Clinical applicability of natural product(s)-containing mouthwashes as adjunctive treatment of biofilm-induced gingivitis: a systematic review

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RESUMO: Aplicabilidade clínica de colutórios à base de produtos naturais como tratamento adjuvante da gengivite induzida por biofilme: uma revisão sistemática. Os produtos naturais têm surgido como alternativa eficaz e de baixo custo para o tratamento de várias doenças da cavidade oral. Objetivou-se avaliar, a partir de revisão sistemática da literatura, se há evidências científicas garantindo a utilização segura e eficaz de antissépticos bucais contendo produto(s) natural(is) como tratamento adjuvante da gengivite induzida por biofilme. Foram realizadas buscas nas bases de dados Medline, SciELO, LILACS e Cochrane Library, através de combinações usando as palavras-chave gengivite/produtos naturais/ fitoterápicos/ bochechos, em Inglês, Português e Espanhol. Consideraram-se os estudos publicados até setembro de 2010. Quatro examinadores analisaram separadamente: desenho e fase do estudo, qualidade metodológica (escala de Jadad - EJ), produto experimental e a concentração, intervalo de administração e tempo de uso, bem como a análise estatística empregada e os resultados clínicos de interesse. Foram encontrados 503 artigos dos quais 08 foram incluídos na revisão final como sendo ensaios clínicos fase II, controlados, randomizados e cegos, marcando 4 (25%) e 5 (75%) na EJ. Os principais produtos naturais avaliados foram *Azadirachta indica, Garcinia mangostana*, *Lippia sidoides, Salvadora persica* e *Sesamum indicum,* cuja concentração, intervalo de administração, tempo de uso, e efeitos adversos, variaram de acordo com cada estudo. Índice de placa e Índice Gengival foram os mais utilizados, bem como α=5% e testes t-pareado, t-Student, Wilcoxon e Mann-Whitney. 62,5% e 50% dos produtos reduziram significativamente a presença de bioflme supragengival e gengivite, respectivamente. Os colutórios contendo o óleo essencial das folhas de *L. sidoides* (1%) e o extrato das folhas de *A. indica* (25%) podem ser indicados como tratamento adjuvante da gengivite induzida por biofilme.

Palavras-chave: produtos naturais, gengivite, biofilmes, ensaios clínicos, enxaguatório bucal

ABSTRACT: Natural products have emerged as an effective and low-cost alternative for treating various diseases of the oral cavity. This study aimed to evaluate, through a systematic literature review, if there is scientific evidence ensuring the safe and effective use of natural product(s)-containing mouthwashes as adjunctive treatment of biofilm-induced gingivitis. Searches were conducted in the databases Medline, SciELO, LILACS and Cochrane Library, by using combinations of the key words gingivitis/natural products/phytotherapy/mouthwash, in English, Portuguese and Spanish. Studies published until September 2010 were considered. Four examiners analyzed independently: study design and phase, methodological quality (*Jadad* scale - JE), experimental product and its concentration, dosing interval and time of usage, as well as employed statistical analysis and clinical outcome of interest. From the 503 articles found, 08 were included in the final review as phase II, controlled, randomized and blind clinical trials, scoring 4 (25%) and 5 (75%) in JE. The main natural products assessed were: *Azadirachta indica, Garcinia mangostana*, *Lippia sidoides, Salvadora persica* and *Sesamum indicum* whose concentration, dosing interval, time of usage and adverse effects varied according to each study. The Plaque and Gingival Index were most employed, as well as α = 5% and paired t, Student's t, Wilcoxon and Mann-Whitney tests. A total of 62.5% and 50% of the products significantly reduced supragingival biofilm and gingivitis, respectively. Mouthwashes containing the essential oil from the leaves of *L. sidoides* (1%) and the extract from the leaves of *A. indica* (25%) can be indicated as adjunctive treatment of biofilm-induced gingivitis.

Key words: natural products, gingivitis, biofilms, clinical trials, mouthrinse

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INTRODUCTION

Chronic periodontal disease (CPD) has affected a large percentage of the population and has been responsible for a significant number of tooth losses, as well as for influencing the development of systemic changes like diabetes (Mealey & Ocampo, 2007), premature newborns (Davernport et al., 2002), brain lesions (Tiejian et al., 2000) and cardiovascular disorders (Genco et al., 2002).

In the United States, the early stages of CPD have affected about 53.1% of the population, whereas moderate or severe stages have been reported for 12.6% of the people (Albandar et al., 1999). In Brazil, the latest epidemiological survey, undertaken in 2003, indicated that 78.1% of adults between 34 and 44 years old showed diseased gingival tissue (Brazil, 2004).

