

Clinical efficacy analysis of the mouth rinsing with pomegranate and chamomile plant extracts in the gingival bleeding reduction



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A B S T R A C T

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Medicinal plants represent important therapeutic resources to health restoration, including the use of herbal products in the mouth conditions treatment. A randomized controlled clinical trial was performed in order to evaluate the effectiveness of mouth rinse with pomegranate and chamomile plant extracts, against chlorhexidine 0.12% in the gingiva bleeding condition. The mouth rinses with the herbal products were effective for this case, showing thus, antimicrobial and anti-inflammatory properties similar to that of chlorhexidine 0.12%.

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1. Introduction

Gingivitis, an inflammatory condition limited to the protection periodontal tissue (marginal and attached gingiva), which is characterized by redness, swelling and bleeding gingiva, and periodontitis, which also involves supporting periodontal tissues (cementum, periodontal ligament and alveolar bone) especially characterized by the presence of periodontal pockets and alveolar bone resorption, have as the primary etiologic agent dental biofilm accumulation, resulting from poor oral hygiene [1,2].

Biofilm is the determining factor for caries and periodontal disease [3], representing a dense, non-calcified mass, composed of micro-organisms, being *Streptococcus mitis* and *Streptococcus sanguis* the pioneers. They are involved in a matrix rich in bacterial extracellular polysaccharides and salivary glycoproteins, firmly attached to the teeth, stones and other hard surfaces of the oral cavity [4]. However, the pathogenesis of periodontal disease is determined not only by the accumulation of dental biofilm, but also by the ratio of their pathogenic potential and quality of immune response of the host organism [5], and other factors such as the morphology of the gingival tissue, the presence of different malocclusion types and factors related to tooth eruption [4].

Until the early '70s, therapies based on the non-specific plaque hypothesis focused on reducing the amount of dental

biofilm. Subsequently, the specific plaque hypothesis established the role of some microorganisms such as *Porphyromonas gingivalis* (Pg), *Prevotella intermedia* (Pi), *Bacteroides forsythus* (Bf), *Agreggatibacter actinomycetemcomitans* (Aa), *Treponema denticola* (Td) and *Fusobacterium nucleatum* (Fn) in different forms of periodontal disease. Recently it was suggested that these periodontal pathogens do not act alone and interactions between species, as the balance between beneficial and pathogenic bacteria, affect disease progression and tissue response to periodontal therapy. Nowadays it is well established that one of the goals of periodontal therapy is controlling these pathogens [6]. Therefore, it is appropriate the use of dental biofilm control measures, being its removal an important factor for the periodontal disease prevention and control.

Brushing, a usual method of biofilm mechanical removal, although practical sometimes becomes difficult, not allowing a reasonable control since it requires time, motivation and manual dexterity [4]. Thus, many patients have to call upon professional practice to remove biofilm and of supra and under-gingival stones. Thus, the procedures for scraping and root planing (SRP) may contain the progression of periodontal disease, providing improvement of their clinical parameters such as reduction in probing depth and clinical attachment gain [7]. However, in some cases, the SRP does not seem to be able to maintain periodontal health, which can be explained by microorganisms' recolonization.

Because of possible after-SRP recolonization of microorganisms, it was proposed the non-surgical periodontal therapy, which consists in mouth cleaning and disinfection in a single stage, with the root surface debridement concept, reducing the microbial load of

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the oral cavity in order to minimize the risk of reinfection of the treated areas [8]. This treatment should promote the elimination and control of periodontal disease risk factors, such step is usually performed with scraping and root planing per quadrant (SRP) or sextant, with a one to two weeks break between sessions. However, the possibility of reinfection of treated sites by periodontal pathogens at sites not yet addressed, and even by pathogens present in other intra-oral niches like tongue, saliva, oral mucosa and tonsils have been the subject of much discussion and, on this basis, a new protocol was proposed, aiming at full mouth disinfection by scraping and root planing in a short period of time [9].

Due to the limitations of mechanical oral hygiene methods, chemical control of dental biofilm acts as a supporting, through the use of antimicrobial agents in mouthwash, reducing the number of pathogenic microorganisms in the oral cavity and aiding in the periodontal diseases prevention and treatment. However, in no case shall such antimicrobial agents replace the mechanical methods of oral hygiene [4].

