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## Evaluation of *Punica granatum* Linn (Pomegranate) Extract in The Postoperative Period of Aesthetic Clinical Crown Augmentation Surgery: A Clinical and Phytochemical Study



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### ABSTRACT

**Objective:** To evaluate the effect of pomegranate aqueous extract on tissue repair and supragingival biofilm control in the postoperative period after periodontal surgery for gingivectomy with sufficiently invasive osteotomy for the aesthetic correction of the smile. **Methodology:** This is a clinical study conducted on 20 patients with indications of pink smile aesthetic correction, divided into two groups according to the product used as a mouth rinse in the postoperative period. The test group used pomegranate aqueous extract, and the control group used the gold standard, 0.12% chlorhexidine digluconate. In both groups, patients rinsed 15 mL of each solution for 1 minute, twice a day, for 15 days. Periodontal clinical parameters such as plaque index (PI), gingival bleeding index (GSS), and probing depth (PS) were collected before surgery and at 7, 15, and 21 days postoperatively. **Results:** Pomegranate extract reduced the amount of tooth surfaces with stained biofilm, reduced gingival bleeding in the operated area, and maintained the probing depth at levels compatible with periodontal health and lower than the control group. **Conclusion:** Pomegranate aqueous extract showed encouraging results as an adjunct to mechanical control of supragingival biofilm in gingivectomy surgical wounds. However, further clinical studies should be conducted especially with a larger number of samples.



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## INTRODUCTION

The desire for an aesthetic and harmonious smile has grown significantly, causing an increase in the demand for dental procedures with aesthetic purposes [1,2]. The gummy smile, defined by excessive exposure of the gums when smiling, is one of the most common aesthetic complaints. Among its several etiologies, altered passive eruption is considered the most prevalent, and its treatment involves surgical interventions by means of gingivectomy procedures with osteotomy, allowing the increase of the clinical crown of teeth, the correction of zenith positioning, and smile harmonization [2,3].

After periodontal surgery, it is common for patients to be unable to maintain good mechanical biofilm control because the surgical wound is still raw. The 0.12% chlorhexidine digluconate is the antimicrobial agent considered the gold standard for this purpose, but most professionals do not recommend its daily and prolonged use because of its adverse effects, such as: change in staining of teeth and restorations, formation of supragingival calculus, loss of taste, burns in the soft tissue, xerostomia, and unpleasant aftertaste in the mouth [4,5].

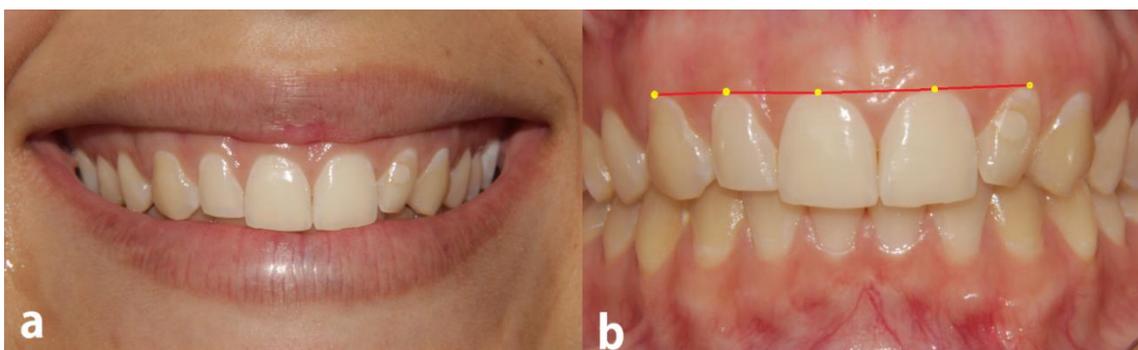
In view of the need for clinical alternatives, medicinal plant extracts have been studied in order to replace chlorhexidine in the chemical control of supragingival biofilm [6]. Among several plants with medicinal potential is *Punica granatum* Linn, popularly known as pomegranate, which represents a phytochemical reservoir of great medicinal value, among which can be mentioned the alkaloids and tannins. Popular for its potent antibacterial, antiviral, antioxidant, and especially anti-inflammatory, probiotic properties, pomegranate has been arousing interest on its effects on periodontal tissues [7,8].