Chronic periodontal involvement is an infectious, irreversible and destructive disease which has the contribution of the host immune system to be developed, since the production of cytokines such as interleukin-1 (IL-1), Tumor Necrosis Factor (TNF- α) and prostaglandins by immune cells promotes bone resorption and destruction of conjunctive tissue by the action of osteoclasts (activated by IL-1 and TNF-α) and metalloproteinases (activated by prostaglandins), respectively (Baker, 2000; Koop & Medzhitov, 2003; Teng, 2006; Alnaeeli et al., 2006).

Etiologically, CPD has been recognized to be caused by biofilm-forming microrganisms in the subgingival area of the teeth (Lovegrove, 2004). In a general way, a microbial community initially consisting of Gram-positive facultative anaerobes becomes, according to the disease progression, a community of Gram-negative strict anaerobes. Such ecological alteration is a result of changes in environmental conditions, caused by reduced availability of oxygen, increased temperature in the gingival sulcus fluid, and establishment of an alkaline environment. Primarily, microrganism colonization on the tooth surface promotes an inflammatory reaction in the adjacent gingival tissues, favoring the clinical condition known as gingivitis, which is mainly characterized by spontaneous or induced gingival bleeding (Sbordone et al., 2000). Thus, the prevention of chronic periodontal disease has been known to require constant removal of dental biofilm, either by mechanical (toothbrush) or by chemical means (mouthwashes or other topical methods).

The main chemical agents currently available are: triclosan, cetylpyridinium chloride, chlorhexidine, and natural products (Nogueira-Filho et al., 2002; Andrade et al., 2009; Yévenes et al., 2009). Hence, natural products have been proposed in an attempt to minimize undesirable effects caused by synthetic products, such as tooth staining, imbalance of the resident microbiota and altered taste, besides representing a new antimicrobial possibility that might be used in a situation of microbial resistance (Gjermo et al., 1970; Torres et al., 2000). Nevertheless, some natural products-containing agents have been evidenced to lack scientific basis supporting their use for preventing and/or removing dental biofilm and for treating induced gingivitis.

In this perspective, considering the broad antimicrobial activity provided by natural products, this study aimed to evaluate, through a systematic literature review, if there is scientific evidence ensuring the safe and effective use of natural product(s)-containing mouthwashes as adjunctive treatment of biofilm-induced gingivitis.

MATERIAL AND METHOD

A systematic review of scientific studies was undertaken according to the methodology proposed by Higgins & Green (2008).

Systematic selection of articles was performed by establishing the following criteria:

Inclusion criteria

- **Study design:** randomized clinical trials and systematic reviews.

- Sample: all ages and both genders.

- Study Product: natural product(s)-containing mouthwashes.

- Intervention: product rinsing for any pre-determined time period, concentration, quantity and dosing interval.

- Primary Outcome of interest: reduction or not in the biofilm-induced gingival inflammation levels.

- Languages: English, Spanish or Portuguese.

Exclusion criteria

- Automatically, all the studies which did not match the search strategies were excluded.

- Studies evaluating synthetic products.

- Studies evaluating other dosage forms such as dentifrice, gel etc instead of mouthwash.

- Studies whose clinical condition did not characterize the context of interest, for instance, gingivitis induced by orthodontic appliances.

Search strategies

Identification of papers was performed through systematic searches in the databases MEDLINE (from 1966); SciELO (Scientific Electronic Library Online); LILACS (Latin American and Caribbean Literature on Health Sciences) and Cochrane Library. All existing articles related to the topic and published up to September 26th 2010 were selected for analysis.

The search strategy in MEDLINE was: (gingivitis AND natural product) OR (gingivitis AND phytotherapy) OR (gingivitis AND mouthwash) OR

(gingivitis AND mouthrinse). To refine the search, the filters *Humans* and *Clinical trial* were activated.

The search strategy in SciELO, LILACS and Cochrane Library was: (gingivitis AND natural product) OR (gengivite E produto natural) OR (gingivitis AND phytotherapy) OR (gengivite E fitoterapia) OR (gingivitis AND mouthwash) OR (gengivite E enxaguatório) OR (gingivitis AND mouthrinse) OR (gengivite E colutório). Due to shortage of articles in Scielo database, the general descriptor (gingivitis) OR (gengivite) was used and all found studies were assessed.

Another strategy consisted in manual search in reference lists from the identified and selected articles.