In order to obtain an antiplaque action, the antimicrobial mouthwash agent should: reduce the bacterial adhesion to the tooth surface, inhibit the growth and proliferation of microorganisms, inhibit the formation of the biofilm intercellular matrix, modify the bacterial biochemistry to reduce the cytotoxic products formation and modify the biofilm ecology to develop a less pathogenic microbiota [4].

Chlorhexidine is well characterized as an excellent antimicrobial agent used in the gingivitis treatment and prevention, and is also used as a reference in efficacy testing studies [10] and considered the most effective chemical agent [11]. Although used in different formulas, in Brazil it is found in 0.12% and 0.20% concentrations [4]. However, it presents local adverse effects when used for prolonged periods [12], including: the teeth and restorations staining, the tongue staining, the oral scaling and sensitivity, and allergic reactions. The bitter taste and interference in the gustatory sense the solution caused some hours, after rinsing, have also been reported [13].

Given the adverse effects of chlorhexidine, one can see the need to develop a substance with potent antimicrobial activity, capable of interfering in the biofilm development and minimize side effects. In this context, there are natural agents that are effective and economically viable alternatives [12], have wide popular acceptance, being used to fight diseases at low cost [14] and contribute to improving people's access to prevention and treatment of periodontal disease [4]. Lastly, the "Health for All" WHO strategy in the year of 2000, finally recognized the need to incorporate in the public health the principles, the resources and the techniques of Natural Medicine, because beyond easing the sickness of millions of people, it is a therapeutic alternative at virtually no cost [15].

So, medicinal plants represent important therapeutic resources for health restoration, including the use of herbal medicines for oral diseases treatment [4]. Among the natural substances used in dentistry, are noteworthy the *Caesalpinia ferrea* ("Jucá"), the Peruvian mastic, ginger, basil, propolis, pomegranate and Cuban oregano (*Plectranthus amboinicus*) that due to its therapeutic properties, have widespread use in folk medicine [12].

The advantages of herbal medicine to justify its use are: the synergistic effect, due to its various phytochemicals that work best in combination, the compounds interaction mechanisms that act on different target molecules, providing diverse actions throughout the body, the low risks of side effects due to low concentrations in which the active ingredients are present in the plants, and also the dose-time correlation and lower research costs, when comparing to the development of a new drug [16].

Punica granatum Linn. species, known as pomegranate, is a shrub of the Lythraceae family, and its parts have different

medicinal importance, with different active ingredients and therapeutic indications [17]. For this reason, it is considered a great potential plant for preventing and fighting various diseases, with antioxidant, hypoglycemic, cholesterol reducer, antiviral, antiparasitic, antifungal and antidiarrhoeal properties, plus cancer preventive, cell differentiation promoter, estrogen enhancer and even antimicrobial (against *Staphylococcus aureus* and *Salmonella typhi*), anti-inflammatory, healing and antiseptic for the large quantity of tannin in the fruit skin [12,14,17,18]. However, due to the presence of alkaloids in its composition, some precautions regarding poisoning should be considered since its use may produce nausea, dizziness and vision problems, and is contraindicated in pregnancy, lactation, children under 5 years, gastritis cases and gastroduodenal ulcer [19].

Matricaria recutita Linn. (Chamomile) is a Europe and western Asia native plant and commonly used by northeastern Brazilian people. Belonging to the Asteraceae family and as a member of the daisy family, chamomile receives names such as: wild chamomile, Hungarian chamomile, pineapple weed (referring to the shape of the inflorescences), and scented mayweed. Chamomile is used forever in popular culture and, therefore, represents one of the few medicinal plants whose chemical constituents have been extensively evaluated pharmacologically, including in clinical trials [20].

Chamomile is known to have a variety of active flavonoids, as well as its volatile oil, which is rich in terpenoids, such as alpha-bisabolol, azulene, matricine and chamazulene. These components provide the anti-inflammatory, antispasmodic and antibacterial activity of the Chamomile [20].

Multiple therapeutic modalities have been tested and used over time, with the purpose of fighting, inhibiting or reducing pathogenic oral microbiota and, therefore, bleeding gingiva, with no real effectiveness to justify an indication to the general population [11]. Therefore, the purpose of this study is to clinically evaluate the effects of herbal mouthwash with extracts of chamomile and pomegranate in reducing the gingival bleeding in periodontal disease, comparing them with one another as well as with the chlorhexidine solution 0.12%.