However, considering that pomegranate has been highlighted in the literature for its power to inhibit bacteria and pro-inflammatory cytokines [7], there are no studies demonstrating its application in biofilm control after periodontal plastic surgery. Therefore, the aim of this study was to evaluate the antimicrobial action and the reparative effect on periodontal tissues of using the extract of *Punica granatum* Linn (pomegranate), during the postoperative period of periodontal surgery, as a substitute for chlorhexidine, due to its local adverse effects.

## METHODOLOGY

### Type, place and ethical aspects of the study

This research is a clinical and phytochemical study carried out from September 2021 to August 2022 at the Clínica-Escola de Odontologia da UFCG/CSTR, where patients in need of aesthetic clinical crown augmentation due to altered gingival contour (Figure 1), who sought care at the Extension Project in Clinical and Surgical Periodontics (PROEPECC), linked to the Academic Unit of Biological Sciences - UACB/CSTR, were selected. This study was approved by the Research Ethics Committee of the Alcides Carneiro University Hospital/UFCG with substantiated opinion no. 3,155,454 and was also registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SisGen under Registration Certificate no. A85A310, in compliance with the provisions of Law no. 13,123/2015 and its regulations.



**Figure No. 1: Clinical pattern of the patients selected for the study: need for aesthetic clinical crown augmentation due to alteration in the gingival apex line.**

### Population

Twenty patients of both genders were selected and randomly assigned to two groups according to the solution used as postoperative rinsing after periodontal surgery for aesthetic crown augmentation. In the positive control group, 10 subjects rinsed with 0.12% chlorhexidine digluconate (Periogard®) and, in the test group, 10 subjects used pomegranate extract.

### Inclusion criteria

Patients in need of clinical crown augmentation due to altered gingival contour between elements 13 and 23 and who met the following criteria were included in the study:

1- Age between 18 and 65 years, both genders; 2- Systematically healthy and without contraindication for periodontal surgery; 3- Not be pregnant or lactating; 4- Present normal lip position, absence of attachment loss, intermediate gingival phenotype or Class A2 of De Rouck *et al.* [9] (2009) (thick gingiva, triangular crown, keratinized mucosa of 3 to 4 mm and long papilla); 5- Non-smoker and 6 - No previous mucogingival surgical involvement.

#### **Exclusion criteria**

1- Gingivitis; 2- Previous periodontitis or abscess formation; 3- Use of medications that interfere with periodontal health; 4- Presence of systemic disease; 5- Patients undergoing orthodontic treatment; 6 - Patients with a thick gingival phenotype (thick gingiva, square crown, keratinized mucosa more than 5mm wide, short papillae, and thick bone plate assessed by palpation); 7 - Patients who do not come for post-operative evaluations; and 8 - Patients who are intolerant to the active ingredient Ibuprofen.

#### **Botanical material and preparation of the extract of *Punica granatum* Linn**

The raw material of *Punica granatum* Linn (Pomegranate) was obtained from various sources in the central public market of the city of Patos-PB/Brazil and the fruits were botanically identified in the Biochemistry Laboratory of the Biological Sciences Unit of UFCG, Patos-PB Campus. To obtain the extract, we used the methodology performed by Carmo, Lima, Takaki [10] (2007) and Dias *et al.* [11] (2000). The fruits were washed with water and then separated into peel and mesocarp, and the peel was the material used in the study. The peel was taken to an oven at 45 °C for 24 h to remove all moisture. After this period, the aqueous extract was obtained by mixing one (01) gram of dry matter with 10 mL of distilled water, extraction was by the macerated method, in which the dry matter was left to rest with distilled water for 24 h and then filtered. The extract was packaged in amber flasks, cleaned, dried, and stored in a cold chamber until it was distributed to the patients.

#### **Phytochemical study of the extract of *Punica granatum* Linn**

The phytochemical analysis of the extract was performed in the Phytochemical Laboratory Prof. Dr. Raimundo Braz Filho (IPeFarM/UFPB) and in the biochemical laboratory of Nova Esperança Colleges with the collaboration of Prof. Dr. Maria Denise Leite Ferreira and Dr. Yuri Manguiera do Nascimento, pharmacists from the Federal University of Paraíba.