Four examiners independently analyzed the methodological quality of the selected articles (title and abstract) to verify if they met the previously established inclusion criteria to be incorporated into the review.

Examiners agreed that in cases that did not allow identifying whether the article should be included or not (only by reading its title and abstract), it would be requested and read in full. The final verdict on which articles would be included in the study was reached by consensus.

Finally, the selected studies were evaluated in accordance with an analysis protocol.

Protocol for analysis of included studies

Table 1 demonstrates the variables that were examined in the included studies.

RESULT

According to the previously set strategy, literature searches resulted in 503 articles. From that total, 18 studies met the inclusion criteria and were selected for analysis. Subsequently, after thorough

TABLE 1. Protocol followed by examiners for analysis of the studies included in the systematic review.

1. Preliminary Analysis
Title; Main Author; Country; Language; Journal/Year of publishing
2. Methodological Analysis
Primary outcome of interest: anti-gingivitis effect
1. Assessment of quality of the trials: *Jadad Scale* (Jadad et al., 1996)
The scale checks the validity of evidence on interventions and evaluates three conditions: Randomization, Blinding and Loss of follow-up. Based on these criteria, scores were assigned to the studies ranging from 0 to 5. Studies reaching a score <3 were considered of poor quality and thus excluded from this review.
1. Methodological design
4. Type of blinding and sampling unit allocation
5. Profile and sample size
6. Sample loss and reasons
7. Masking of product color, taste and/or smell
8. Presence and characterization of Placebo or Control Group
9. Comparability between the experimental and control groups at the beginning of the study: group description in order to evaluate the equivalence between them at baseline
10. Citation of a pilot study
11. Quality of outcome's measurement: intra or inter-examiners calibration
12. Indexes used to assess gingival condition and amount of biofilm
13. Statistical analysis and significance level employed
14. Type of clinical trial (Phase I, II, III or IV) according to Lapa et al. (2007)
2.1. Intervention Analysis
1. Experimental product: tincture, essential oil, extract etc.
2. Product concentration
3. Dosing interval: quantity and frequency per day; at which time the product had been used.
4. Time of usage (e.g.: 2 weeks)
5. Assessment intervals of gingival condition and amount of biofilm
6. Treatment adherence and adverse effects: patient's complaints about side effects or discomfort caused by the product
3. Analysis of Results
Verification of accuracy according to the confidence interval and sample size
4. Analysis of Conclusions
Attesting if the conclusion met the aims of the study

analyses, 08 high-quality studies were included in the final review (Figure 1).

In the Cochrane library, no systematic review relating to the topic was found. Nevertheless, 22 records of controlled trials which had already been included from other sources were identified and 02 articles not yet counted in the search were considered for analysis.

Data Synthesis

The included clinical trials were conducted in six countries: India, Nepal, Thailand, USA, New Zealand and Brazil. The years of their publications ranged from 2004 to 2009 (Table 2).

As seen in Table 2, all studies were well designed and predominantly classified as phase II, randomized, blind trials, controlled, placebo-controlled or having a comparative group. All of them were parallel studies, except for the study of Khalessi et al. (2004), designed as crossover. *Jadad* Scale scores were "4" in 25% of the studies and "5" in 75%, which reflected high methodological quality.

Identification of potentially relevant studies - reading the titles and abstracts - performed by four examiners

The size of samples differed substantially in each study, without equivalence in the distribution of