2. Method

A comparative clinical study was performed, a randomized controlled trial, double blind, interventional, experimental, longitudinal and prospective, with an inductive approach, all data being recorded in specific forms.

The universe of this study involves patients usually treated at the Department of Clinical Dentistry at the Paraíba State University of Brazil. The sample is composed by individuals of both sexes, aged over 18 years old, during October 2010 to June 2011 that after clinical examination were diagnosed as having periodontal disease (gingivitis or chronic periodontitis), met the other criteria for inclusion in the study (absence of periodontal treatment and antibiotics in the last three months) and were in accordance with the participation in it. We excluded patients with healthy periodontium, users of braces and patients with diseases or systemic conditions with periodontal repercussions requiring antibiotic prophylaxis for the periodontal therapy completion as well as patients with diabetes mellitus, immunocompromised individuals, pregnant women, nursing mothers and drug users with periodontal repercussions.

Following the precepts of the CNS/MS 196/96 resolution which regulates the research ethics involving human subjects in Brazil, a total of 55 Brazilian adults with gingivitis [31] and chronic periodontitis [24] participated voluntarily in the study, in compliance with the protocol (CAAE: 0076.0.133.000-10) approved by the Paraíba State University Ethics Committee.

The sample was randomly divided into three [3] groups – each one of them containing individuals suffering from chronic gingivitis and periodontitis – which showed equivalence in number, sex, and age of its components, divided as follows, according with the mouthwash type used:

- G1 (control group) – patients using chlorhexidine 0.12% solution mouthwash ($n = 18$)
- G2 – patients using chamomile extract mouthwash ($n = 19$)
- G3 – patients using pomegranate extract mouthwash ($n = 18$)

The ethanol extracts were prepared at the Pharmacy Department Phytochemistry Laboratory, Paraíba State University. The Pomegranate (*Punica granatum L.*) and Chamomile (*Matricaria recutita L.*) specimens were purchased dried at Sao Paulo Flora Santos Company (Brazil), reference in medicinal species sale, under IBAMA registration No. 35867. The plant extracts were prepared with 100 g of herbal powder from each drug separately, and adding 900 g of ethanol 96%. The extraction begun by turbolysis, with a 10 min shaking, divided into 5 min intervals, with a rest period of 5 min between them. Then the material was packed in a container with a screw cap and left in the maceration process for 5 days. After that time, the plant extracts were obtained after filtration. At first, two experiments were conducted *in vitro*: 1) a screening to check the extract with the best antimicrobial activity and 2) testing of the extracts with Minimum Inhibitory Concentration (MIC) in 100%, 50%, 25%, 12.5%, 6.25% and 5% dilutions. The 5% Minimum Inhibitory Concentration (MIC) of the extract was used according to Srivastava and Sanjay¹⁵. The extracts were then diluted in sterile distilled water.

The instrument used for clinical assessment of gingival bleeding was the gingival bleeding index (GBI) of Ainamo and Bay (1975), which corresponds to the investigation of bleeding based on the bleeding/no bleeding on probing after 10 s of measurement. According to this index, for a bleeding occurring after 10 s of probing along the gingival mouth and tongue margin (gingival units) a positive score (+) is assigned to the bleeding face, otherwise a negative score (–) is assigned. The positive values of gingival units, related to bleeding faces, are added together and divided by the total number of gingival units evaluated, and this result multiplied by 100 to express the patient's GBI as a percentage.

The percentages of the GBI are grouped in the following scores: 1 – no bleeding; 2–10 to 10% of bleeding faces; 3–11 to 25%; 4–26 to 50%; 5–51 to 75%; and 6–over 75% of bleeding faces. Considered to be with discrete gingival inflammation patients with score 2, those with moderate gingival inflammation, score 3, and severe those with scores above 3.

After defining the periodontal diagnosis and the groups distribution, all patients were subjected, on the very first experiment day, to the following procedures: completing the Ainamo and Bay gingival bleeding index (GBI); Dental Hygiene Orientation (DHO); mouth scraping and root planing (SRP) all in a single session, using McCall universal curettes, and, finally, rinse with the substance for the correspondent group, and the data obtained in the first assessment were recorded in specific forms.