The extract was evaporated at a rotary evaporator and the screening of the secondary metabolites present occurred according to the methodology recommended by Silva *et al.* [12] (2010); Nascimento Júnior [13] (2020); as described below:

### **Flavonoids Test**

The cyanidin or Shinoda (concentrated HCl and magnesium) test was performed. Where, 5mg of the extract was added, to approximately 0.5 cm of magnesium strip with 2 ml of concentrated hydrochloric acid. The end of the reaction was verified by the termination of effervescence. The appearance of brown or red color indicates the presence of flavonoids in the extract.

### **Test for tannins**

In a test tube containing 2 ml of the test extract (solubilized in organic solvent), three drops of alcoholic FeCl<sub>3</sub> solution were added and shaken strongly to observe any color variation. If a blue precipitate is formed, there is the presence of hydrolyzable tannins, and green, the presence of condensed tannins.

### **Test for alkaloids**

About 5 mg of the test extract was heated to boiling with 30 ml of dilute hydrochloric acid. It was then filtered and divided into 4 test tubes. In 3 of these tubes three drops of the Dragendorff, Mayer and Bertrand reagents respectively were added. If turbidity and/or precipitate formation occurs, this is a positive reaction for alkaloids.

### **Saponins test**

To 5 mg of the extract, 2 ml of chloroform and 5 ml of distilled water were added, then filtered into a test tube, and the solution was stirred permanently for 3 minutes to see if foam was formed. The presence of foam after the process indicates the presence of saponins in the extract.

### **Clinical and periodontal parameters evaluated preoperatively and postoperatively**

Some periodontal clinical parameters were observed in the preoperative evaluation and at 7, 15 and 21 days after the surgical procedure to analyze the effect of 0.12% chlorhexidine and pomegranate extract on biofilm control and gingival inflammation (Figure 2).



**Figure No. 2: Patient with altered gingival contour on elements of the 2nd sextant (a), immediate postoperative (b) and 21-day postoperative (c).**

To evaluate biofilm control (plaque index - PI), gingival inflammation (gingival bleeding index - GSS), and probing depth - PS in the elements involved in the surgical area, a dichotomous evaluation was used (yes/no), with which the proportion of sites with stimulated gingival bleeding was calculated, as recommended by Chapple *et al.* [14] (2018) when the PC-15 periodontal probe (North Carolina) was inserted with controlled force into the gingival sulcus at four sites, three of which were buccal (mesial, buccal, and distal) and the palatal face. Similarly, the proportion of biofilm adhered to the tooth surface was calculated. The dental elements were brushed with plaque evidencer (Eviplac®), washed thoroughly with water to remove the excess and then the index was calculated. The probing depth was evaluated by measuring the distance between the gingival margin and the apical end of the gingival sulcus with the same probe reported above. All exams were performed by a previously calibrated examiner and recorded in a specific chart for the research. After each evaluation, prophylaxis was performed to remove the stain from the surface of the teeth used to show the biofilm.

### **Surgical procedure**

Since the elective patients for the study had an intermediate phenotype, without excessive gingival exposure, and the goal of periodontal surgery was to correct only the gingival contour, the technique chosen was gingivectomy with sufficiently invasive osteotomy when necessary according to the methodology used by Cristovám *et al.* [1] (2019).

The surgical protocol was divided into the following steps: **1-** Evaluation of the ISG; **2-** Evaluation of the IP; **3-** Polishing of the stained crowns with prophylactic paste and rubber cup; **4-** Extra and intraoral antiseptics with chlorhexidine digluconate at 2% and 0.12%, respectively; **5-** Terminal infiltrative anesthesia using the anesthetic Mepivacaine with vasoconstrictor; **6-** Marking of points by probing with the aid of periodontal probe PC-15 in the region where the incision will be made (three points were marked on each tooth using the CEJ as a reference point); **7-** Union of the previously marked points using a fine line made

with the 15c scalpel; **8-** Incision with the 15c scalpel without bevel; **9-** Intrasulcular incision to loosen the gingival fibers; **10-** Removal of soft tissue and granulation tissue with Gracey curettes (5/6); **11-** Sufficiently invasive osteotomy with Fedi microcinzel; **12-** Plenty of saline solution irrigation; **13-** Hemostasis; **14-** Postoperative instructions.