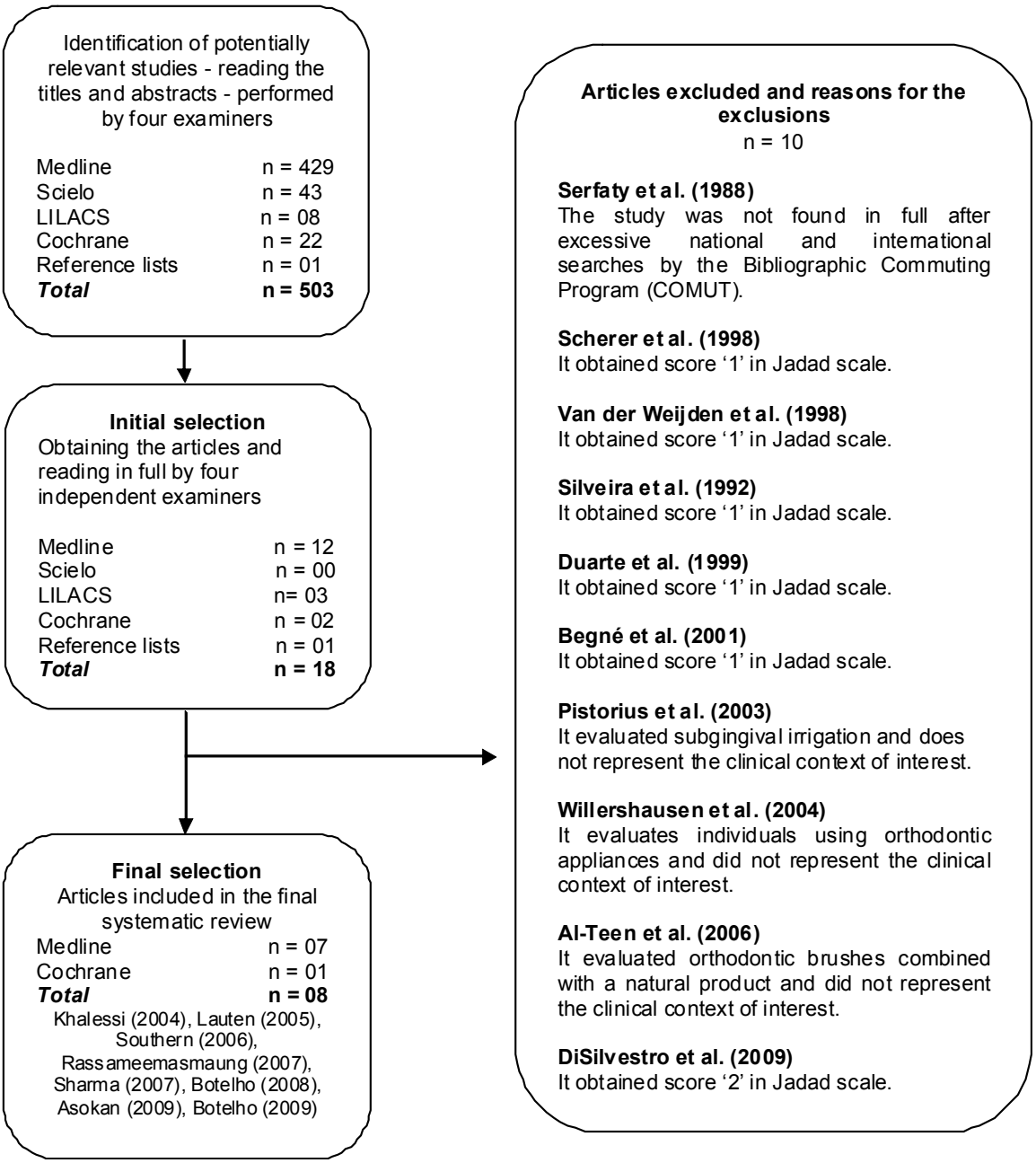

FIGURE 1. Flowchart of the search strategy.

gender and age (ranged from 12 to 69 years old). All studies included men and women in their samples, except the study of Asokan et al. (2009), in which only men were included (Table 3).

It was verified that 50% of the studies had no sample loss, whereas the other ones had a sample loss varying from 4.76% to 29.0% of the total sample size. Table 3 shows the reasons why participants withdrew from the studies.

The investigation of a calibration of the study's examiners in order to verify inter- or intra-examiner agreement indicated that 50% of them did not mention a calibration process; 25% cited an intra-examiner calibration but did not mention the index-value and, finally, 25% mentioned an intra-examiner calibration, reaching a *k* statistics of 0.86.

As regards statistical analysis, significance level was unanimously set at 5% and the mostly used tests were Paired t, Student's t, Wilcoxon and Mann-Whitney (Table 3).

Table 4 shows the intervention profile, including plant species, forms of masking, plaque and gingival indexes employed, assessment intervals, adverse effects and outcomes of interest.

The period of product use varied a lot in each study, oscillating from 10 to 84 days (12 weeks), and assessment intervals were consequently diversified. With respect to evaluation measures, Plaque (Silness & Löe, 1964) and Gingival Index (Löe, 1967) were most employed.

The main adverse effects reported by the participants related to product use were bitter taste, burning sensation and mild nauseas.

Even though the studies of Khalessi et al. (2004), Southern et al. (2006), Rassameemasmaung et al. (2007) and Asokan et al. (2009) did not mention the concentration of the experimental product, mouthwashes containing *A. indica*, *G. mangostana*, *L. sidoides* and *S. indicum* were capable of reducing both the amount of biofilm and the gingival inflammation levels. Moreover, the alcoholic extract from *Salvadora persica* was capable of reducing gingivitis but did not hamper biofilm accumulation.