After the treatment, patients were advised to make two daily, 1 min long, mouthwashes with 10 ml of the solution corresponding to their group, 30 min after morning and night brushing, and for a 15 days period. It was given to all patients in each group, individually, a recipe containing the prescription, how to use and dosage of the substances used as a mouthwash.

After seven and 15 days, patients returned for the second and third evaluations in which the gingival bleeding index (GBI) was repeated, and the Dental Hygiene Orientation (DHO) was

Table 1
Sociodemographic profile of the studied population.

Variable	N	%
Total	55	100.0
• Age (years old)		
Up to 29	25	45.5
30 to 39	14	25.5
40 or over	16	29.1
• Genre		
Male	9	16.4
Female	46	83.6
• Race		
White	26	47.3
Not White	29	52.7

reinforced. All data obtained from the second and third assessments were also recorded in the sheets drawn up for this work.

We used the F (ANOVA) tests with Tukey comparisons, when the hypothesis of equal variances was verified; the Tamhane's T2, when this hypothesis was rejected, the t-Student test with equal variances and t-Student with unequal variances. The verification of the equal variances hypothesis was performed using the Levene's F test. All tests were performed in the a statistical software[#], with a 5% significance level.

[#] SPSS (Statistical Package for Social Sciences for Windows 15).

3. Results

The age of respondents ranged from 18 to 56 years old, with a 33 years old average, 31 years old median and a standard deviation of 10.76 years old, with most of these (45.5%) were aged from 18 up to 29 years old, while 25.5% corresponded to the 30–39 years old range and 29.1% aged 40 or more years old. The female were more prevalent (83.6%) and 47.3% of the patients were white (Table 1).

Regarding the assessment of sample's gingival bleeding index (GBI), Table 2 shows the statistical results for evaluation time and group as well as the differences (absolute changes between the evaluation times) between the groups. In the different periods of assessment, all studied groups had statistically significant GBI reduction ($p < 0.05$) and, as the Chart 1 also reveals, all averages were higher in the initial evaluation than in the following two. Comparing the groups, it is seen in Table 2 that the higher average

Table 2
GBI statistics by evaluation periods according to the group.

Evaluation	Groups			p value
	G1 Average ± SD (Median)	G2 Average ± SD (Median)	G3 Average ± SD (Median)	
0 days	65.50 ± 21.96 (67.37) ^(A)	66.71 ± 24.50 (76.08) ^(A)	62.84 ± 25.07 (65.44) ^(A)	p^a = 0.882
7 days	26.75 ± 20.08 (24.16) ^(B)	38.51 ± 29.15 (23.21) ^(B)	34.89 ± 24.44 (33.50) ^(B)	p^a = 0.350
15 days	20.25 ± 19.86 (9.93) ^(C)	31.55 ± 21.87 (26.68) ^(B)	23.60 ± 17.07 (18.93) ^(C)	p^a = 0.210
p value	p^b < 0.001*	p^b < 0.001*	p^b < 0.001*	
Absolute difference				
0–7 days	38.75 ± 21.00 (34.27)	28.20 ± 24.81 (25.00)	27.95 ± 27.08 (23.44)	p^a = 0.323
0–15 days	45.25 ± 23.20 (44.65)	35.16 ± 16.94 (35.70)	39.23 ± 26.39 (36.86)	p^a = 0.396
7–15 days	6.50 ± 9.97 (5.05)	6.96 ± 19.31 (6.89)	11.29 ± 14.83 (6.22)	p^a = 0.586

Obs.: If all the letters in parentheses are distinct, a significant difference is confirmed between the corresponding measurements by Bonferroni pairwise comparisons.

(*) Significant difference at the 5.0% rate.

^a Through the F (ANOVA) test.

^b Through the F (ANOVA) test for repeated measurements.

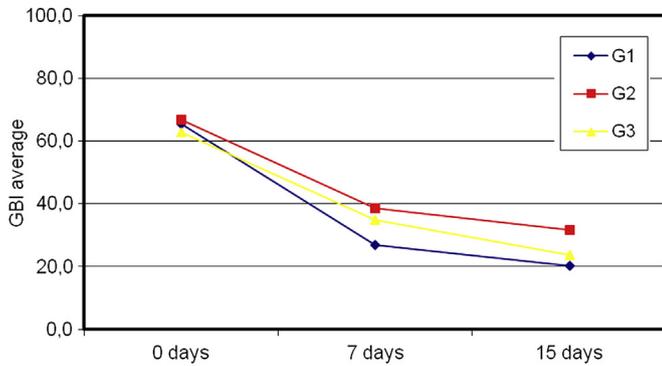


Chart 1. Gingival bleeding index according to the group and evaluation period.

within the three evaluation times occurred in G2 and that from 0 to 7 days the greater reduction was in G1 and from 7 to 15 days in G3. However, considering the 0 (zero) and 15 days periods, the best effect in reducing the gingival bleeding was in G1.