### **Postoperative management**

The study participants were informed about possible postoperative problems such as bleeding, pain and discomfort and were prescribed Ibuprofen 600 mg tablets to be taken only in case of pain. In the positive control group (G1), patients were instructed to swish 15 ml of chlorhexidine digluconate 0.12% for 1 minute twice a day for 15 days. In the test group (G2), the subjects rinsed with 15 ml of *Punica granatum* Linn (pomegranate) extract, twice a day, for 1 minute, for 15 days. For both groups, the first mouth rinse was performed immediately after surgery in the presence of the researcher, the remaining mouth rinses were performed 30 min after morning and evening brushing for a period of 15 days. All patients were individually given a prescription for how to use the mouth rinses. During home use, the pomegranate extract was stored in a refrigerator at a temperature of 15°C.

### **Data analysis**

Data analysis was performed using the IBM SPSS Statistics software (SPSS for Windows, Version 20.0. Armonk, NY: IBM Corp). Initially, descriptive statistics were performed to characterize the sample, which corresponded to the calculation of measures of central tendency and variability for quantitative variables. The assumption of data normality for quantitative variables was not confirmed after employing the Shapiro-Wilk test. Therefore, nonparametric tests were used to determine the significance of intergroup and intragroup differences, according to Hannigan, Lynch [15] (2013). The Mann-Whitney test was used to determine significant intergroup differences in relation to the clinical parameters (ISG, PI and PS). The significance level was set at  $p < 0.05$ . An indication of the magnitude of statistical variation was assessed by estimating the effect size (ES) according to Cohen [16] (1994) and Wasserstein, Lazar [17] (2016). The ranking of ES values was done according to Cohen [16] (1994):  $\leq 0.20$  indicative of a small effect; 0.30-0.70 indicative of a moderate effect; and  $\geq 0.80$  indicative of a large effect.

## RESULTS

### Phytochemical results

The phytochemical study, performed with the extract of *Punica granatum* Linn (pomegranate) by means of classical techniques and analytical-qualitative reactions, revealed the presence of flavonoids, tannins, alkaloids and saponins (Table 1).

**Table No. 1: Phytochemical prospection of the extract of *Punica granatum* Linn (pomegranate)**

CHEMICAL CONSTITUENT	RESULT
Flavonoids	+
Alkaloids	-
Tannins	+
Saponins	+

“+”: present, “-”: absent.

### Clinical results

Twenty patients with a need for aesthetic clinical crown augmentation due to altered gingival contour participated. Seventeen patients (85%) were female and 3 (15%) were male. In the chlorhexidine group, 80% were in the age group between 22 and 31 years old and 20% between 18 and 21 years old. While in the pomegranate group, the prevalent age group was 22 to 31 years (100%).

The comparative analysis of periodontal clinical parameters, evaluated in both groups according to the preoperative and postoperative periods, can be seen in Table 2.

**Table No. 2: Comparative analysis of the clinical parameters in the groups (chlorhexidine vs. pomegranate) according to the evaluated times.**