TABLE 2. General aspects, methodological design and quality of the studies included in the systematic review.

Main Author	Country	Language	Year of Publishing	Journal	Study Design	ClinicalTrial's Phase	*Jadad* Scale
Khalessi AM	New Zealand	English	2004	Int Dent J	Randomized crossover placebo-controlled double-blind clinical trial	Phase II	4
Lauten JD	USA	English	2005	Phytotherapy Res	Randomized placebo-controlled double-blind clinical trial	Phases I and II	5
Southern EM	USA	English	2006	JDent Hygiene	Randomized placebo-controlled double-blind clinical trial	Phase II	5
Rassameemasmaung S	Thailand	English	2007	J Int Acad Periodontol	Randomized placebo-controlled double-blind clinical trial	Phase II	4
Sharma S	Nepal	English	2007	J Clin Ped Dent	Randomized double-blind clinical trial	Phase II	5
Botelho MA	Brazil	English	2008	J. Med. Plant. Res.	Randomized controlled double-blind clinical trial	Phase II	5
Asokan S	India	English	2009	Indian J Dent Res	Randomized controlled triple-blind clinical trial	Phase II	5
Botelho MA	Brazil	English	2009	Phytotherapy Res	Randomized controlled double-blind clinical trial	Phase II	5

TABLE 3. Characterization of the study population, sample loss, examiner calibration and statistical analysis of the studies included in the systematic review.

Main Author	SampleSize (age/gender) - All arms	Sample Loss/ Reasons	Examiners calibration	Pilot Study	Statistical Analysis
Khalessi AM	28 individuals(18- 42 years / 13 Menand 15 Women)	No sample loss	Intra-examiner. Index was not mentioned	This study is mentioned as a pilot study	Paired t test and Wilcoxon.P<.05 / Stata statistical software (Stata corporation)
Lauten JD	20 individuals(Over 18 years / 3 Menand 17 Women)	03 Individuals / Adverse effect (1); Withdrawal for participation in another study (1) and Required treatment with antibiotics (1).	Not mentioned	This study is mentioned as a pilot study	ANCOVA (co- analysis of variance).P<.05 / SASR8.2
Southern EM	63 individuals(Average 25 years/ 35 Men and 28 Women)	03 individuals / Personal reasons	Intra-examiner. Index was not mentioned	Not mentioned	ANOVA, followed by Tukey's test.P<.05 / Program was not mentioned
Rassameemasmaung S	60 individuals(17-37 years / 48 Menand 12 Women)	No sample loss	Not mentioned	Not mentioned	Paired t test, Wilcoxon and Mann-Whitney test.P<.05 / Program was not mentioned
Sharma S	80 individuals(12-20 years / 42 Menand 38 Women)	No sample loss	Not mentioned	Not mentioned	Chi-square test, Student's t test, F-test and WilcoxonP<.05 / SPSS (Inc., Chicago)
Botelho MA	54 individuals(17-65 years / 33 Menand 21 Women)	05 individuals / They did not follow the study protocol	Intra-examiner. *k* statistics: 0.86	Not mentioned	Fischer's exact test, Mann-Whitney test and Wilcoxon.P<.05 / Stata statistical software (Stata corporation)
Asokan S	20 individuals(16-18 years / All Men)	No sample loss	Not mentioned	Not mentioned	Paired t test and Student's t test. P<.05 / SPSS (Inc., Chicago)
Botelho MA	55 individuals(18-69 years / 29 Menand 26 Women)	16 individuals / They did not follow the study protocol or were not found in follow-up visits	Intra-examiner. *k* statistics: 0.86	This study is mentioned as a pilot study	Fischer's exact test, Mann-Whitney test and Wilcoxon.P<.05 / Stata statistical software (Stata corporation)

TABLE 4. Characterization of products, forms of masking, indexes employed, assessment intervals and outcomes of interest of the studies included in the systematic review.