4. Discussion

Dental biofilm has an important role in the etiology of diseases affecting the oral cavity, with major implications for the quality of life and impact on systemic health. The first theories that oral diseases were associated only to the amount of biofilm colonizing bacteria have been replaced by the realization that specific pathogens could induce the disease [21]. Knowledge of the biofilm structure and composition, as well as about the main factors involved in the periodontal disease pathogenesis, resulted in improvements in traditional approaches to control biofilm; encouraging the development of new preventive and therapeutic strategies [22].

Several authors [4,23–28] are unanimous in stating that the biofilm is the primary etiologic factor for the development of periodontal disease, and its control being indispensable for its prevention. However, it is important to highlight the difficulties exhibited by patients to maintain an adequate mechanical control of dental biofilm [4,26,29,30].

Chaves et al. (2000) [31] and Golub et al. (2001) [32] agree with the statement that conventional mechanical periodontal treatment usually does not completely eradicate the Periodontopathic bacteria, since sites as periodontal pockets, grooves, furcations and concavities are difficult areas to access with periodontal instruments. Therefore, several therapeutic modalities have been proposed and studied in order to combat and control periodontal microbiota [10,11,33]. So, antimicrobial substances in the mouthwash form can be used as adjuvants in an attempt to compensate for these difficulties [4,26,29,30].

In accordance with Bugno et al. (2006) [34], the mouth rinses have been used in chemical control of dental biofilm, as well as being facilitators for the placement of active compounds for specific conditions treatment. However, for Rojas, Santos and Alemany (2005) [35] and Marine and Araujo (2007) [4], these rinses should never override the mechanical removal of biofilm, but act as its assistants.

Several antimicrobial agents are used in the biofilm chemical control in dentistry, including chlorhexidine, which has produced good results in biofilm reducing and in periodontal disease treatment [10,11,33].

Although it is scientifically proved that chlorhexidine significantly reduces and controls the gingival inflammation in patients exhibiting a poor oral hygiene, through its antibacterial action [33],

the study by Lucena et al. (2009) [36] to assess, in a comparative way, its anti-inflammatory effect with the chamomile, it was found similar results regarding the efficacy of both substances in the dental biofilm chemical control.

Chlorhexidine has a broad spectrum, acting against Gram-positive and Gram-negative bacteria. At low concentrations, it has a bacteriostatic effect, at high concentrations, has a bactericidal effect, causing cell lysis [4,37]. Complementing, Bastos et al. (2004) [38] reported that chlorhexidine is the product that has demonstrated the best clinical outcomes in the biofilm control compared to other topical antimicrobial agents being used prophylactically or therapeutically in gingivitis and chronic periodontitis. In addition, because of its high effectiveness, chlorhexidine is usually the standard product [4]. However, as noted by Smith et al. (2006) [12] and Vasconcelos et al. (2001) [13], the undesirable side effects of the chlorhexidine use, such as the teeth, tongue and restorations staining, and the loss of taste sensitivity, its long term use is limited.

Within this context, phytotherapy has been highly publicized for therapeutic purposes because of its low cost, easy access, the economic situation of the population, the absence of apparent adverse effects and the search for a better life quality, besides the broad popular acceptance and the World Health Organization (WHO) support, motivating scientific research [4,14,17]. Plus, Drummond et al. (2004) [26] and Marine and Araujo (2007) [4] state that about 20% of 250000 medicinal species, cataloged by UNESCO, are located in Brazil, facilitating the use of the plants curative potential for the treatment of diseases, including dentistry.

All these facts may also be associated with the indiscriminate use of herbal medicine, since the data from WHO (2006) show that approximately 85% of the world population has used some plant for some painful or unpleasant symptoms, but only 30% under physician orientation. Herbal medicine exists mainly in the informal market, representing a great danger to public health due to the marketing of vegetal drugs with no control, either phytosanitary, whether of identity and/or purity [4,17].