Variables	G1 (chlorhexidine)					G2 (pomegranate)					p-value <sup>(1)</sup>	ES
	M	SD	Me	IIQ		M	SD	Me	IIQ			
				P25	P75				P25	P75		
<b>ISG</b>												
Before	20,80	17,11	16,60	11,45	29,15	27,48	16,81	29,10	8,30	37,50	0,403	0,39
7 days later	34,55	18,43	31,20	23,95	48,93	37,06	19,00	39,55	19,75	50,00	0,820	0,13
15 days later	19,55	10,94	16,60	11,45	27,08	13,32	9,38	12,50	8,30	15,63	0,142	0,61
21 days later	13,71	12,27	10,40	4,10	18,70	18,72	10,61	18,70	8,30	30,15	0,209	0,44
Reduction in % (T0 - T15)	14,86	25,83	0,00	0,00	33,98	45,14	36,43	53,52	8,40	71,44	<b>0,036*</b>	0,96
Reduction in % (T0 - T21)	39,29	39,22	39,32	0,00	80,94	24,72	31,55	5,60	0,00	51,43	0,451	0,41
<b>IP</b>												
Before	54,56	25,49	52,05	28,08	76,03	57,05	31,60	60,40	31,22	82,23	0,791	0,09
7 days later	41,55	13,76	39,15	29,10	56,20	50,39	21,01	52,05	38,48	59,38	0,225	0,50
15 days later	41,24	24,86	35,40	16,75	65,63	50,39	19,18	58,33	30,18	66,60	0,344	0,41
21 days later	36,21	13,47	31,20	25,00	45,78	44,54	20,02	43,70	32,25	66,60	0,160	0,49
Reduction in % (T0 - T15)	26,33	33,82	10,15	0,00	62,65	19,90	27,69	7,90	0,00	37,81	0,716	0,21
Reduction in % (T0 - T21)	29,57	28,45	27,83	0,00	58,80	25,76	23,41	24,86	0,00	50,02	0,757	0,15
<b>PS</b>												
Before	1,45	0,51	1,40	1,04	2,03	1,74	0,45	1,70	1,46	2,03	0,173	0,60
7 days later	1,67	0,53	1,75	1,08	2,00	1,74	0,59	1,77	1,15	2,29	0,596	0,13
15 days later	1,03	0,27	1,05	0,93	1,23	1,36	0,48	1,23	0,98	1,63	0,208	0,85
21 days later	0,89	0,14	0,95	0,70	1,00	1,28	0,34	1,16	1,00	1,50	<b>0,001*</b>	1,50
Reduction in % (T0 - T15)	27,01	21,44	18,33	11,85	46,97	24,17	21,72	23,40	4,51	44,44	0,495	0,13
Reduction in % (T0 - T21)	32,91	25,50	33,33	5,56	59,11	25,41	18,38	18,93	11,15	44,44	0,544	0,34

*Note. M = mean; SD = standard deviation; Me = Median; IIQ = interquartile range (25th - 75th percentile). (1) Mann-Whitney test.*

**\* p < 0,05.**

**ES = effect size (Cohen's)**

In both groups, during the period of use of the postoperative mouthrinses, there was an increase in the GSI in the 7-day period after surgery. However, at the 15-day evaluation, the pomegranate group showed a significant reduction in gingival bleeding on probing ( $p = 0.036$ ) compared to the chlorhexidine group.

With respect to PI, a reduction was observed between the baseline and 7-day periods. Even with the surgical wound in the initial phase of tissue repair, this index remained at lower values than those observed in the first evaluation before periodontal surgery. After suspension of the mouth rinses, there was an increase in PI only in the pomegranate group, but with values below the baseline period and without significant difference from the chlorhexidine group.

The probing depth of all research participants was reduced after periodontal surgery, remaining below 2 mm, and there was no difference between groups. In the 21-day period, the clinical gingival sulcus showed less depth in the chlorhexidine group ( $p = 0.001$ ).

None of the participants in this study used Ibuprofen, as they reported no need for it due to the absence of post-surgical pain. The presence of supragingival calculus was observed in patients who used 0.12% chlorhexidine, while in the pomegranate group there was no such occurrence.

## DISCUSSION

Under normal conditions, the gingival sulcus has a depth less than or equal to 3 mm, however, situations may occur where this depth is greater, characterizing the formation of a periodontal pocket. It is observed in table 2, the average in the probing depth among the test group of 1.7 at the initial moment and after 21 days this same average was 1.16, meaning that the pomegranate extract has anti-inflammatory and healing activity, helping to maintain the periodontium within the standards.

A reduction of this index was observed in the period, with a better result in the pomegranate group, although not statistically different from the chlorhexidine group. The chlorhexidine digluconate 0.12% is considered the priority choice in the postoperative period, however, the average GSS of 15 days in the control group was 14.86 while in the test group it was 45.14. Thus, these results highlight the healing and anti-inflammatory role of pomegranate.

The search for periodontal plastic surgery due to complaints of "excessive gum disease when smiling" has been constant in clinical dental practice, especially among women, who seek aesthetic procedures more frequently and, consequently, are more interested in a harmonious smile, corroborating the data found in this clinical study, in which 85% of the patients who sought the surgical procedure were female [18, 19].

After the procedure, due to the surgical wound, the mechanical control of dental biofilm becomes difficult, and it is necessary to use a coadjuvant therapy during the postoperative period. Chlorhexidine is a broad-spectrum bisbiguanide that acts on gram-positive and gram-negative bacteria, fungi and yeasts. It is considered the gold standard chemical agent for controlling supragingival biofilm and inflammation, and has been used as an adjunctive therapy in periodontics since the 1970s [20, 21].