Main Author	Product / Concentration	Control / Placebo or Comparative Group(s)	Masking (matching the products)	Dosing Interval	Time of Usage	Adverse Effects regarding the test product	Indexes Employed	Assessment Intervals	Reduction* in the AB and GI, respectively, when compared to baseline
Khalessi AM	Commercial product containing alcoholic extract from *Salvadora persica* (PursinaLtda, Teheran, Iran). Concentration was not mentioned	Placebo:Not specified	Not mentioned	2 x day, 20sec.15 drops at every 15mL of water	6 weeks, (3 weeks before and 3 after a *washout* period of 8 weeks	Not mentioned	Plaque index (Silness & Löe, 1964)Gingival index (Löe, 1967)	*Baseline,* after 03 weeks e 03 weeks after a washout period	**- / +**
Lauten JD	Essential oils from *Melaleucaalternifolia* (0.67%), *Leptospermum scoparium* (0.33%), and extracts from *Calendula officinalis* (1%) and *Camellia sinensis* (0.5%) plus ethanol in water (12.8%)	Placebo: 12.8% of ethanol in water	Not mentioned	2 x day, 30sec.15mL	12 weeks	Lighthea-dedness within 30 minutes of rinsing	Plaque index (Quigley & Hein, 1962) Gingival index (Löe, 1967)	*Baseline*, after 06 weeks and 12 weeks	**- / -**
Southern EM	Commercial product containing Echinacea, goldenseal, calendula,aloe, bloodroot, grapefruit seed extract, citric acid, mint oil, peppermint oil and cinnamon / Concentration was not mentioned	Placebo: Not specified	Not mentioned	2 x day, -15mL	12 weeks	Not mentioned	Plaque index (Silness & Löe,1964) Gingival index (Löe,1967) Bleeding on Probing index (Greenstein, 1984)	*Baseline,* after 01 month, 02 months and 3 months	**- / -**
Rassameemasmaung S	Extract from the pericarp of *Garcinia mangostana L* / Concentration was not mentioned	Placebo :Not specified	Not mentioned	2 x day, 1 min. 15mL	14 days	No adverse effect	Plaque index (Silness & Löe, 1964) Papillary Bleeding index (Saxer & Muhlëmann, 1975)index (Löe, 1967)	*Baseline* andafter 14 days	**+ / +**

Statistically significant reduction (P>.05) in the amount of biofilm and / or gingival inflammation levels, respectively, promoted by the experimental product when compared to the initial condition (baseline). The symbol (+) represents a statistically significant reduction, whereas the symbol (-) represents no reduction in the values. AB: Amount of Biofilm; GI: Gingival Inflammation.

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Sharma S	Extract from *Azadirachta indica* / 1%	Comparative Groups- Listerine (Davis Ltd., Hyderabad, India)- Povidone (1%) (Win Medicare, New Delhi, India)- Chlorhedixine (0.2%), (ICPA Health Products Ltd.,Ankleshwar, India)	Not mentioned	2 x day, 1 min.10mL	14 days	Bitter taste	Plaque index (Silness & Löe,1964)Gingival index (Löe,1967)	*Baseline* andafter 14 days	- / - + / +
Botelho MA	Extract from leaves of *Azadirachta indica* (25%), Saccharin (20%), Peppermint oil (<0.1%) as flavor and amaranth red color	Control: Chlorhexidine 0.12% (Periogard, Colgate, Brazil)	Color, taste and smell	2 x day, 30sec. 15mL	1 week of use and 30 days of assessment	Burning sensation andmild nausea	Plaque index (Silness & Löe, 1964) Gingival Bleeding index (Ainamo & Bay, 1975)Gingival index (Löe, 1967)	*Baseline*, after 01 week and 01 month	+ / + + / +
Asokan S	Commercial product containing the oil from *Sesamumindicum* (Idhayam Oil, VVV Sons India) /Concentration was not mentioned	Control:Mouthwash of Chlorhexidine 0.12% (Rexidine, Warren India)	Not mentioned	1 x day, 1 min.-	10 days	Not mentioned	Plaque index (Silness & Löe, 1964)Modified Gingival index (Lobene, 1986)	*Baseline* andafter 10 days	
Botelho MA	Essential oil from the leaves of *Lippia sidoides* (without alcohol)1%	Control:Chlorhexidine 0.12% (Periogard, Colgate, Brazil)	Color, taste and smell	2 x day, 30sec.15mL	1 week of use and 30 days of assessment	Burning sensation, bad taste and mild nausea	Plaque index (Silness & Löe, 1964) Gingival Bleeding index (Ainamo & Bay, 1975)Gingival	*Baseline*, after 01 week and 01 month	

Statistically significant reduction (P>.05) in the amount of biofilm and / or gingival inflammation levels, respectively, promoted by the experimental product when compared to the initial condition (baseline). The symbol (+) represents a statistically significant reduction, whereas the symbol (-) represents no reduction in the values. AB: Amount of Biofilm; GI: Gingival Inflammation.