Herbal medicine has been used to treat various dental conditions. Cloves, pomegranate, common mallow, plantain, mulberry, sages, chamomile, among others, have been used in cases of gum disease, mouth abscesses, inflammation and ulcers. In accordance with Oliveira et al. (2007) [3], *Salvia officinalis* L. (sage), the *Punica granatum* L. (pomegranate), the *Althaea officinalis* L., *Calendula officinalis* L., *Malva sylvestris* L. (mauve) and *Plantago major* L. were the most cited in the literature surveyed, all widely used in folk medicine.

Thus, this work seeks to examine the effects of herbal mouthwash in reducing gingival bleeding in periodontal disease, comparing it with the effect of 0.12% chlorhexidine solution, being noteworthy that none of the study participants developed adverse or allergic reactions to the used rinse, even those belonging to the group of chlorhexidine mouthwash, which according to Vasconcelos et al. (2003) [13], is usually followed by side effects when used for a period exceeding 15 days, which in fact did not occur in the experiment in question. That may be the reason for the absence of such effects.

Among the plants used to obtain extracts and chemical groups surveyed in this study, the *Matricaria recutita* Linn (chamomile) species was positive for flavonoids and essential oils and the *Punica granatum* Linn (pomegranate) species for the presence of tannins, which agrees to the description of other studies [20].

Chamomile has been used for centuries to treat various inflammatory diseases of the gastrointestinal tract, mucous membranes and skin, as it has analgesic, antioxidant, antiparasitic, anticancer and immunoregulatory properties [20].

In the oral cavity, multiple studies have been developed using chamomile with different purposes, such as the treatment of thrush, mucositis, gingivitis and other [39]. Specifically for periodontal diseases, Pourabbas et al. (2005) [40] and Lucena et al. (2009) [36], by using chamomile to treat chronic gum disease, found a reduction in the gingiva bleeding index, confirming the findings of this study, in which the mouthwash using *Matricaria recutita* Linn. (chamomile) extract also reduced the bleeding index, both in gingivitis and in chronic periodontitis, showing statistically significant results ($p < 0.05$) in the different assessment periods (0, 7 and 15 days).

Literature points *P. granatum* (pomegranate) as a plant with great potential for preventing and fighting various diseases, highlighting its anti-inflammatory and antimicrobial properties. However, most studies of this plant were carried out *in vitro* or *in vivo*. Being a non-native and non-arable plant, certainly the use of pomegranate may arouse the interest of pharmaceutical and food companies. The use of pomegranate and particularly its fruit is relatively easily, without compromising its therapeutic properties [14].

Pomegranate (*Punica granatum*) has been used by several populations in the treatment of oral diseases [3,41,42]. In recent ethnobotanical studies, the pomegranate was the most often cited plant by users and recommended by health professionals for the treatment of several pathologies [14,43].

Regarding the reduction in the gingival bleeding index in the groups of patients with gingivitis and chronic periodontitis, who used pomegranate extract mouthwash, in the experiment in question, results were as statistically significant ($p < 0.001$) as those observed in groups of chamomile and chlorhexidine 0.12%, confirming the anti-inflammatory potential of pomegranate described in other studies [3,14,42].

Comparing the effects of the substances used to reduce the bleeding index in patients with periodontal disease, it was found that both the 0.12% chlorhexidine, and chamomile and pomegranate were effective, demonstrating statistically significant and similar results ($p < 0.001$), suggesting that any of these three substances can be used to control bleeding gums in periodontal disease.

Thus, considering that the chamomile and pomegranate extracts were as effective as the solution of chlorhexidine 0.12% in reducing the bleeding index in subjects with chronic gingivitis and periodontitis, it can be inferred, sometimes, replacing the solution of 0.12% chlorhexidine by the herbal products tested or their alternative use to control bleeding gums in periodontal disease.

5. Conclusion

Chamomile and pomegranate extracts mouthwashes were effective in reducing gingival bleeding in periodontal disease, suggesting that both extracts have anti-inflammatory and antimicrobial actions similar to those of the chlorhexidine 0.12%, and thus can also be used as additional therapeutic agents to reestablishment and maintenance of periodontal health.

Conflict of interest

None declared.

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