However, the literature has shown some undesirable side effects of its use, such as: loss of taste, changes in the color of teeth, restorations and tongue, precipitation of supragingival calculus, burns in the soft tissue, xerostomia and unpleasant aftertaste in the mouth. For this reason, the search for alternative methods capable of reducing side effects in patients has influenced the use of natural products and, in this context, phytotherapies have been standing out and occupying more and more space [22, 5, 6].

In periodontics, research has increasingly focused on *in vitro* and *in vivo* studies of plant extracts because of their antibacterial, anti-inflammatory and healing actions on the microbiota that cause periodontal disease [23-27]. Thus, the aim of this research was to study, as a therapeutic alternative to chlorhexidine, the clinical effects of the extract of *Punica granatum* Linn (pomegranate) after clinical crown augmentation surgery, in which postoperative biofilm control is important for the aesthetic result.

*Punica granatum* Linn was chosen because it is very popular, has a low cost, and is found throughout the country. The extract used was the aqueous type, because it presents a simple extraction method and can be reproduced for domestic use. Moreover, the selection of this plant is associated to the fact that several recent studies have proven its beneficial effects in periodontics, such as: antimicrobial action against bacteria from dental biofilm, antifungal and anti-inflammatory effects, inhibition of cyclooxygenase (COX) and lipoxygenase (LOX)

enzymes, and improvement in clinical signs of periodontitis and in the level of interleukin 1 (IL-1) in patients under maintenance therapy [28,24,25,29,30,22,26,13,32].

Such therapeutic properties are attributed to the chemical compounds present in pomegranate, especially in its peel. This corresponds to about 50% of the total weight of the fruit and is an important source of bioactive constituents that have antimicrobial, antioxidant, anti-inflammatory, and healing activity. Among these are flavonoids, tannins, alkaloids, and saponins [33], as shown in the phytochemical results of the *Punica granatum* Linn extract used in this research.

As for the alkaloids, the test showed a negative result, which does not agree with the literature of the species. Araújo *et al.* [33] performed a phytochemical analysis of pomegranate peel tea to determine its constituents and among the compounds present the alkaloids were identified. However, it is worth noting that many factors can influence the extraction and/or identification of these metabolites, such as the part of the plant material used, its origin, how the extraction was performed, the degree of processing, the solvent used and its concentration, temperature, polarity and seasonal variations [34] and even the methodology used in the research [13].

The antimicrobial property of pomegranate extract on supragingival biofilm bacteria is well studied and several studies have shown its effect against bacteria belonging to the genus *Streptococcus*, more specifically *S. mutans*, *S. sanguis*, *S. mitis*, *S. sobrinus* and the species *Lactobacillus casei* [27,30,35,36]. In the present study, this effect was evaluated only clinically by staining the biofilm adhered to the tooth surface. The parameter used was the plaque index (PI), evaluated dichotomously (yes/no) only of the area involved in the surgical procedure, to thus evaluate the effect of the extract in the postoperative period. Gradual reductions in mean PI values were observed in postoperative evaluations, which corroborates the results of the double-blind randomized controlled clinical trial published by Nóbrega *et al.* [27] (2015).

After stopping the mouthwash with pomegranate, the PI increased, but remained at a lower level than the baseline. These data confirm the references obtained in the literature, which show antimicrobial activity of the pomegranate against the supragingival biofilm and reduction in PI after rinsing with mouth rinses containing this phytotherapeutic [28,27,37]. When comparing the initial PI with the postoperative values, an increase was observed at 7 days, followed by a

reduction at 14 days. The increase in the index in the first days after surgery is related to the difficulty of hygiene due to the surgical wound is in the early stage of healing.

This significant antibacterial property is mainly due to the phytoconstituents of the extract, especially the tannins, which, according to the literature, may inhibit the action of some important bacterial enzymes for the mechanism of union between other bacteria and the tooth surface, leading to decreased biofilm formation, enabling its control [24,38,36]. Among the main tannins present in pomegranate is punicalagin, an ellagic tannin derived from the pomegranate fruit. With anti-inflammatory, antifungal and antioxidant effects, this is probably one of the main antimicrobial constituents of this fruit [39]. This is justified in the phytochemical analysis of the pomegranate extract, which tested positive for tannins.