DISCUSSION

Systematic reviews have been recommended as the best source of evidence to guide clinical decisions (Lavis et al., 2004). A synthesis by this type of study leads to a comprehensive answer to clinical questions, with least possible systematic errors and with high internal validity (Mickenautsch, 2010). In this perspective, there is a clear need to verify if there is scientific evidence to indicate natural product(s)-containing mouthwashes as adjunctive treatment against biofilm-induced gingivitis.

The use of antimicrobial mouthrinses as an adjuvant to mechanical methods for controlling dental biofilms and gingival inflammation is well established (Cortelli, 2010; Gunsolley, 2010; Marsh, 2010). In addition, some clinical trials have demonstrated that the clinical value of a mouthrinse may meet or exceed the value of flossing for interproximal cleaning (Sharma et al., 2002; Bauroth, 2003).

A recent systematic review aimed at evaluating the efficacy of anti-plaque and anti-gingivitis mouthrinses and at determining the clinical relevance of this evidence found that the clinical benefits of mouthrinses containing chlorhexidine and essential oils are similar to the benefits of oral prophylaxis and oral hygiene instructions at six-month recall appointments (Gunsolley, 2010).

Among the products that presented significant anti-gingivitis activity are two commercial products containing oil of *S. indicum* and extract of *S. persica* and three products prepared by the own researchers or collaborators from extracts of *A. indica*, *G. mangostana* and from the essential oil of *L. sidoides.*

It is noteworthy that the type of plant byproduct (crude extract, essential oil, fractions etc), as well as the used part of the plant, influences the biological activity of the product. Of the studies that showed satisfactory results, only Rassameemasmaung et al. (2007), Botelho et al. (2008) and Botelho et al. (2009) specify which part of the plant was used in the mouthrinse formulation.

Sesame indicum oil is relatively high in unsaponifiable substances. The unsaponifiable fraction, a class of substances not found in other fats (sesamin or sesamolin) can probably protect the oral cavity from infection and inflammation by its antioxidant property (Asokan et al., 2009). By performing a microbiological assessment, the authors found a considerable reduction in the colony counting of aerobic microrganisms promoted by the product (Asokan et al., 2009).

Sesame oil compounds have multiple physiological functions, such as estrogenic activity, providing anti-inflammatory functions, decreasing blood lipids and arachidonic acid levels (Hirata et al., 1996; Kita,1998) and increasing antioxidant capability and ã-tocopherol bioavailability (Jannat et al., 2010).

Salvadora persica has been known to contain several biologically active chemical constituents such as fluoride, volatile oils, flavonoids, alkaloids, steroids, terpenoids, saponins, and carbohydrates (Wolinsky & Sote, 1984; Abdillahi et al., 2010). It was demonstrated that the extract from *S. persica* promoted significant reductions in gingival bleeding (Table 4) and in the carriage of cariogenic bacteria (Khalessi et al., 2004).

Azadirachta indica presents a vast array of biologically active compounds that are chemically diverse and structurally complex. All parts of the plant - leaves, flowers, seeds, fruits, roots and bark – have been traditionally used for the treatment of inflammation, infections, fever, skin diseases and dental disorders (Subapriya & Nagini, 2005). A study recorded the properties of 150 triterpenoids from *A. indica* leaves (Akhila & Rani, 1999).

The extract from the leaves of *A. indica* at 25% was tested, reaching significant reductions in the amount of biofilm and gingivitis when compared to the baseline (Botelho et al. 2008). The same result was not found at a concentration of 1% (Sharma et al., 2007).

Garcinia mangostana L. has many pharmacologically active compounds, especially its pericarp. It is rich in a variety of oxygenated and prenylated xanthones (Peres & Nagem, 1997; Peres et al., 2000). This species has been considered to have antioxidant, anti-inflammatory, cytotoxic, and antifungal activities (Jung, 2006). Recently, three new compounds trivially named garcimangosxanthone A–C (1-3) have been isolated along with fourteen known xanthones from the pericarp of this plant (Zhang et al., 2010).

Besides its anti-gingivitis efficacy, the mouthwash of *G. mangostana* L. pericarp was also confirmed to significantly reduce the levels of volatile sulfur compounds when compared to the baseline and to a placebo. Thus, such product has also been considered an alternative option in treating malodor (Rassameemasmaung et al., 2007).

A study investigated the chemical composition and the antimicrobial activity of the essential oil from *L. sidoides*. Thymol has been the main component found and might be associated with the antimicrobial activity presented by that natural product (Fontenelle et al., 2007). Botelho et al. (2009) also demonstrated that the essential oil from the leaves of *L. sidoides* (1%) is responsible for a decrease in the levels of salivary mutans streptococci.

