:540–549. 30446138 10.1016/j.carbpol.2018.10.057
- Du X, Wang J, Niu X, Smith D, Wu D, Meydani SN. Dietary wolfberry supplementation enhances the protective effect of flu vaccine against influenza challenges in aged mice. *J Nutr* 2014;144: 224–9.35.
- Ren Z, Na L, Xu Y, Rozati M, Wang J, Xu J, et al. Dietary supplementation with lacto-wolfberry enhances the immune response and reduces pathogenesis to influenza infection in mice. *J Nutr* 2012;142:1596–602.

There is no discussion regarding the very short follow-up period.

Response: We agree with the reviewer and we have added these points as the limitations of the study

Competing Interests: No potential conflict of interest to be reported

Reviewer Report 06 June 2023

<https://doi.org/10.5256/f1000research.142608.r173857>

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Abstract:

In abstract, results have been mentioned as significant in both the groups, however the p values mentioned don't corroborate the significant results. Please do the necessary corrections.

Introduction:

It needs to be brief & relevant. The objectives of the study may be omitted in the introduction.

Methodology

Please describe in detail the method of randomization of 57 participants in test group(30) and control group(27).

Were the participants recruited on consecutive order or in pooled manner?

Out of 30 participants in test group, 15 were dropped out, only 15 were included in statistical analysis. Sample size calculation was 27 patients in each group. Rationale of sample size calculation is not served in this study. 'Around' 60 participants were recruited; Please avoid vague terms like 'around'. Please mention 57.

Out of 27 allocated to control group, 16 were lost to follow up hence only 11 are left for follow up completion, however in the flow chart it is mentioned, 14 participants were analyzed. Please look into it.

Results

In the result section, demographic data is with reference to patients recruited whereas all other data is with reference to patients who completed the study. Similar data may be included in the result section, either all of the patients recruited or only the patients who have completed the follow up.

All the results have been presented in overall average values. PPD is an important parameter influencing the outcomes of periodontal intervention. Data needs to be segregated as per PPD.

Discussion:

Please focus the discussion on the results of the overall study period of one month, unless there is some interesting finding otherwise.

It takes 6-8 weeks for healing to complete after SRP, Results of SRP are required to be estimated after this period. However the follow up of study period was one month; Please comment on it.

A significant part of discussion is only review of literature with reference to Guji berry; Please avoid it as much as possible. Comment on the findings of your study may be the focus of discussion .

Conclusion:

Conclusions are not based on the results of the study. For example "L.barbarum is a promising superfood with many potential health benefits", is not the conclusion of the study.

L. barbarum has been investigated as an adjunct to SRP. Negative control is missing in the study. Hence the results can't be attributed exclusively to L.barbarum.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

Partly

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Periodontitis, periodontal medicine

We confirm that we have read this submission and believe that we have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however we have significant reservations, as outlined above.

Author Response 06 Nov 2023

Aditi Chopra

Firstly, we would like to thank all the reviewers and the editor for spending their valuable time and providing us with their valuable comments and suggestions. We truly appreciate your guidance in improving our work. We have tried to address the comments and revised the manuscript accordingly. The changes in the manuscript have been highlighted in track change mode in the revised manuscript.

Reviewer 1

- Abstract: In the abstract, results have been mentioned as significant in both the groups, however the p values mentioned don't corroborate the significant results. Please do the necessary corrections.

Response: We thank the reviewers for their valuable insight. We have now revised the abstract and interpretation of the results.

- Introduction: It needs to be brief & relevant. The objectives of the study may be omitted in the introduction.

Response: We thank the reviewer for their suggestion. We have now revised and shortened the introduction/background section in the abstract.

- Methodology: Please describe in detail the method of randomization of 57 participants in test group(30) and control group(27). Were the participants recruited on consecutive order or in pooled manner?

Response: We have now added the method of randomization (computer-generated random sequence) in the abstract. The participants were recruited in as when they come to the OPD and randomized once the consequent order.

- Out of 30 participants in test group, 15 were dropped out, only 15 were included in the statistical analysis. The sample size calculation was 27 patients in each group. The rationale of sample size calculation is not served in this study. 'Around' 60 participants were recruited; Please avoid vague terms like 'around'. Please mention 57.

Response: We thank the reviewer for their valuable suggestion. We have now revised paper accordingly and removed the word around along number of participants

- Out of 27 allocated to control group, 16 were lost to follow up hence only 11 are left for follow up completion, however in the flow chart it is mentioned, 14 participants were analyzed. Please look into it.

Response: We thank the reviewer for their valuable suggestion. We have now revised paper accordingly and provided the corrected CONSORT flow diagram

Results

- In the result section, demographic data is with reference to patients recruited whereas all other data is with reference to patients who completed the study. Similar data may be included in the result section, either all of the patients recruited or only the patients who have completed the follow up.

Response: We thank the reviewer for their valuable suggestion. We have now revised paper accordingly and included the data related to the patients who were recruited

- All the results have been presented in overall average values. PPD is an important parameter influencing the outcomes of periodontal intervention. Data needs to be segregated as per PPD.

Response: We thank the reviewers for their valuable insight. However, since it study was conducted on a small number of participant where segregation of PPD was not possible due to similarity in the amount of PPD, we have mentioned the data as combine reduction in PPD from baseline, moreover with segregation we found that all the patient fall under 1-3 mm of PPD and CAL loss due to recession of 1-2 mm .

Discussion:

- Please focus the discussion on the results of the overall study period of one month, unless there is some interesting finding otherwise. It takes 6-8 weeks for healing to complete after SRP, Results of SRP are required to be estimated after this period. However, the follow-up of study period was one month; Please comment on it.

Response: We thank the reviewer for the critical appraisal of these key findings. We have now added these points to the limitations of the study. We have kept the follow-up at 1 month as long-term use of chlorhexidine mouthwash is not recommended owing to the development of various side effects

- A significant part of discussion is only review of literature with reference to Goji berry; Please avoid it as much as possible. Comment on the findings of your study may be the focus of discussion.

Response: We thank the reviewers for their valuable insight. We have now revised the manuscript accordingly. We have shortened the evidence on goji berry and discussed the clinical implications of our results and limitations of the study

Conclusion:

- Conclusions are not based on the results of the study. For example "L.barbarum is a promising superfood with many potential health benefits", is not the conclusion of the study.

Response: We thank the reviewers for their valuable insight. We have revised the conclusion and removed the line suggested above.

- L. barbarum has been investigated as an adjunct to SRP. Negative control is missing in the study. Hence the results can't be attributed exclusively to L.barbarum.

Response: We thank the reviewers for their valuable insight and agree with fact that the results are due to the combined effects of SRP with goji berry mouthwash. Since SRP is primary and the fundamental step that should be performed and matched in both the group we aimed to compared goji berry mouthwash with chlorhexidine. We have added these points to our discussion as you have suggested.

Competing Interests: nil

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