This finding corroborates the study of Nóbrega [39] (2012) who evaluated a mouthwash containing *Punica granatum* Linn, in order to study the clinical efficacy in controlling dental biofilm and gingival inflammation in comparison with 0.12% chlorhexidine. The results showed that mouthrinses of *Punica granatum* reduced the mean values of PI and ISG, but with no significant difference between them. More recently, Hernawati and Soesilawati [25] (2020) reported the potential effect of pomegranate extract against *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, which are common gram negative bacteria in the microbiota of gingival and periodontal diseases.

According to Pereira *et al.* [37] (2005), who analyzed the effect of a herbal toothpaste, *Punica granatum* Linn, they observed a reduction in bleeding on probing in patients. In the present study, in both groups, the SDI increased at the 7-day postoperative evaluation when compared to the SDI at baseline. This can be discussed and justified by the fact of the short period of repair. However, 15 days after the surgical procedure, the group that used pomegranate extract as a postoperative rinse showed a significant reduction in bleeding on probing similarly to the 0.12% chlorhexidine digluconate group, admitted as the gold standard group.

Batista *et al.* [24] (2014) and Nóbrega *et al.* [27] (2015) who compared pomegranate extract with 0.12% chlorhexidine digluconate, also observed a reduction in ISG and attributed antimicrobial and anti-inflammatory activities to the herbal medicine, which may be related to its ability to inhibit NF-K $\beta$  (nuclear factor Kappa Beta), COX 1 and 2 and IL-1 $\beta$  [26]. The inhibitory capacity

of the oxidant enzymes COX and LOX may be related to the large amount of flavonoids present in pomegranate peel [37], which matches the positive result for flavonoids in the extract. This is known to present several biological activities, among them the ability to act on inflammation [40] and the rich antioxidant activity, which may be due to its ability to sequester OH<sup>-</sup> and O<sub>2</sub>, to exert a synergistic effect with other antioxidant metabolites [33].

Furthermore, in this study, it was found that the SES increased after suspension of the mouth rinse, showing the ability of the aqueous extract of pomegranate to positively impact the control of gingival inflammation in the operated region, leading to believe that it may be an alternative therapeutic adjunct to brushing during the postoperative period of periodontal surgery for aesthetic clinical crown augmentation [26]. Furthermore, Batista *et al.* [24] (2014) evaluated pomegranate extract in patients with gingivitis and periodontitis, in which they also observed a reduction in GSI.

In this context, the mouth rinsing with pomegranate extract maintained PS at levels compatible with gingival health and, on average, with greater reduction than the chlorhexidine digluconate group, showing the anti-inflammatory and healing effect of pomegranate, as well as its antibacterial action against the supragingival biofilm, acting mainly against gram-negative bacteria, preventing gingival edema [28,24-26,31,41]. Pomegranate also stands out for its antifungal action, the saponins present in the extract stand out for this property, as well as for its cytotoxic activity acting against tumor cells [39]. The presence of this metabolite is confirmed in research conducted by Soares *et al.* [42] (2018) in which chemical and physicochemical characterizations of fluid extracts of the pomegranate tree were performed with extracts of the pomegranate peel, seeds and leaf and the phytochemical result of the peel extract showed a positive result for saponins.

Because this is a clinical study, the main limitation of this study is the lack of microbiological data, which would be important to support the clinical findings, especially to compare with PI. However, pomegranate extract showed promising results and very similar to chlorhexidine, which is already an established antimicrobial substance in the literature and in clinical practice. Thus, it is essential that further clinical studies are conducted to strengthen this clinical indication of pomegranate in surgical periodontics. Thus, the breadth of research can safely enable its use by the population.

## CONCLUSION

Compared with 0.12% chlorhexidine digluconate, the use of *Punica granatum* Linn (pomegranate) extract as a mouth rinse in the postoperative periodontal plastic surgery of gingivectomy to correct the red esthetics of the smile showed satisfactory results, such as a reduction in the amount of dental surfaces with the presence of stained biofilm, reduction of gingival bleeding in the operated area and maintenance of the probing depth at levels compatible with health. The clinical and phytochemical results demonstrated that the secondary metabolites of *Punica granatum* have properties relevant to periodontal health.

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