Limitations could be found in the studies of Khalessi et al. (2004), Southern et al. (2006), Rassameemasmaung et al. (2007) and Asokan et al. (2009) as the concentration of the experimental products were not cited. Thus, it is inappropriate to indicate these products without knowing their

concentrations, since the concentration of the product may influence its biological action. Another shortcoming relates to the absence of a standard mouthwash protocol, hindering inter-study comparisons, with regard to the quantity of product to be rinsed, and the rinsing time and frequency.

The names of the herbaria where the voucher specimens were deposited, as well as their registration numbers, were not reported in the methodologies of the reviewed articles, except for the studies of Lauten et al. (2005), Botelho et al. (2008) and Botelho et al. (2009). Such information is important in view of the need of highlighting data of the studied species, once the plant needs to be reliably identified.

Concerning the safety for clinical use, it is emphasized that only the study of Lauten et al. (2005) used phase-I experimental clinical model to verify possible side effects of the product in healthy individuals, as seen in Table 2. This step must be considered indispensable for the development of subsequent clinical trials, such as phase II, guaranteeing thus the development of an effective and safe product.

It is considered that for phase II clinical trials the sample must consist of at least 30 individuals in each group (experimental and control or placebo arms) (Lapa et al., 2007); however, three out of the eight studies did not obey this sample size criterion (Table 3). Ideally, clinical trials should be large enough to reliably detect the smallest possible differences in the primary outcome with treatment that are considered clinically worthwhile (Kirby et al., 2002).

Nonetheless, the criteria for sample exclusion in the studies were well designed, such as individuals who made use of systemic antibiotics or non-steroidal anti-inflammatory drugs or used mouthwashes prior to the study, with complicating systemic conditions or hormonal alteration (e.g. pregnant women), had less than a determined number of dental elements, used orthodontic brackets, among others. This selection becomes crucial since it is important to prevent any type of bias that could influence the results.

Study subjects can modify their behavior or their way of reporting outcomes (including adverse effects) systematically if they are aware of the treatment they are going to receive (Panutti, 2009). Thus, the masking of products as regards color, taste, smell and administration methods is very important. This procedure ensures that neither the subject under study nor the examiner identifies the intervention arm and therefore be biased. In this sense, only the studies of Botelho et al. (2008) and Botelho et al. (2009) masked the products (Table 4).

Besides this possibility of bias, the existence of the Hawthorne effect (Panutti, 2009) should still be considered; it is a form of reactivity whereby subjects improve or modify an aspect of their behavior being experimentally measured simply in response to the fact that they are being studied. In this case, there would be an improvement in the oral hygiene, interfering in the results. This effect was considered by Southern et al. (2006), Rassameemasmaung et al. (2007) and Botelho et al. (2009).

Examiner reliability is a very significant issue in research. Different measures of reliability may be used to assess intra- or inter-examiner agreement. In spite of that, four out of the eight studies did not mention any calibration process in order to assure the reproducibility of measurements.

An inferential statistical analysis to evaluate which product was most effective against gingivitis would be ideal. However, due to those differences in methodological criteria, such as interval and quantity of the product to be rinsed, time of usage, different forms of assessment, among others, equivalence of contexts for analysis could not be detected, which might reflect biases.

Jadad Scale was adopted in this review as it checks the validity of evidence on interventions and evaluates methodological quality, allowing thus an objective assessment (Jadad et al., 1996). Several studies, including systematic reviews, have already embraced this validated evaluation tool (Linde et al., 2001; Hu et al., 2002; Beek et al., 2004; Huntley & Ernst, 2004).

Summarizing, *S. indicum*, *G.mangostana,* and *S. persica* showed reduction in the amount of biofilm and gingivitis levels. Nevertheless, this review cannot indicate with certainty their general use as adjunctive treatment for biofilm-induced gingivitis since the authors did not mention the concentration and/or the used part of the natural product, even though two of these products have already been marketed.

However, there is strong evidence to indicate the essential oil from the leaves of *L. sidoides* (1%) and the extract from the leaves of *A. indica* (25%) to significantly reduce biofilm and gingival inflammation levels.

It has been recommended the conduction of other clinical trials in this line of research evaluating different concentrations and time of usage, relating to the products reported in this study, proposing a protocol for standardization of interventions (quantity, frequency and duration of rinsing) and, finally, investigating adverse effects arising from prolonged use on the teeth and oral mucosa, as well as substantivity and inactivation in the gastrointestinal tract.

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